Eleventh Edition

AVERY'S DISEASES of the NEWBORN

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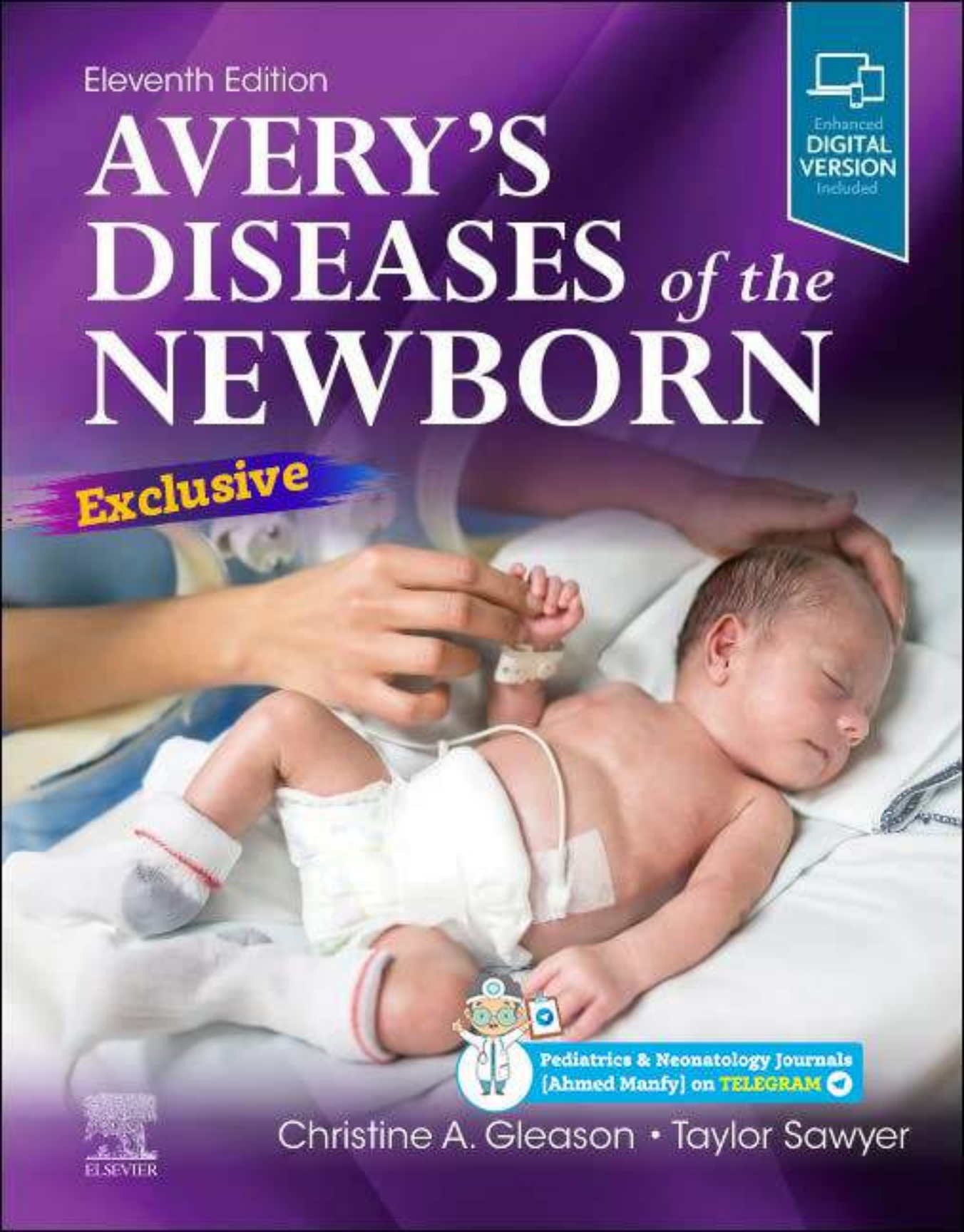

Christine A. Gleason • Taylor Sawyer

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Eleventh Edition

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AVERY'S DISEASES OF THE NEWBORN, ELEVENTH EDITION **ISBN: 978-0-323-82823-9**

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The Publisher

Publisher: Sarah Barth
Senior Content Development Manager: Meghan Andress
Content Development Specialist: Erika Ninsin
Publishing Services Manager: Catherine Jackson
Senior Project Manager: John Casey
Design Direction: Brian Salisbury

Printed in India 9 8 7 6 5 4 3 2 1

To the babies—our patients—who humble and inspire us.
To their families, who encourage us to keep moving our field forward.
To neonatal caregivers everywhere, with gratitude for all you do.

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Preface

"The neonatal period therefore represents the last frontier of medicine, territory which has just begun to be cleared of its forests and underbrush in preparation for its eagerly anticipated crops of saved lives."

From the Introduction to the 1st edition of *Diseases of the Newborn*

The History of *Diseases of the Newborn*

Diseases of the Newborn was one of the first books dedicated to the diagnosis and treatment of problems of the neonate. The 1st edition was published in 1960 by Dr. Alexander Schaffer, a well-known Baltimore pediatrician who first coined the terms *neonatology* and *neonatologist*. He described neonatology as an emerging pediatric subspecialty concentrating on the "art and science of diagnosis and treatment of disorders of the newborn infant," and a neonatologist as a "physician whose primary concern lay in that specialty." Dr. Schaffer served as sole author for both the 1st and 2nd editions (1966) of the book. Dr. Mary Ellen Avery joined Dr. Schaffer as a co-author for the 3rd edition in 1971. Drs. Avery and Schaffer recognized that their book needed multiple contributors with subspecialty expertise as they developed the 4th edition in 1977, and they became co-editors, rather than co-authors. Dr. Schaffer died in 1981 and Dr. H. William Taeusch joined Dr. Avery in 1984 as co-editor for the 5th edition. Dr. Roberta Ballard joined Drs. Taeusch and Avery for the 6th edition in 1991, then titled, *Schaffer & Avery's Diseases of the Newborn*. The 7th edition, edited by Drs. Taeusch and Ballard, was published in 1998, and was entitled *Avery's Diseases of the Newborn*, in recognition of Dr. Avery's diligent work on the book through four editions over 20 years. Dr. Christine Gleason joined Drs. Taeusch and Ballard in 2005 as editors for the 8th edition. In 2009, Drs. Avery, Taeusch, and Ballard retired from editing *Avery's*, and became "editors emeriti." Sadly, Dr. Avery passed away in 2011. Her legacy lives on, however, in the title of this book. Dr. Sherin Devaskar joined Dr. Gleason in 2012 as co-editor for the 9th edition—the first edition with accompanying online content. For the 10th edition, Dr. Sandra "Sunny" Juul teamed with Dr. Gleason as co-editor, marking the first time since the 5th edition that all editors were faculty at the same institution. For this new, 11th edition, Dr. Taylor Sawyer, also on the faculty at Dr. Gleason's institution, joins as co-editor. This edition marks the fourth that Dr. Gleason has co-edited, making her the longest serving editor since Dr. Avery.

The 1st edition of *Diseases of the Newborn* was used mainly for diagnosis, but also included descriptions of early neonatal therapies that had led to a remarkable decrease in the infant mortality rate in the United States: from 47 deaths per 1000 live births in 1940 to 26 per 1000 in 1960. However, a pivotal year for the fledgling subspecialty of neonatology came in 1963, 3 years after the first publication of *Diseases of the Newborn*, with the birth of President John F. Kennedy's son, Patrick Bouvier Kennedy. Patrick was a preterm infant, born at 34-35 weeks' gestation, and his death at 3 days of age from complications of respiratory distress syndrome accelerated the development of infant ventilators, which, coupled with micro-blood gas analysis and the use of umbilical artery catheterization, led to the development of newborn intensive care in the late 1960s.

Advances in neonatal surgery and cardiology, along with ongoing technological innovations, stimulated the development of neonatal intensive care units and regionalization of care for sick newborn infants over the next several decades. These developments were accompanied by an explosion of research that improved our understanding of the pathophysiology and genetic basis of diseases of the newborn. This in turn led to spectacular advances in neonatal diagnosis and therapeutics—particularly in the care of preterm infants. Combined, these advances have resulted in significant reductions in infant mortality worldwide: from 6.45% in 1990 to 2.82% in 2019. Current research efforts are focused on decreasing the unacceptable regional, ethnic, and global disparities in infant mortality, improving neonatal long-term outcomes, advancing neonatal therapeutics, preventing newborn diseases, and finally—teaming with our obstetrical colleagues—preventing prematurity. This edition tries—as all prior editions have—to translate the findings of ongoing research into practical advice for use at the bedside by neonatal caregivers.

What's New and Improved About This Edition?

Perhaps the most significant change to this edition is what was removed rather than what was added. We carefully reviewed the 10th edition's table of contents, examining each chapter with a keen eye on keeping the book targeted on diseases of the newborn, bringing the content more in line with the original editions. Thus, several chapters that were not specifically disease-focused were archived, while chapters in some sections were subdivided into new chapters focused on disease-specific content.

This book continues to be thoroughly (and sometimes painfully) revised and updated by some of the best clinicians and investigators in their fields—several of whom are new contributors. Some chapters required more extensive updates than others. For all chapters, however, we challenged authors to decrease the word count, use boxes, tables, and figures to break up dense text, and to do their best to make the content as disease-focused as appropriate. This resulted in a more concise, readable, and hopefully, clinically helpful text. We are so grateful to our authors for their contributions and hope readers appreciate their work.

Do We Still Need Textbooks?

With the incredible amount of information immediately available on the internet, what's the value of a textbook? We believe that textbooks, such as *Avery's Diseases of the Newborn*, will always be needed by clinicians striving to provide state-of-the-art neonatal care, by educators working to train the next generation of caregivers, and by investigators diligently advancing neonatal research and scholarship. A textbook's content is only as good as its contributors. This book, like in previous editions, has awesome contributors.

The authors were chosen for their expertise and ability to integrate their knowledge into a comprehensive, readable, and useful chapter. They did this in the hope that their syntheses could, as Ethel Dunham wrote in the foreword to the 1st edition, "spread more widely what is already known … and make it possible to apply these facts."

We are grateful that the online content of this textbook enjoys increasing popularity. However, we still find printed copies of this and other books lying dog-eared, coffee-stained, annotated, and broken-spined in places where neonatal caregivers congregate.

With each subsequent edition, the authors of *Diseases of the Newborn* help fulfill Dr. Schaffer's vision of clearing the underbrush from the last frontier of medicine in preparation for its eagerly anticipated crops of saved neonatal lives. Textbooks connect us to the past, bring us up to date on the present, and prepare and excite us for the future. We will always need them, in one form or another. To that end, we have challenged ourselves to meet, and hopefully exceed, that need—for our field, for our colleagues, and for the babies entrusted to our care.

Acknowledgments and Gratitude

We wish to thank some key staff at Elsevier— our Content Development Specialist, Erika Ninsin; our Senior Content Development Manager, Meghan Andress; our Publishing Services Manager, Catherine Jackson; our Senior Project Manager, John Casey; our Design Director, Brian Salisbury; and our Publisher, Sarah Barth. They demonstrated patience, guidance, and persistence; without them, we would still be hard at work, trying to make this book a reality! We also wish to thank our colleagues at our academic institution, the University of Washington, especially our Division Chief, Sunny Juul, and our Department Chair, Leslie Walker-Harding, whose leadership and unwavering support have meant so much to us both. We are deeply indebted to our chapter authors, who wrote the book and did so willingly, enthusiastically, and (for the most part) in a timely fashion—despite myriad other responsibilities in their lives, and a worldwide COVID-19 pandemic! Finally, we are extremely grateful for the support of our families throughout the long, and often challenging, editorial process.

Christine Gleason and **Taylor Sawyer**

Contents

1 Neonatal and Perinatal Epidemiology

NIGEL PANETH, SIMRAN PATEL, AND THOMAS MICHAEL O'SHEA JR.

KEY POINTS

- Maternal and child health in the population have traditionally been assessed by monitoring two key statistics—the maternal mortality ratio and the infant mortality rate. The infant mortality rate is the sum of the neonatal mortality and post-neonatal mortality rates.
- Due to improvements in income, housing, birth spacing, and nutrition, along with public health interventions to produce cleaner food and water, improve maternal and infant nutrition, and immunize mothers and infants against infectious diseases, maternal mortality and infant mortality declined steadily through the 20th century. By 2000, neonatal mortality had declined by 90% from its 1915 value, postneonatal mortality by 93%, and maternal mortality by 98%.
- In high-income countries, the leading causes of neonatal mortality are preterm birth and congenital anomalies. The leading cause of postneonatal mortality is sudden infant death syndrome.
- Health disparities are especially prominent in the perinatal period. Even as rates of infant mortality decline in both Black and White babies, infant mortality among Black babies remains about twice that of White infant mortality in the United States.
- Despite comparable, or lower, birthweight-specific infant mortality rates, the United States has one of the highest infant mortality rates among high-income countries. This surprising phenomenon is due to the striking excess of preterm births in the United States, as compared with other high-income countries.
- Notable improvements in health outcomes resulting from epidemiologic research include reductions in neural tube defects (reduced by prenatal folate), sudden infant death syndrome (reduced by supine infant sleeping), and cerebral palsy among preterm infants (reduced by maternal magnesium sulfate).

Introduction—Epidemiologic Approaches to the Perinatal and Neonatal Period

The period surrounding the time of birth, the *perinatal* period, is a critical window in human development, as the infant makes the transition from its dependence upon maternal and placental support—oxidative, nutritional, and endocrinologic—to establishing independent life. That this transition is not always successful is signaled by an annualized mortality rate in the neonatal period that is not exceeded until age 85 and older,[1] and risks for damage to organ systems, most notably the brain, that can be lifelong. However, years must pass before the effects on higher cortical functions of insults and injuries occurring during the perinatal period can reliably be detected. Epidemiologic approaches to the perinatal period must therefore be bidirectional—looking backward to examine the causes of adverse health conditions that arise during the perinatal period and looking forward to seeing how these conditions shape disorders of health found later in life.

Traditionally the perinatal period was described as extending from 28 weeks of gestation until 1 week of life, but in 2004 the World Health Organization (WHO) antedated the onset of the perinatal period to 22 weeks.[2] For the purposes of this discussion we will define perinatal more expansively, as including the second half of gestation (by which time most organogenesis has occurred, but growth and maturation of many systems has yet to occur) and the first month of life. The *neonatal* period, usually considered as the first month of life, is thus included in the term perinatal, reflecting the view that addressing the problems of the neonate requires an understanding of intrauterine phenomena.

Health Disorders of Pregnancy and the Perinatal Period

Key Population Mortality Statistics

Maternal and child health in the population have traditionally been assessed by monitoring two key statistics—the maternal mortality ratio and the infant mortality rate. A maternal death is defined by the WHO as the death of a woman during pregnancy or within 42 days of pregnancy.[3] Because maternal deaths are not part of the denominator of births, the resulting fraction is referred to as the **maternal mortality ratio**. When the cause of death is attributed to a pregnancy-related cause, it is described as *direct*. When pregnancy has aggravated an underlying health disorder present before pregnancy, the death is termed an *indirect* maternal death. The WHO recommends that both direct and total (direct plus indirect) maternal mortality rates be monitored. Typically, the ratio is indexed to 100,000 births.

Because pregnancy can contribute to deaths beyond 42 days, the term "late maternal death" has been used to describe the death of a woman from direct or indirect obstetric causes more than 42 days but less than 1 year after termination of pregnancy.

These later deaths are not usually included in tabulations of maternal mortality in vital data,[4,5] although they are included in "pregnancy-associated mortality" as defined by the Centers for Disease Control and Prevention (CDC).[6]

Deaths unrelated to pregnancy, but occurring within 42 days of pregnancy, are termed *incidental* maternal deaths and are not included in maternal mortality.[7] However, even incidental deaths may bear a relation to pregnancy; for example, homicide and suicide are more common in pregnancy and shortly thereafter and might not be entirely incidental to it.[8,9]

In most geographic entities, infant mortality is defined as all deaths occurring from birth to 365 days of age. The infant mortality rate is the number of infant deaths in a calendar year divided by the number of births occurring in the same year. This approach makes for imprecision because some deaths in the examined year occurred to the previous year's birth cohort, and some births in the examined year will die as infants in the following year. In recent years, birth-death linkage has permitted vital registration areas in the United States to provide infant mortality rates that avoid this imprecision. The standard infant mortality rate reported by the National Center for Health Statistics (NCHS) links *deaths* for the index year to all births to whom the death occurred, including births that took place the *previous* year. This form of infant mortality is termed *period* infant mortality. An alternative procedure is to take births for the index year and link them to infant deaths, including those taking place the *following* year. This is referred to as *birth cohort* infant mortality and is not used for regular annual comparisons because it cannot be completed in as timely a fashion as can period infant mortality.[10] The denominator for all forms of infant mortality is 1000 live births.

Infant deaths are conventionally divided into deaths in the first 28 days of life (*neonatal* deaths) and deaths later in the first year *(postneonatal* deaths). Neonatal deaths, which are largely related to preterm birth and birth defects, tend to reflect the circumstances of pregnancy and birth; postneonatal deaths, when high, are largely from infection, often in the setting of poor nutrition. In high-income countries (HICs), neonatal deaths have for many years made up a larger proportion of infant mortality than postneonatal deaths. This has been true of the United States since 1921, and in recent years the ratio of neonatal to postneonatal deaths in the United States has consistently been approximately 2 to 1. Until quite recently, postnatal deaths outnumbered neonatal deaths in low- and middle-income countries (LMICs), but in 2019, infant mortality was 28.2/1000 live births in LMICs while neonatal mortality was 17.9/1000 live births, indicating that infant mortality in LMICs is beginning to resemble patterns seen in HICs.[11]

Perinatal mortality is a term used for a rate that combines stillbirths and neonatal deaths in some fashion.[2] Stillbirth reporting prior to 28 weeks is probably incomplete, even in the United States, where such births are required to be reported in every state.[12] Nonetheless, stillbirths continue to be reported at a level not much lower than that of neonatal deaths, and our understanding of the causes of stillbirth remains very uncertain.[13]

Sources of Information on Mortality—Vital Data

All US mortality data depend upon the collection of information about all births and deaths. Routinely collected vital data are the nation's key resource for monitoring progress in caring for mothers and children. Annual counts of births and deaths collected by the 52 vital registration areas of the United States (50 states, District of Columbia [DC], and NYC) are assembled into national data sets by the NCHS and described under the heading of National Vital Statistics Reports (NVSRs).[14] Unlike data collected in hospitals or clinics, or even from nationally representative surveys, birth and death certificates are required by law to be completed for each birth and death. Birth and death registration have been virtually 100% complete for all parts of the United States since the 1950s. The universality of this process renders many findings from vital data analyses stable and generalizable, although formatting changes recommended in 2003, affecting both the birth and death certificates, have created some difficulties in interpretation because the NCHS can only *recommend* format revisions in vital data certificates; each state is free to adopt them or not.

The 2003 revision of the birth certificate emphasized recording of data from medical records rather than maternal interview and recommended the reformatting of some elements, such as date of first prenatal visit, in ways that produced differences in findings compared to an earlier revision made in 1989. To complicate matters further, states adopted the 2003 revision at different times, and for much of the next decade, both versions of the birth certificate—1989 and 2003 revisions—were in use, leading to the NCHS deciding not to issue national data for several years for the prevalence of gestational diabetes, gestational hypertension, and gestational age at initiation of prenatal care. This problem has now been resolved because, as of 2016, all 50 states, the DC, Puerto Rico, Guam, Commonwealth of the Northern Marianas, and US Virgin Islands reported data based on the 2003 US Certificate of Live Birth. American Samoa continues to report based on the earlier 1989 birth certificate revision.[15]

In 2003, the NCHS also recommended revisions to the US Standard Certificate of Death,[16] including a special checkbox for identifying whether the decedent, if female, was pregnant or had been pregnant in the previous 42 days. As with the birth certificate, this revision was variably followed by states, and it has been found that the number of deaths recognized as maternal in states that adopted the checkbox is higher than in those that did not (Fig. 1.1).[17]

The limitations of vital data are well known. Causes of death are subject to certifier variability and, perhaps more importantly, to professional trends in diagnostic categorization. The accuracy of recording of conditions and measures on birth certificates is often uncertain and variable from state to state and hospital to hospital. Yet the frequencies of births and deaths in subgroups defined objectively and recorded consistently, such as birthweight and mode of delivery, are likely to be valid.

Time Trends in Mortality Rates of the Perinatal Period in the United States

Maternal mortality and infant mortality declined steadily through the 20th century; by 2000, neonatal mortality had declined by 90% from its 1915 value, postneonatal mortality by 93%, and maternal mortality by 98%. These extraordinary and unprecedented changes are the product of a variety of complex social factors including improvements in income, housing, birth spacing, and nutrition, as well as ecological-level public health interventions that produced cleaner food and water.[18] Public health action at the individual level, including targeted maternal and infant nutrition programs and immunization programs have made a lesser, but still notable contribution. Medical care per se was, until recently, less critically involved in these declines, with the exception of the decline in maternal mortality, which was very sensitive to the developments in blood banking and antibiotics that began in the 1930s.

MOTHER

29a. DATE OF FIRST PRENATAL CARE VISIT
_____/_____/_____ No Prenatal Care
M M D D YYYY

29b. DATE OF LAST PRENATAL CARE VISIT
_____/_____/_____
M M D D YYYY

30. TOTAL NUMBER OF PRENATAL VISITS FOR THIS PREGNANCY
_____ (If none, enter A0".)

31. MOTHER'S HEIGHT
_____ (feet/inches)

32. MOTHER'S PREPREGNANCY WEIGHT
_____ (pounds)

33. MOTHER'S WEIGHT AT DELIVERY
_____ (pounds)

34. DID MOTHER GET WIC FOOD FOR HERSELF DURING THIS PREGNANCY? Yes No

35. NUMBER OF PREVIOUS LIVE BIRTHS (Do not include this child)

35a. Now Living
Number _____
None

35b. Now Dead
Number _____
None

36. NUMBER OF OTHER PREGNANCY OUTCOMES (spontaneous or induced losses or ectopic pregnancies)

36a. Other Outcomes
Number _____
None

37. CIGARETTE SMOKING BEFORE AND DURING PREGNANCY
For each time period, enter either the number of cigarettes or the number of packs of cigarettes smoked. IF NONE, ENTER A0".

Average number of cigarettes or packs of cigarettes smoked per day.

	# of cigarettes		# of packs
Three Months Before Pregnancy	_____	OR	_____
First Three Months of Pregnancy	_____	OR	_____
Second Three Months of Pregnancy	_____	OR	_____
Third Trimester of Pregnancy	_____	OR	_____

38. PRINCIPAL SOURCE OF PAYMENT FOR THIS DELIVERY
Private Insurance
Medicaid
Self-pay
Other
(Specify) _____

35c. DATE OF LAST LIVE BIRTH
_____/_____
MM Y Y Y Y

36b. DATE OF LAST OTHER PREGNANCY OUTCOME
_____/_____
MM Y Y Y Y

39. DATE LAST NORMAL MENSES BEGAN
_____/_____/_____
M M D D YYYY

40. MOTHER'S MEDICAL RECORD NUMBER

MEDICAL AND HEALTH INFORMATION

41. RISK FACTORS IN THIS PREGNANCY (Check all that apply)
Diabetes
Prepregnancy (Diagnosis prior to this pregnancy)
Gestational (Diagnosis in this pregnancy)

Hypertension
Prepregnancy (Chronic)
Gestational (PIH, preeclampsia)
Eclampsia

Previous preterm birth

Other previous poor pregnancy outcome (Includes perinatal death, small-for-gestational age/intrauterine growth restricted birth)

Pregnancy resulted from infertility treatment-If yes, check all that apply:
Fertility-enhancing drugs, Artificial insemination or Intrauterine insemination
Assisted reproductive technology (e.g., in vitro fertilization (IVF), gamete intrafallopian transfer (GIFT))

Mother had a previous cesarean delivery
If yes, how many _____

None of the above

42. INFECTIONS PRESENT AND/OR TREATED DURING THIS PREGNANCY (Check all that apply)
Gonorrhea
Syphilis
Chlamydia
Hepatitis B
Hepatitis C
None of the above

43. OBSTETRIC PROCEDURES (Check all that apply)
Cervical cerclage
Tocolysis

External cephalic version:
Successful
Failed

None of the above

44. ONSET OF LABOR (Check all that apply)
Premature Rupture of the Membranes (prolonged, ∃12 hrs.)
Precipitous Labor (<3 hrs.)
Prolonged Labor (∃ 20 hrs.)
None of the above

45. CHARACTERISTICS OF LABOR AND DELIVERY (Check all that apply)
Induction of labor
Augmentation of labor
Non-vertex presentation
Steroids (glucocorticoids) for fetal lung maturation received by the mother prior to delivery
Antibiotics received by the mother during labor
Clinical chorioamnionitis diagnosed during labor or maternal temperature ≥38°C (100.4°F)
Moderate/heavy meconium staining of the amniotic fluid
Fetal intolerance of labor such that one or more of the following actions was taken: in-utero resuscitative measures, further fetal assessment, or operative delivery
Epidural or spinal anesthesia during labor
None of the above

46. METHOD OF DELIVERY
A. Was delivery with forceps attempted but unsuccessful?
Yes No
B. Was delivery with vacuum extraction attempted but unsuccessful?
Yes No
C. Fetal presentation at birth
Cephalic
Breech
Other
D. Final route and method of delivery (Check one)
Vaginal/Spontaneous
Vaginal/Forceps
Vaginal/Vacuum
Cesarean
If cesarean, was a trial of labor attempted?
Yes
No

47. MATERNAL MORBIDITY (Check all that apply) (Complications associated with labor and delivery)
Maternal transfusion
Third or fourth degree perineal laceration
Ruptured uterus
Unplanned hysterectomy
Admission to intensive care unit
Unplanned operating room procedure following delivery
None of the above

NEWBORN

NEWBORN INFORMATION

48. NEWBORN MEDICAL RECORD NUMBER

49. BIRTHWEIGHT (grams preferred, specify unit)
_____
9 grams 9 lb/oz

50. OBSTETRIC ESTIMATE OF GESTATION:
_____ (completed weeks)

51. APGAR SCORE:
Score at 5 minutes:_____
If 5 minute score is less than 6,
Score at 10 minutes: _____

52. PLURALITY - Single, Twin, Triplet, etc.
(Specify)_____

53. IF NOT SINGLE BIRTH - Born First, Second, Third, etc. (Specify) _____

54. ABNORMAL CONDITIONS OF THE NEWBORN (Check all that apply)
Assisted ventilation required immediately following delivery
Assisted ventilation required for more than six hours
NICU admission
Newborn given surfactant replacement therapy
Antibiotics received by the newborn for suspected neonatal sepsis
Seizure or serious neurologic dysfunction
Significant birth injury (skeletal fracture(s), peripheral nerve injury, and/or soft tissue/solid organ hemorrhage which requires intervention)
9 None of the above

55. CONGENITAL ANOMALIES OF THE NEWBORN (Check all that apply)
Anencephaly
Meningomyelocele/Spina bifida
Cyanotic congenital heart disease
Congenital diaphragmatic hernia
Omphalocele
Gastroschisis
Limb reduction defect (excluding congenital amputation and dwarfing syndromes)
Cleft Lip with or without Cleft Palate
Cleft Palate alone
Down Syndrome
Karyotype confirmed
Karyotype pending
Suspected chromosomal disorder
Karyotype confirmed
Karyotype pending
Hypospadias
None of the anomalies listed above

56. WAS INFANT TRANSFERRED WITHIN 24 HOURS OF DELIVERY? 9 Yes 9 No
IF YES, NAME OF FACILITY INFANT TRANSFERRED
TO:_____

57. IS INFANT LIVING AT TIME OF REPORT?
Yes No Infant transferred, status unknown

58. IS THE INFANT BEING BREASTFED AT DISCHARGE?
Yes No

Mother's Name _____ Mother's Medical Record No. _____

• **Fig. 1.1** US Birth and Death Certificates. (A) US national standard birth certificate, 2003 version. (B) US national standard death certificate, 2003 version.

U.S. STANDARD CERTIFICATE OF DEATH

LOCAL FILE NO. STATE FILE NO.

NAME OF DECEDENT
For use by physician or institution
To Be Completed/ Verified By: FUNERAL DIRECTOR:

1. DECEDENT'S LEGAL NAME (Include AKA's if any) (First, Middle, Last) | 2. SEX | 3. SOCIAL SECURITY NUMBER

4a. AGE-Last Birthday (Years) | 4b. UNDER 1 YEAR: Months, Days | 4c. UNDER 1 DAY: Hours, Minutes | 5. DATE OF BIRTH (Mo/Day/Yr) | 6. BIRTHPLACE (City and State or Foreign Country)

7a. RESIDENCE-STATE | 7b. COUNTY | 7c. CITY OR TOWN

7d. STREET AND NUMBER | 7e. APT. NO. | 7f. ZIP CODE | 7g. INSIDE CITY LIMITS? ☐ Yes ☐ No

8. EVER IN US ARMED FORCES? ☐ Yes ☐ No | 9. MARITAL STATUS AT TIME OF DEATH ☐ Married ☐ Married, but separated ☐ Widowed ☐ Divorced ☐ Never Married ☐ Unknown | 10. SURVIVING SPOUSE'S NAME (If wife, give name prior to first marriage)

11. FATHER'S NAME (First, Middle, Last) | 12. MOTHER'S NAME PRIOR TO FIRST MARRIAGE (First, Middle, Last)

13a. INFORMANT'S NAME | 13b. RELATIONSHIP TO DECEDENT | 13c. MAILING ADDRESS (Street and Number, City, State, Zip Code)

14. PLACE OF DEATH (Check only one: see instructions)

IF DEATH OCCURRED IN A HOSPITAL: ☐ Inpatient ☐ Emergency Room/Outpatient ☐ Dead on Arrival
IF DEATH OCCURRED SOMEWHERE OTHER THAN A HOSPITAL: ☐ Hospice facility ☐ Nursing home/Long term care facility ☐ Decedent's home ☐ Other (Specify):

15. FACILITY NAME (If not institution, give street & number) | 16. CITY OR TOWN , STATE, AND ZIP CODE | 17. COUNTY OF DEATH

18. METHOD OF DISPOSITION: ☐ Burial ☐ Cremation ☐ Donation ☐ Entombment ☐ Removal from State ☐ Other (Specify):_____ | 19. PLACE OF DISPOSITION (Name of cemetery, crematory, other place)

20. LOCATION-CITY, TOWN, AND STATE | 21. NAME AND COMPLETE ADDRESS OF FUNERAL FACILITY

22. SIGNATURE OF FUNERAL SERVICE LICENSEE OR OTHER AGENT | 23. LICENSE NUMBER (Of Licensee)

To Be Completed By: MEDICAL CERTIFIER

ITEMS 24-28 MUST BE COMPLETED BY PERSON WHO PRONOUNCES OR CERTIFIES DEATH | 24. DATE PRONOUNCED DEAD (Mo/Day/Yr) | 25. TIME PRONOUNCED DEAD

26. SIGNATURE OF PERSON PRONOUNCING DEATH (Only when applicable) | 27. LICENSE NUMBER | 28. DATE SIGNED (Mo/Day/Yr)

29. ACTUAL OR PRESUMED DATE OF DEATH (Mo/Day/Yr) (Spell Month) | 30. ACTUAL OR PRESUMED TIME OF DEATH | 31. WAS MEDICAL EXAMINER OR CORONER CONTACTED? ☐ Yes ☐ No

CAUSE OF DEATH (See instructions and examples)

Approximate interval: Onset to death

32. **PART I.** Enter the chain of events--diseases, injuries, or complications--that directly caused the death. DO NOT enter terminal events such as cardiac arrest, respiratory arrest, or ventricular fibrillation without showing the etiology. DO NOT ABBREVIATE. Enter only one cause on a line. Add additional lines if necessary.

IMMEDIATE CAUSE (Final disease or condition ----------> resulting in death)
a._____ Due to (or as a consequence of):

Sequentially list conditions, if any, leading to the cause listed on line a. Enter the **UNDERLYING CAUSE** (disease or injury that initiated the events resulting in death) **LAST**
b._____ Due to (or as a consequence of):
c._____ Due to (or as a consequence of):
d._____

PART II. Enter other significant conditions contributing to death but not resulting in the underlying cause given in PART I

33. WAS AN AUTOPSY PERFORMED? ☐ Yes ☐ No

34. WERE AUTOPSY FINDINGS AVAILABLE TO COMPLETE THE CAUSE OF DEATH? ☐ Yes ☐ No

35. DID TOBACCO USE CONTRIBUTE TO DEATH? ☐ Yes ☐ Probably ☐ No ☐ Unknown

36. IF FEMALE: ☐ Not pregnant within past year ☐ Pregnant at time of death ☐ Not pregnant, but pregnant within 42 days of death ☐ Not pregnant, but pregnant 43 days to 1 year before death ☐ Unknown if pregnant within the past year

37. MANNER OF DEATH ☐ Natural ☐ Homicide ☐ Accident ☐ Pending Investigation ☐ Suicide ☐ Could not be determined

38. DATE OF INJURY (Mo/Day/Yr) (Spell Month) | 39. TIME OF INJURY | 40. PLACE OF INJURY (e.g., Decedent's home; construction site; restaurant; wooded area) | 41. INJURY AT WORK? ☐ Yes ☐ No

42. LOCATION OF INJURY: State: City or Town: Street & Number: Apartment No.: Zip Code:

43. DESCRIBE HOW INJURY OCCURRED: | 44. IF TRANSPORTATION INJURY, SPECIFY: ☐ Driver/Operator ☐ Passenger ☐ Pedestrian ☐ Other (Specify)

45. CERTIFIER (Check only one):
☐ Certifying physician-To the best of my knowledge, death occurred due to the cause(s) and manner stated.
☐ Pronouncing & Certifying physician-To the best of my knowledge, death occurred at the time, date, and place, and due to the cause(s) and manner stated.
☐ Medical Examiner/Coroner-On the basis of examination, and/or investigation, in my opinion, death occurred at the time, date, and place, and due to the cause(s) and manner stated.

Signature of certifier:_____

46. NAME, ADDRESS, AND ZIP CODE OF PERSON COMPLETING CAUSE OF DEATH (Item 32)

47. TITLE OF CERTIFIER | 48. LICENSE NUMBER | 49. DATE CERTIFIED (Mo/Day/Yr) | 50. **FOR REGISTRAR ONLY**- DATE FILED (Mo/Day/Yr)

To Be Completed By: FUNERAL DIRECTOR

51. DECEDENT'S EDUCATION-Check the box that best describes the highest degree or level of school completed at the time of death.
☐ 8th grade or less
☐ 9th - 12th grade; no diploma
☐ High school graduate or GED completed
☐ Some college credit, but no degree
☐ Associate degree (e.g., AA, AS)
☐ Bachelor's degree (e.g., BA, AB, BS)
☐ Master's degree (e.g., MA, MS, MEng, MEd, MSW, MBA)
☐ Doctorate (e.g., PhD, EdD) or Professional degree (e.g., MD, DDS, DVM, LLB, JD)

52. DECEDENT OF HISPANIC ORIGIN? Check the box that best describes whether the decedent is Spanish/Hispanic/Latino. Check the "No" box if decedent is not Spanish/Hispanic/Latino.
☐ No, not Spanish/Hispanic/Latino
☐ Yes, Mexican, Mexican American, Chicano
☐ Yes, Puerto Rican
☐ Yes, Cuban
☐ Yes, other Spanish/Hispanic/Latino (Specify) _____

53. DECEDENT'S RACE (Check one or more races to indicate what the decedent considered himself or herself to be)
☐ White
☐ Black or African American
☐ American Indian or Alaska Native (Name of the enrolled or principal tribe) _____
☐ Asian Indian
☐ Chinese
☐ Filipino
☐ Japanese
☐ Korean
☐ Vietnamese
☐ Other Asian (Specify)_____
☐ Native Hawaiian
☐ Guamanian or Chamorro
☐ Samoan
☐ Other Pacific Islander (Specify)_____
☐ Other (Specify)_____

54. DECEDENT'S USUAL OCCUPATION (Indicate type of work done during most of working life. DO NOT USE RETIRED).

55. KIND OF BUSINESS/INDUSTRY

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• Fig. 1.1, cont'd

Cause-of-death – Background, Examples, and Common Problems

Accurate cause of death information is important
•to the public health community in evaluating and improving the health of all citizens, and
•often to the family, now and in the future, and to the person settling the decedent's estate.

The cause-of-death section consists of two parts. **Part I** is for reporting a chain of events leading directly to death, with the **immediate cause** of death (the final disease, injury, or complication directly causing death) on line a and the **underlying cause** of death (the disease or injury that initiated the chain of events that led directly and inevitably to death) on the lowest used line. **Part II** is for reporting all other significant diseases, conditions, or injuries that contributed to death but which did not result in the underlying cause of death given in **Part I. The cause-of-death information should be YOUR best medical OPINION.** A condition can be listed as "probable" even if it has not been definitively diagnosed.

Examples of properly completed medical certifications

CAUSE OF DEATH (See instructions and examples)

32. **PART I.** Enter the chain of events--diseases, injuries, or complications--that directly caused the death. DO NOT enter terminal events such as cardiac arrest, respiratory arrest, or ventricular fibrillation without showing the etiology. DO NOT ABBREVIATE. Enter only one cause on a line. Add additional lines if necessary.

		Approximate interval: Onset to death
IMMEDIATE CAUSE (Final disease or condition ---------> resulting in death)	a. Rupture of myocardium	Minutes
	Due to (or as a consequence of):	
Sequentially list conditions, if any, leading to the cause listed on line a. Enter the **UNDERLYING CAUSE** (disease or injury that initiated the events resulting in death) **LAST**	b. Acute myocardial infarction	6 days
	Due to (or as a consequence of):	
	c. Coronary artery thrombosis	5 years
	Due to (or as a consequence of):	
	d. Atherosclerotic coronary artery disease	7 years

PART II. Enter other significant conditions contributing to death but not resulting in the underlying cause given in PART I

Diabetes, Chronic obstructive pulmonary disease, smoking

33. WAS AN AUTOPSY PERFORMED? ■ Yes □ No

34. WERE AUTOPSY FINDINGS AVAILABLE TO COMPLETE THE CAUSE OF DEATH? ■ Yes □ No

35. DID TOBACCO USE CONTRIBUTE TO DEATH? ■ Yes □ Probably □ No □ Unknown

36. IF FEMALE: ■ Not pregnant within past year □ Pregnant at time of death □ Not pregnant, but pregnant within 42 days of death □ Not pregnant, but pregnant 43 days to 1 year before death □ Unknown if pregnant within the past year

37. MANNER OF DEATH ■ Natural □ Homicide □ Accident □ Pending Investigation □ Suicide □ Could not be determined

CAUSE OF DEATH (See instructions and examples)

32. **PART I.** Enter the chain of events--diseases, injuries, or complications--that directly caused the death. DO NOT enter terminal events such as cardiac arrest, respiratory arrest, or ventricular fibrillation without showing the etiology. DO NOT ABBREVIATE. Enter only one cause on a line. Add additional lines if necessary.

		Approximate interval: Onset to death
IMMEDIATE CAUSE (Final disease or condition ---------> resulting in death)	a. Aspiration pneumonia	2 Days
	Due to (or as a consequence of):	
Sequentially list conditions, if any, leading to the cause listed on line a. Enter the **UNDERLYING CAUSE** (disease or injury that initiated the events resulting in death) **LAST**	b. Complications of coma	7 weeks
	Due to (or as a consequence of):	
	c. Blunt force injuries	7 weeks
	Due to (or as a consequence of):	
	d. Motor vehicle accident	7 weeks

PART II. Enter other significant conditions contributing to death but not resulting in the underlying cause given in PART I

33. WAS AN AUTOPSY PERFORMED? ■ Yes □ No

34. WERE AUTOPSY FINDINGS AVAILABLE TO COMPLETE THE CAUSE OF DEATH? ■ Yes □ No

35. DID TOBACCO USE CONTRIBUTE TO DEATH? □ Yes □ Probably ■ No □ Unknown

36. IF FEMALE: □ Not pregnant within past year □ Pregnant at time of death □ Not pregnant, but pregnant within 42 days of death □ Not pregnant, but pregnant 43 days to 1 year before death □ Unknown if pregnant within the past year

37. MANNER OF DEATH □ Natural □ Homicide ■ Accident □ Pending Investigation □ Suicide □ Could not be determined

38. DATE OF INJURY (Mo/Day/Yr) (Spell Month): August 15, 2003

39. TIME OF INJURY: Approx. 2320

40. PLACE OF INJURY (e.g., Decedent's home; construction site; restaurant; wooded area): road side near state highway

41. INJURY AT WORK? □ Yes ■ No

42. LOCATION OF INJURY: State: Missouri City or Town: near Alexandria Street & Number: mile marker 17 on state route 46a Apartment No.: Zip Code:

43. DESCRIBE HOW INJURY OCCURRED: Decedent driver of van, ran off road into tree

44. IF TRANSPORTATION INJURY, SPECIFY: ■ Driver/Operator □ Passenger □ Pedestrian □ Other (Specify)

Common problems in death certification

The **elderly decedent** should have a clear and distinct etiological sequence for cause of death, if possible. Terms such as senescence, infirmity, old age, and advanced age have little value for public health or medical research. Age is recorded elsewhere on the certificate. When a number of conditions resulted in death, the physician should choose the single sequence that, in his or her opinion, best describes the process leading to death, and place any other pertinent conditions in Part II. If after careful consideration the physician cannot determine a sequence that ends in death, then the medical examiner or coroner should be consulted about conducting an investigation or providing assistance in completing the cause of death.

The **infant decedent** should have a clear and distinct etiological sequence for cause of death, if possible. "Prematurity" should not be entered without explaining the etiology of prematurity. Maternal conditions may have initiated or affected the sequence that resulted in infant death, and such maternal causes should be reported in addition to the infant causes on the infant's death certificate (e.g., Hyaline membrane disease **due to** prematurity, 28 weeks **due to** placental abruption **due to** blunt trauma to mother's abdomen).

When **SIDS** is suspected, a complete investigation should be conducted, typically by a medical examiner or coroner. If the infant is under 1 year of age, no cause of death is determined after scene investigation, clinical history is reviewed, and a complete autopsy is performed, then the death can be reported as Sudden Infant Death Syndrome.

When processes such as the following are reported, additional information about the etiology should be reported:

Abscess
Abdominal hemorrhage
Adhesions
Adult respiratory distress syndrome
Acute myocardial infarction
Altered mental status
Anemia
Anoxia
Anoxic encephalopathy
Arrhythmia
Ascites
Aspiration
Atrial fibrillation
Bacteremia
Bedridden
Biliary obstruction
Bowel obstruction
Brain injury
Brain stem herniation
Carcinogenesis
Carcinomatosis
Cardiac arrest
Cardiac dysrhythmia
Cardiomyopathy
Cardiopulmonary arrest
Cellulitis
Cerebral edema
Cerebrovascular accident
Cerebellar tonsillar herniation
Chronic bedridden state
Cirrhosis
Coagulopathy
Compression fracture
Congestive heart failure
Convulsions
Decubiti
Dehydration
Dementia (when not otherwise specified)
Diarrhea
Disseminated intra vascular coagulopathy
Dysrhythmia
End-stage liver disease
End-stage renal disease
Epidural hematoma
Exsanguination
Failure to thrive
Fracture
Gangrene
Gastrointestinal hemorrhage
Heart failure
Hemothorax
Hepatic failure
Hepatitis
Hepatorenal syndrome
Hyperglycemia
Hyperkalemia
Hypovolemic shock
Hyponatremia
Hypotension
Immunosuppression
Increased intra cranial pressure
Intra cranial hemorrhage
Malnutrition
Metabolic encephalopathy
Multi-organ failure
Multi-system organ failure
Myocardial infarction
Necrotizing soft-tissue infection
Old age
Open (or closed) head injury
Paralysis
Pancytopenia
Perforated gallbladder
Peritonitis
Pleural effusions
Pneumonia
Pulmonary arrest
Pulmonary edema
Pulmonary embolism
Pulmonary insufficiency
Renal failure
Respiratory arrest
Seizures
Sepsis
Septic shock
Shock
Starvation
Subdural hematoma
Subarachnoid hemorrhage
Sudden death
Thrombocytopenia
Uncal herniation
Urinary tract infection
Ventricular fibrillation
Ventricular tachycardia
Volume depletion

If the certifier is unable to determine the etiology of a process such as those shown above, the process must be qualified as being of an unknown, undetermined, probable, presumed, or unspecified etiology so it is clear that a distinct etiology was not inadvertently or carelessly omitted.

The following conditions and types of death might seem to be specific or natural but when the medical history is examined further may be found to be complications of an injury or poisoning (possibly occurring long ago). Such cases should be reported to the medical examiner/coroner.

Asphyxia
Bolus
Choking
Drug or alcohol overdose/drug or alcohol abuse
Epidural hematoma
Exsanguination
Fall
Fracture
Hip fracture
Hyperthermia
Hypothermia
Open reduction of fracture
Pulmonary emboli
Seizure disorder
Sepsis
Subarachnoid hemorrhage
Subdural hematoma
Surgery
Thermal burns/chemical burns

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• Fig. 1.1, cont'd

Maternal mortality remains a major public health problem in much of the world, and such manageable complications as hemorrhage and infection continue to account for a large fraction of the world's maternal deaths.[19]

A notable feature of the last half of the 20th century was the sharp decline in all three mortality rates beginning in the 1960s, following a period of stagnation in the 1950s. The decline began with maternal mortality, followed by postneonatal and then neonatal. The contribution of medical care of the neonate was most clearly seen in national statistics in the 1970s, a decade that witnessed a larger proportional decline in neonatal mortality than in any previous decade of the century. All of the change in neonatal mortality between 1950 and 1975 was in mortality for a given birthweight; no improvement was seen in the birthweight distribution.[20] This finding suggested the effectiveness of newborn intensive care, whose impact on mortality in very small babies has been striking. In 1960, shortly before the development of newborn intensive care, survival of an infant with birthweight of 1000 g was no more than 5%. Forty years later, survival at that birthweight was 95%.[21] In retrospect, several factors seem to have played critical roles in the rapid development of the newborn intensive care programs that largely accounted for this rapid decline in birthweight-specific neonatal mortality. Perhaps the most important was the provision of more than nursing care to marginal populations such as the premature infant. Although the death of the mildly premature son of President Kennedy in 1963 provided a stimulus to the development of newborn intensive care,[22] it should be noted that the decline in infant mortality that began in the 1970s was paralleled by a similar decline in mortality for the extremely old,[23] perhaps an indicator that the availability of federal funding through Medicare and Medicaid enabled previously underserved populations at the extremes of age to receive greater medical attention than before. The Medicaid program, adopted in 1965, may have made it feasible for the first time to pay for the intensive care of premature newborns, among whom the medically indigent are overrepresented. While financial support for newborn intensive care may have been a necessary ingredient in its development, finances would not have been sufficient to improve neonatal mortality had not new medical technologies, especially those supporting ventilation of the immature newborn lung, been developed at about the same time.[24]

Advances in newborn care have ameliorated the impact of premature birth and birth defects on mortality. Progress has come from improved medical care of the high-risk pregnancy and the sick infant, rather than through understanding and prevention of the disorders themselves. Unfortunately, the frequencies of underlying disorders that drive perinatal mortality have shown less improvement.

With the very important exception of neural tube defects, the prevalence of which has declined with folate fortification of flour in the United States and programs to encourage intake of folate in women of child-bearing age,[25] prevalence rates of the major causes of death—preterm birth and birth defects—have not declined. The incidence of cerebral palsy, the major neurodevelopmental disorder that can be of perinatal origin, was remarkably stable for decades,[26] notwithstanding advances in obstetric and neonatal care. However, there are now suggestions from some parts of the world that the birth prevalence of this disorder is on the decline.[27]

The pace of decline in infant, neonatal, and postneonatal mortality in the United States began to slow in 1995 and changed little in the following decade. However, a decline of nearly 20% in both neonatal and postneonatal mortality has been seen since 2005 (Table 1.1). For infants weighing 501 to 1500 g at birth, data from the Vermont Oxford Neonatal Network encompassing more than a quarter of a million newborns from hundreds of largely North American neonatal units showed a decline in mortality of 12.2% in the final decade of the 20th century[28] and a further decline of 13.3% from 2000 to 2009.[29] For infants at the threshold of viability (born at 22 to 24 weeks), the large multicenter National Institute of Child Health and Human Development (NICHD) neonatal network has reported that mortality declined by 12.6% between 2000 and 2011.[30]

These declines are more modest than in the early days of newborn intensive care. From 1960 to 1985, a greater than 50% decline in mortality for infants weighing 501 to 1500 g at birth was recorded in national data,[31,32] even though much of the first decade of that interval preceded the use of newborn intensive care technology in all but a few pioneering centers. The pace of advances in newborn medicine and the expansion of newborn intensive care to populations previously underserved, factors that have exerted a constant downward pressure on infant mortality since the 1960 s, have lessened in the past two decades or so.

Reported maternal mortality has actually climbed substantially in recent years, but this almost certainly reflects the effect of improved reporting. The CDC has a special unit dedicated to the problem of maternal mortality, the Pregnancy Mortality Surveillance System (PMSS).[33] Established in 1987, its counts of "pregnancy-related" deaths, based on more in-depth exploration than is possible from a vital registration system alone, have provided consistently higher estimates of maternal mortality than data reported by the HCHS, as shown in Fig. 1.2, in part because the CDC count includes deaths occurring up to 1 year after delivery.

The major reason for the increase in reported maternal mortality was the recommendation by the NCHS in 2003 that all death certificates to females include a checkbox indicating whether the decedent had been pregnant in the prior year. This recommendation was initially adopted by some states and not others, producing considerable variability across states' reported maternal mortality ratios. The inconsistency led the NCHS to not report on maternal mortality ratios in the United States from 2008 to 2017, as seen in Fig. 1.2.[34]

Inasmuch as use of the checkbox has now been adopted by all states, the NCHS resumed reporting maternal mortality ratios in 2018 and has provided a detailed overview of issues in defining this important health parameter in vital data.[35] The checkbox on the death certificate has proven to be a mixed blessing. While it uncovers many otherwise unknown maternal deaths, it also produces a small number of false positives. For example, in 2013, seven births were reported to women in their 60s, yet 53 death certificates for women of that age had indicated a recent pregnancy. The careful assessments by NCHS of the procedures for recording maternal deaths may account for a welcome convergence of estimates of maternal mortality from the two systems. PMSS estimated the maternal mortality ratio at 17.3/100,000 in 2017, and the NCHS estimated it at 17.4 in 2018. However, the NCHS reported an increase in the maternal mortality ratio to 20.1/100,000 in 2019, although with continued cautions about data quality.[36]

The risk of preterm birth (<37 weeks' gestation) increased steadily in the first years of the present century, peaked in 2007, and has declined since. These changes occurred largely in moderately preterm babies and likely reflected a period in which obstetricians became more comfortable with earlier inductions of labor as well as the increased prevalence of twins and triplets, who are

TABLE 1.1 US Perinatal Mortality, Morbidity, Interventions, and Pregnancy Health Conditions and Behaviors, 2000–2019

	2000	2005	2010	2019	Net Change (2000–2019) (%)
Mortality					
Maternal mortality per 100,000 live births	13.2	15.2	17.8	20.1	+52.3
Infant mortality per 1000 live births	6.9	6.9	6.1	5.7	−17.7
Neonatal mortality per 1000 live births	4.6	4.5	4.0	3.8	−18.0
Postneonatal mortality per 1000 live births	2.3	2.3	2.1	1.9	−17.4
Morbidity (percentage of live births)					
Preterm birth (<37 weeks)	11.6%	12.7%	12.0%	10.2%	−11.8
Very preterm birth (<32 weeks)	1.9%	2.0%	2.0%	1.6%	−16.3
Extremely preterm birth (<28 weeks)	0.7%	0.8%	0.7%	0.7%	−5.7
Moderately low birthweight	7.1%	7.3%	6.7%	6.9%	−2.4
Low birthweight	7.6%	8.2%	8.2%	8.3%	+9.3
Very low birthweight	1.4%	1.5%	1.5%	1.4%	−1.4
Pregnancy-associated hypertension[1,2]	3.9%	4.0%	–	8.1%	+106.9
Diabetes in pregnancy[1]	2.9%	3.8%	5.1%	6.9%	+138.3
Interventions (percentage of live births)					
Cesarean section	22.9%	30.3%	32.8%	31.7%	+38.4
Induction of labor	19.9%	22.3%	23.4%	29.4%	+47.7
Health Behaviors (percentage of live births)					
Smoking	12.2%	10.7%	–	6.0%	−50.8
Late or no prenatal care	3.9%	3.5%	–	6.4%	+64.1
Unmarried	33.2%	36.9%	40.8%	40.0%	+20.5
Multiple births	3.1%	3.4%	3.4%	3.3%	+6.4
Fertility rate (women 15–44)	6.8%	6.7%	6.4%	5.8%	−13.6

[1]Birth certificate data underreports medical complications of pregnancy.
[2]Data includes preeclampsia, pregnancy-induced hypertension, and eclampsia values combined.
All rates denominatored to 1000 unless specified.

generally born preterm, resulting from in vitro fertilization. The newer data suggest some modifications of these earlier practices.

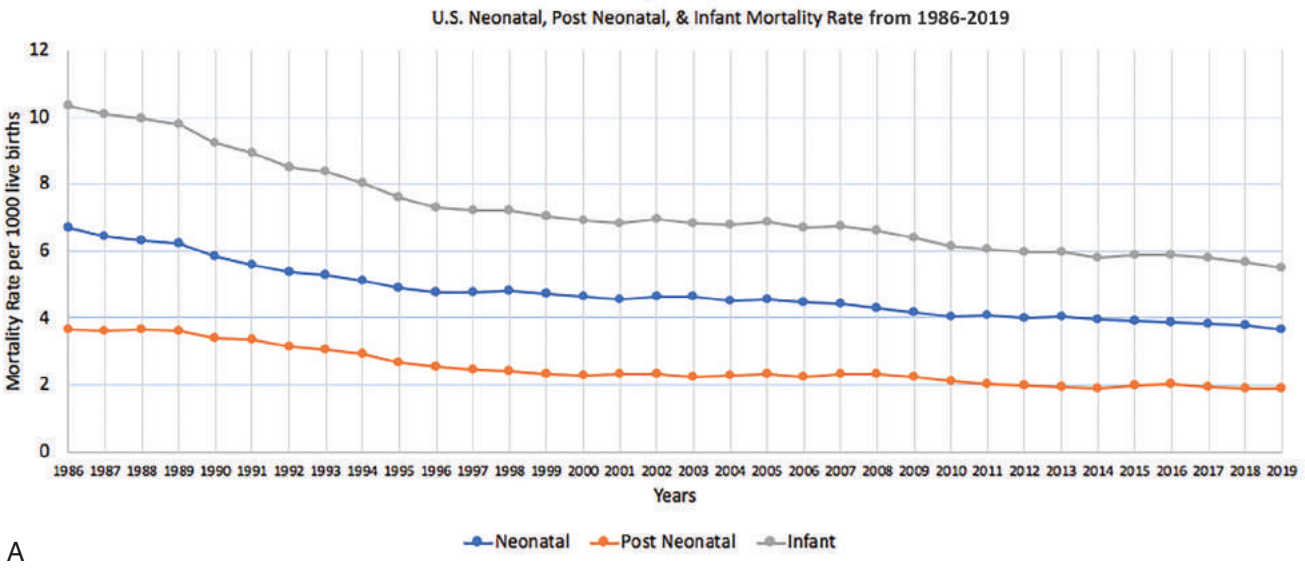
The recording of diabetes in pregnancy on birth certificates has increased substantially in the 21st century, with the 2019 prevalence 2.5 times higher than in 2000 (Table 1.2).[37] It is possible that some of this increase might reflect more complete reporting on the 2003 birth certificate revision, but the obesity epidemic has likely contributed to increased prevalence of both prenatal and gestational diabetes.[38]

The cesarean section rate continues its long-term increase, from 5% in 1970 to 23% in 1990, peaking at nearly 33% in 2010, with a faint decline to 31.7% in 2019. The reasons for this long-term increase are multifactorial and include pressures from patients, physicians, and the medical malpractice system. The steady reduction in smoking in pregnancy, which is now reported to be just 6%, is encouraging and parallels the decline in smoking in the general population but may unfortunately also reflect the uncertainty of medical records from which this information is obtained since the 2003 birth certificate revision. The proportion of mothers unmarried when they gave birth peaked at more than 50% in 2007 to 2008 but declined to 40% in 2019.

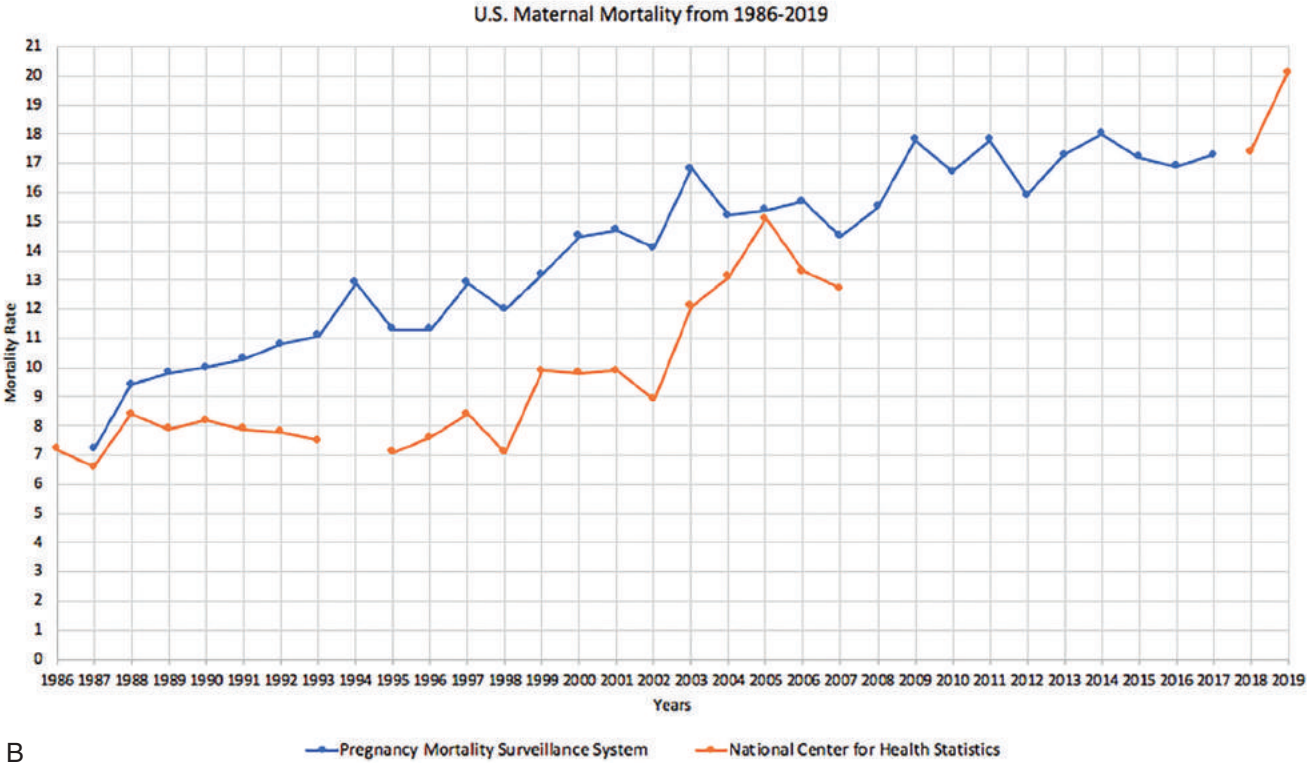
The long-term decline in the US fertility rate was accentuated in 2020, the year of the COVID-19 pandemic, when it was lower than at any time since the Depression. The number of US births declined by 4% in 2020.[39] The preterm birth rate declined as well, by slightly more than 1%, with a suggestion that the decline in births before 34 weeks was slightly greater.

International Comparisons

The relatively high rate in the United States, in comparison with other HICs, is well known. The United States ranked 33rd of



A



B

• **Fig. 1.2** Maternal, Neonatal, and Postneonatal Mortality Rates. (A) Neonatal, post-neonatal and infant mortality rates, 1986–2019. (B) Maternal mortality rates, 1986–2019.

36 countries in the Organisation for Economic Co-operation and Development (OECD) countries in the 2019 annual report.[40] Only Chile, Turkey, and Mexico had higher infant mortality rates among OECD countries. This surprising phenomenon, in light of more favorable socioeconomic and medical care circumstances in the United States than in many nations with lower infant mortality, cannot be attributed to inferior neonatal care because mortality rates for low birthweight infants are generally lower in the United States than in European and Asian nations.

A likely contributor is the more complete reporting of marginally viable small newborns as live births in the United States.[41] It is likely that the recording of such infants as live births rather than stillbirths is more pronounced in the United States than in Europe.[42] While this practice makes a contribution to our higher prematurity and infant mortality rates, it cannot fully explain them.

The key driver of the relatively high US infant mortality rate is that our nation suffers from a striking excess of premature births. While the US Black population is especially vulnerable to prematurity and especially severe prematurity, prematurity rates are also considerably higher in White Americans than in most European populations.

TABLE 1.2 Ethnic Disparities in Key Perinatal Outcomes and Exposures in 2019

	Non-Hispanic White	African - American	RR Compared With White	Hispanic	RR Compared With White
Mortality					
Maternal mortality per 100,000 live births	17.9	44.0	2.5	12.6	0.70
Infant mortality per 1000 live births	4.6	10.8	2.3	4.9	1.1
Neonatal mortality per 1000 live births	3.0	7.1	2.4	3.4	1.1
Postneonatal mortality per 1000 live births	1.6	4.1	2.6	1.4	0.88
Morbidity (percentage of live births)					
Preterm birth (<37 weeks)	9.3%	14.4%	1.6	10.0%	1.1
Very preterm birth (<32 weeks)	1.2%	3.2%	2.6	1.5%	1.2
Extremely preterm birth (<28 weeks)	0.4%	1.5%	3.5	0.6%	1.4
Moderately low birthweight	5.9%	11.2%	1.9	6.3%	1.1
Low birthweight	6.9%	14.2%	2.1	7.6%	1.1
Very low birthweight	1.0%	2.9%	2.9	1.3%	1.2
Pregnancy-associated hypertension[1,2]	8.6%	9.8%	1.1	6.7%	0.78
Diabetes in pregnancy[1]	6.3%	5.4%	0.86	7.4%	1.2
Overweight or obese (BMI of 25.0 or greater)	52.2%	65.9%	1.3	63.2%	1.2
Interventions (percentage of live births)					
Cesarean section	30.7%	35.9%	1.2	31.3%	1.0
Induction of labor	32.7%	27.7%	0.85	24.9%	0.76
Health Behaviors (percentage of live births)					
Late or no prenatal care	4.5%	9.6%	2.1	8.2%	1.8
Unmarried	28.2%	70.0%	2.5	52.1%	1.8
Fertility rate (women 15–44)	5.5%	6.1%	1.1	6.5%	1.2
Multiple births	3.4%	4.2%	1.2	2.5%	0.74

[1]Birth certificate data underreports medical complications of pregnancy.
[2]Data includes preeclampsia, pregnancy-induced hypertension, and eclampsia values combined.
All rates denominatored to 1000 unless specified.

Premature birth, fetal growth restriction, and infant mortality are tightly linked, in every setting in which they have been studied, to most measures of social class, especially to maternal education. However, uncovering precisely what it is about lower social class that drives these important differences in pregnancy outcomes has been elusive. Factors such as smoking have at times been implicated but can explain only a small fraction of the social class effect. It is unlikely that this situation will change until a better understanding of the complex social, environmental, and biologic roots of preterm birth is achieved.

Health Disparities in the Perinatal Period

In 2019, 51.1% of all US births were to non-Hispanic White mothers, 23.7% were to Hispanic mothers, 14.6% were to African-American (AA) mothers, 6.4% were to Asian mothers, and the remainder were to mothers of other ethnic groups or of multiple ethnicities or of unknown ethnicity.[43] Health disparities are especially prominent in the perinatal period, with AA infant mortality stubbornly remaining about double that of White infant mortality in the United States, even as rates decline in both populations. Preterm birth is the central contributor to this racial disparity in infant mortality, and the more severe the degree of prematurity, the higher the excess risk for AA infants. In 2019, the risk of birth before 32 weeks of gestation was 1.6 times higher in AA infants than in non-Hispanic Whites, the risk of birth before 34 weeks was 2.6 times higher, and for births before 28 weeks, the risk for AA mothers was 3.5 times higher. Reduction in infant mortality disparities in the United States thus requires better understanding of the etiology and mechanisms of preterm birth. Birth defect mortality shows a less pronounced gradient by ethnic group and does not contribute in a major way to overall infant mortality disparities.[44]

The "Hispanic paradox" is a term often used to describe the observation that infant mortality is the same or lower in US citizens classified as Hispanic than in non-Hispanic Whites, in spite of the generally lower income and education levels of US Hispanics.[45] The infant mortality experience of Hispanics in the United States reflects the principle that premature birth and low birthweight are key determinants of infant mortality, because the rates of these outcomes are also lower among Hispanics. Smoking is much less common among Hispanics in the United States, but this factor alone does not fully explain the paradox.

Major Causes of Death

Cause-of-death analysis, a staple of epidemiologic investigation, has limitations when applied to the perinatal period. Birth defect mortality is relatively accurate, but causes of deaths among preterm infants are divided among categories such as respiratory distress syndrome, immaturity, and a variety of complications of prematurity. The choice of which particular epiphenomenon of preterm birth should be listed as the primary cause of death is to some extent arbitrary. Some maternal complications, such as preeclampsia, that can lead to preterm birth are also occasionally listed as causes of newborn death.

Postmortem pathologic examination (autopsy) of the deceased fetus or infant can provide valuable information about cause of death. However, the rate at which parents provide consent for autopsy has declined over the past several decades so that alternative methods have been proposed, such as ultrasound, magnetic resonance imaging, and needle biopsy.[46]

In the period before prenatal ultrasound permitted reasonably accurate gestational age estimation, a high fraction of neonatal deaths was attributed to low birthweight, but most of these deaths occurred in premature infants, because premature birth is much more important as a cause of death than is fetal growth restriction. Extreme prematurity makes a contribution to infant mortality well beyond its frequency in the population. The 1.6% of births born prior to 32 weeks in 2018 accounted for 51% of all infant deaths.[10]

After premature birth, the next most important group of causes of death is congenital anomalies. With the signal exception of the folate-neural tube association, we have no clearly effective primary prevention program for any birth defect. For very severe defects, pregnancy screening and termination is an option, and noninvasive prenatal screening may have increased the reach of this therapeutic option.[47]

The major postneonatal cause of death since approximately the 1970s in the United States is the sudden infant death syndrome (SIDS). Although this cause of death has declined substantially in the United States in parallel with successful public health efforts to discourage prone sleeping in infancy,[48] sudden unexpected death in infancy (which includes suffocation and unknown causes along with SIDS) has been stable for the past two decades.[49]

Factors Affecting Perinatal Health

Health States in Pregnancy

The major causes of neonatal morbidity—prematurity and birth defects—generally occur in pregnancies free of antecedent complications. Having a previous birth with an anomaly or a previous preterm birth raises the maternal risk for recurrence of the condition. Indeed, for preterm birth, no other known risk factor carries as much risk as having previously delivered preterm.[50]

More than a quarter of preterm births are iatrogenic, the result of induced labor in pregnancies in which the mother's and/or fetus' health is severely compromised. In general, the reason is preeclampsia with attendant impairments in uterine blood flow and poor fetal growth, but poor uterine blood flow and impaired fetal growth can also occur independently of diagnosed preeclampsia. The other major complication of pregnancy is diabetes, most often gestational, but at times preexisting. Hyperglycemia in the mother promotes the same elevation in the fetus, and typically the infant of the diabetic mother is large for gestational age. However, severe diabetes can be accompanied by fetal growth restriction.

Health Behaviors

The most carefully studied and well-established health behavior affecting newborns is maternal cigarette smoking, which more than doubles the likelihood of intrauterine growth restriction and increases the risk of premature birth by 20% to 50%.[51] Infants with growth restriction from smoking paradoxically survive slightly more often than do infants of the same weight whose mothers did not smoke, but the net effect of smoking is to increase perinatal mortality. The risk of SIDS is also increased in the babies of smoking mothers.[52] Although the subject is much debated, it has not been conclusively shown that prenatal smoking has independent long-term effects on child cognitive capacity.[53]

Mothers who drink alcohol heavily in pregnancy are at risk of having infants with the cluster of defects known as the fetal alcohol syndrome.[54] Cocaine use in pregnancy is a restrictor of fetal growth[55] and may affect neonatal behavior, but the long-term effects of this exposure on infant cognition and behavior are not as great as initially feared.[56]

The frequency of marijuana use during pregnancy has increased substantially in recent years, in parallel with legalization in several states. Between 2002 and 2017, self-reported cannabis use more than doubled, from 3.4% to 7.0%, among pregnant participants in the National Survey on Drug Use and Health (NSDUH),[57] and pregnant women in states with legalized recreational cannabis are twice as likely to use cannabis than their counterparts in states where cannabis remains illegal.[58] In addition, the potency of recreational and medicinal cannabis, measured by the concentration of tetrahydrocannabinol (THC), the main psychoactive constituent of cannabis, appears to have tripled in recent decades.[59] THC crosses the human placenta, with greater transfer during the early stages of pregnancy when the fetus is most susceptible to the adverse effects of drug use.[60] Although the evidence that marijuana use during pregnancy adversely affects neurodevelopment of the offspring is inconclusive,[61] the American College of Obstetricians and Gynecologists recommends against use by women who are considering pregnancy or who become pregnant.[62]

Perinatal Medical Care

In light of the potent effects of medical care on the neonate, it has been important to develop systems of care that ensure, or at least facilitate, provision of care to neonates in need. This concept was first promoted by the March of Dimes Foundation, which in its committee report of 1976 recommended that all hospitals caring for babies be classified as either Level 1 (care for healthy and mildly ill newborns), Level 2 (care for most sick in-born newborns but not accepting transfers), and Level 3 (regional centers caring for complex surgical disease and receiving transfers).[63] This concept of a regional approach to neonatal care, with different

hospitals playing distinct roles in providing care, was endorsed by organizations such as American College of Obstetricians and Gynecologists and the American Academy of Pediatrics and by many state health departments. While it is important to transfer sick babies to Level 3 centers when needed, it is preferable, if at all possible, to transfer mothers at risk of delivering prematurely or of having a sick neonate, because transport of the fetus in utero is far superior to any form of postnatal transport. Birth at a level 3 center has consistently been shown to produce lower mortality rates for small or premature infants than birth in other levels of care,[64] although this reduction in mortality is not clearly seen for infants born at term of normal weight.[65]

Epidemiologic Study Designs in the Perinatal Period

Epidemiologic studies have contributed substantially to better understanding of patterns of risk and prognosis in the perinatal period, to tracking patterns of mortality and morbidity, to assessing regional medical care, to identifying pre- and perinatal hazards, and to evaluating the efficacy of treatments. The use of vital data to provide *descriptive* epidemiology of the health of mothers and infants and to monitor time trends has already been mentioned, but *analytic* epidemiology, drawing on cohort, case-control and randomized trial designs, has been essential to advances in neonatology.

Cohort Studies Beginning in Pregnancy or at Birth

Studies that follow populations of infants over time, beginning at birth or even before birth, continuing to hospital discharge, to early childhood or even into adult life, are the leading sources of information about perinatal risk factors for disease and adverse outcomes. As with all observational studies, cohort studies produce associations of exposures and outcomes whose strength and consistency must be carefully judged in the light of other biologic evidence and with attention to confounding and bias. Collaborations across centers in assembling such data are very valuable. One such notable collaboration is the Vermont-Oxford Network, which provides continuous information on the frequency of conditions observed and diagnoses made in hundreds of US and overseas hospitals, with a particular emphasis on using these data for improving care.[66] Another is the NICHD-supported Neonatal Research Network, which has not only been a rich source of randomized clinical trials but also has produced observations about prognosis based on very large samples of low birthweight babies.[67]

Multicenter cohort studies focusing on diagnosis and follow-up of brain injury in preterm infants have contributed much to our understanding of the prognostic value of brain injury imaged by ultrasound in the neonatal period because they include follow-up to age 2 or later. Of particular value have been regional or population-wide studies of low birthweight infants with follow-up to at least school age, among which are included the NBH study from the United States and also important studies from France,[68] Germany,[69] Holland,[70] Great Britain,[71] and Canada.[72]

Newborn intensive care has been in place long enough that the first reports of adult outcomes in very small infants have been published,[73] and they paint a picture that is perhaps less dire than many had anticipated. The Adults Born Preterm International Collaboration is an expanding resource for epidemiologic studies on adult outcomes after preterm birth.[74]

From 1959 to 1966, the National Collaborative Perinatal Project assembled data on approximately 50,000 pregnancies in 12 major medical centers and followed them to age 7.[75] This highly productive exercise, one of whose major contributions was to show that birth asphyxia is a rare cause of cerebral palsy, has now been followed by the development of even larger pregnancy cohort studies. These studies, all of which archive biologic material such as blood and/or urine in pregnancy, should, in principle, permit us to learn a great deal about the unrecognized pregnancy factors that lead to adverse perinatal and child health outcomes. For reasons not entirely clear, a sample size of 100,000 has been universally adopted in studies in Norway,[76] Denmark,[77] and, most recently, Japan.[78] Efforts to mount a similar study in the United States have not been successful, but the recently launched National Institutes of Health (NIH)-sponsored Environmental Influences on Child Health Outcomes Program holds is beginning to create a useful database of prenatal, perinatal, and postnatal data, with assessments of neurodevelopmental outcomes, obesity, and asthma in childhood.[79]

Case-Control Studies

A notable contribution using the case-control approach was the 1971 discovery that diethylstilbestrol (DES) is a prenatal carcinogen. Notably, this study was based on only 8 cases of vaginal clear cell adenocarcinoma in adolescents and young women, but the linkage to exposure to DES in utero was unmistakable (7 of 8 cases, none of 32 controls).[80] The US Food and Drug Administration (FDA) banned the use of DES in pregnancy 5 months after the publication of the study, and a very large decline in the incidence of vaginal clear cell adenocarcinoma has since been documented.[81]

As noted previously, the United States has witnessed a large decline in the incidence of SIDS. This decline is almost certainly owed to the discovery, in two well-done case-control studies, one in Holland the other in New Zealand, that prone sleeping is a strong risk factor for SIDS.[82,83] The decline in SIDS incidence closely paralleled remarkably rapid changes in maternal behavior, with the placing of babies in the prone position for sleep declining steeply in just a few short years.[84]

Randomized Controlled Trials

Few areas of medicine have adopted the randomized trial as wholeheartedly as newborn medicine. The number of trials mounted has been large and their influence on practice strong. A notable influence on this field has been the National Perinatal Epidemiology Unit (NPEU) at Oxford University, established in 1978, which prioritized randomized trials among their several investigations of perinatal care practices and other circumstances affecting maternal and newborn outcomes. The NICHD neonatal research network was established in 1986, principally to support trials in newborns. Hundreds of trials have been mounted by just these two organizations alone, but many other centers have contributed to the trial literature.

Trials in pregnancy or in labor have also been supported by the NPEU and by a network of obstetric centers supported by NICHD, the maternal-fetal research network, established, like the neonatal network, in 1986 but by and large involving different medical centers. These trials have often had important implications for newborns and for mothers, most notably, the one trial

that has thus far successfully reduced the risk of preterm birth, the administration of 17-OH hydroxyprogesterone caproate in midgestation to high-risk women.[85] Vaginal progesterone may also be effective.[86]

Most newborn trials have focused on outcomes evident in the newborn period, such as mortality, chronic lung disease, brain damage visualized on ultrasound, duration of mechanical ventilation, and/or hospital stay. Recently, however, trials extending into infancy or even to early childhood that incorporate measures of cognition or neurologic function have been a welcome addition to the trial arena. In the past two decades, we have learned from such trials that induced hypothermia can reduce mortality and brain damage in asphyxiated term infants[87] and that both caffeine for apnea treatment[88] and magnesium sulfate administered in labor may reduce the risk of cerebral palsy.[89]

Trials in which both mortality and later outcome are combined raise complex methodologic issues. Imbalance in the frequency of the two outcomes being combined can result in random variation in the commoner outcome overwhelming a significant finding in the other. Precisely how best to conduct such dual or multioutcome trials is the subject of discussion and debate in the neonatal and epidemiologic communities.

As trials multiply, not all of them sufficiently powered, the methodology for summarizing them and drawing effective conclusions has become increasingly important to neonatologists. The terms "systematic review" and "meta analysis" have firmly entered the research lexicon, especially the randomized trial literature. The Cochrane collaboration is an international organization that uses an army of volunteers to systematically review trial results in all fields of medicine. The collaboration, established in 1993, began in the field of perinatal medical trials. Systematic reviews of neonatal trials reviewed by the Cochrane Collaboration are hosted on the website of the NICHD.[90]

In a particularly valuable experiment, women at high risk of having a child with a neural tube defect were randomized to receive 4 mg folate or placebo, showing conclusively that folate administration in the preconceptional period prevented neural tube defects.[91] A trial in unselected women also showed a beneficial effect but was underpowered.[92] Major efforts to promote folate intake in women of childbearing age have been followed by reductions in the birth prevalence of neural tube defects, especially in countries, which, like the United States, have also mandated fortification of flour with folic acid.[93]

Summary and Conclusions

The patterns of disease, mortality, and later outcome in the perinatal period are complex. Some factors, such as the long-term trends in preterm birth and birthweight, are reasonably stable, whereas others, such as the rates of cesarean section and twinning, can undergo rapid change. The success of newborn intensive care is well established. No other organized medical care program, targeted at a broad patient population, has had such remarkable success in lowering mortality rates in such a short period of time. Much of that success is owed to the evidence-based nature of neonatal practice.

Nonetheless, this success has opened the door to new problems as survivors of intensive care face the challenges of the information age. Resource allocations similar to those that permitted the development of newborn intensive care are now needed to address the educational and rehabilitative needs of survivors. A hopeful sign is the success of some recently studied interventions in reducing the burden of brain damage.

On the nontechnologic front, targeted epidemiologic efforts to address perinatal disorders have yielded progress as well. Careful study of the circumstances surrounding infant sleep patterns led to active discouragement of prone sleeping, which has produced a halving of mortality from SIDS.[47] Observational research, followed by two important randomized trials in Europe, led to interventions that increased folate intake in women of child-bearing age and a substantial reduction in the birth prevalence of neural tube defects.

The population-level study of health events occurring in pregnancy and infancy, their antecedents, and long-term consequences has been an important component of the success of newborn care. Careful self-evaluation through monitoring of vital data and of collaborative clinical data, rigorous assessment of new treatments through randomized trials, and alertness to opportunities to implement prevention activities following discovery of important risk factors should continue to guide care of the newborn.

Acknowledgments

We thank Ban Al-Sahab, PhD for information about cannabis use in the United States and Tracy Thompson, MPH for data on maternal mortality.

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2

Ethics, Data, and Policy in Newborn Intensive Care

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KEY POINTS

- Increasingly available data in neonatal intensive care may help guide ethical decision-making about sick infants.
- Despite better ability to predict outcomes for sick infants, prognostic efforts are imperfect.
- When using data to guide decision-making in the neonatal intensive care unit, it is important to choose appropriate outcomes that match families' values.
- There are policy implications to both limited data, as in predicting outcomes for preterm infants, and to increasingly available data, as in newer advances in genetic screening.

Background

Ethics in the neonatal intensive care unit (NICU), as in all clinical contexts, starts with the traditional triangular framework of principles. Autonomy means to do what the patient, or in this case the parent, thinks is right; paternalism means to do what the doctor thinks is best; beneficence and nonmaleficence mean to do what is most beneficial and least harmful. These principles are widely accepted as the basis of ethical medical practice. Their applications occur daily. The problem with using these widely accepted principles to guide practice is they sometimes conflict with one another. Then one must decide quickly which compromises are best. What is the right thing? What facts should be brought to bear in the decision? What weight should be given to each fact? Whose opinion should count?

Many decisions in the NICU evoke and reflect these principles. Sometimes, neonatologists make relatively straightforward decisions not to resuscitate babies born at 21 weeks, or to provide obligatory support to babies born at 28 weeks, or to not perform cardiopulmonary resuscitation on infants with lethal anomalies. In more difficult cases, when ethical principles are in conflict, their application to a particular case is not straightforward. Faced with a difficult case, it is rare that simply applying principles will help to devise a solution.

Applying ethical principles requires attention to the specific details of each case. We must ground our concerns in context, take data into account, and be sympathetic to patient preferences when the balance of benefits and burdens is not clear. In the NICU, health professionals are constantly and anxiously aware that the burdens of treatment are real, immediate, and significant, whereas the benefits of NICU interventions are distant, statistical, and unpredictable. Babies must undergo months of painful procedures such as intubation, ventilation, and intravenous catheterization in the hope that everything will turn out well in the end. However, sometimes outcomes are ambiguous. Babies are left with lifelong sequelae. How should we decide whether we did the right thing? How should we decide whether, in similar circumstances, we should again do the same thing?

To make good decisions, we need good data. We need to know (1) where to get data; (2) how to decide which data matters; (3) the implications of limited data; and (4) the implications of increasingly available data. These questions are the focus of this chapter.

Getting Data

Neonatologists have a number of repositories through which data are collected and analyzed. The Vermont Oxford Network (VON) and the National Institute of Child Health and Human Development (NICHD) Neonatal Research Network Generic Database use prespecified data elements to describe outcomes of preterm infants.[1,2] Similar databases exist to describe outcomes of other neonatal populations. These include the Extracorporeal Life Support Organization database and the Congenital Diaphragmatic Hernia Study Group.[3,4] For all these biorepositories, data are gathered using trained abstractors and ongoing quality assurance regarding data collection. Each has discrete variables related to the population of interest. Secondary analysis of electronic health record (EHR) data is a more feasible option than previous manual chart review, following meaningful use incentives for implementation of EHRs.[5] Administrative data from billing claims are another commonly used source of outcomes information; examples include the Pediatric Health Information System from children's hospitals, the National Inpatient Sample and its associated Kids Inpatient Database from the Agency for Healthcare Research and Quality, or the Kaiser Permanente Neonatal Minimum Data Set.[6–8] From the standpoint of understanding outcomes to inform decision-making for sick infants, each type of data has benefits and drawbacks. Benefits of abstracted data include ongoing quality assurance for variables related to the disease of interest. Limitations include differences in definitions between data sources, and the way that individual data are aggregated into discrete variables. EHR data have the benefit of extreme

detail and potential flexibility in defining measures of interest, but they are limited by the accuracy of the recording clinician. Claims data have the benefit of understanding costs of care but are limited by the accuracy of coding and discrepancies between clinical diagnosis and coding terminology for common diseases.

These multiple potential sources of information offer opportunities for those who want to better understand the determinants of outcomes, but the plethora of information is a mixed blessing. Databases can be local, regional, national, or international. They can gather data on all babies or only on specific subpopulations of babies. These variations lead to different outputs. Caregivers must then decide which database to use and how to interpret and apply the results.

Getting Data That Matters

What kind of information would parents, physicians, or judges need to decide when treatment should be obligatory and when it should be optional?

One essential truth at the intersection of NICU epidemiology and ethics is that gestational age—particularly at the margins of viability—is a powerful predictor of survival. In the United States, as in virtually all the industrialized world, infants born after 24 weeks' gestation have survival rates greater than 80%. This is high enough so that treatment is generally considered obligatory. For these infants, the ethical principle of best interest requires their resuscitation, in the same way that sick children born at term deserve resuscitation. Conversely, for infants born before 22 weeks' gestation, survival is essentially zero. Consequently, these infants and their parents deserve our compassion but not necessarily our interventions, on the ethical grounds of strict futility.

In between, spanning approximately 3 weeks of gestational age, from 22 to 24 weeks, we require not only data but also its interpretation. Tyson et al., using the NICHD Neonatal Research Network, attempted to quantify additional risk factors for both mortality and neurologic morbidity among infants born on the cusp of viability.[9] Their analysis revealed that, for babies at the same gestational age, singleton status, birth weight, antenatal steroids, and female gender all improve the likelihood of survival and intact neurodevelopmental outcome. By considering these factors—which are available at the time of birth—caregivers can more accurately estimate the chances that a baby will survive or that those survivors will have neurodevelopmental impairment. This predictive model has been turned into a publicly available and widely used calculator, which is used in many locations to counsel families at risk of delivering a baby in this gestational age range. The model has been updated recently to include more modern neonatal and obstetric outcomes and to provide a range to account for the wide center variability in outcomes.[10,11]

However, two problems remain. First, for many infants, the algorithm's predictive value is still not very good—some lower-risk patients will die, and some of the highest-risk patients will survive. Second, by only using data available at the time of birth, the algorithm ignores a potentially important feature of NICU care—time. It does not account for prognostic features that might become available as the infant's course unfolds in the NICU. This is an important limitation that has ethical implications.

There are distinct advantages to making decisions over the first few days of an infant's NICU stay rather than in the delivery room at the time of birth. The first is emotional. Parents often appreciate the opportunity to get to know their baby as an individual, as opposed to making decisions based only on anonymous population-based prognostications that are available at the time of birth. Second, there is valuable information to learn while the baby is in the NICU. Several time-sensitive prognostic features have been evaluated for infants born at the border of viability. These include illness severity scores such as the Score for Neonatal Acute Physiology (SNAP), cranial ultrasound results in the first week or two of life, and caregiver intuitions that the patient would "die before NICU discharge."[12,13] Unfortunately, although illness severity scores on the first day of life have good prognostic power for death or survival, their power diminishes over time. Intriguingly, serial intuitions that an individual baby will die before discharge—offered by medical caregivers for infants who require mechanical ventilation and for whom there is an ethical alternative to continued ventilation, namely extubation and palliative care—are remarkably accurate in predicting a combined outcome of either death or survival with neurodevelopmental impairment (mental developmental index [MDI] or psychomotor developmental index [PDI] <70). Babies with abnormal results from a cranial ultrasound examination whose doctors agree with one another that the babies are likely to die have a less than 5% chance of surviving with both MDI and PDI greater than 70 at 2 years, independent of their gestational age. The predictive power of these data, acquired over time during an individual infant's NICU course, although not perfect, is greater than any algorithm available at the time of birth.

What do prospective parents consider when asked to decide whether to resuscitate their micropreemie? They may not want the precise prognostic estimates that we try to offer. For many parents, the death of their baby in the NICU is not necessarily the worst outcome. Instead, for many parents, it may be emotionally more difficult to not try to save their baby than it is to try and fail. The decision not to try may leave parents with a lifetime of self-doubt about whether, had they only tried, their baby might have survived. The old adage, "It is better to try and fail than not to try at all" seems to summarize these attitudes.

If trying and failing is seen as a more acceptable option than not trying at all, that could help us figure out which outcome statistic is most relevant to parents. Many studies based on database information, including that of the NICHD, try to predict how many babies either die or survive with severe neurodevelopmental impairment. This approach implicitly considers both severe disability and death to be equivalent and equally bad outcomes. The implicit assumption is that, if there is a high likelihood of death, then we should not attempt resuscitation or provide life-sustaining interventions. By contrast, if trying and failing is better than not trying at all, then perhaps we should combine different variables—reporting the percentage of survivors who are neurologically intact while leaving the babies who die out of the denominator. Understanding outcomes as a fraction of infants who survive to discharge is often obscured in research reports of preterm infant outcomes, due to epidemiologic concerns about "competing outcomes."[14] Because an infant who does not survive to NICU discharge cannot have developmental delays at age 2, these two very different outcomes—death or severe disability—are reported together. This is problematic for two reasons. First, while combining competing outcomes makes sense from a statistical perspective, from a family or clinician's perspective, death in the NICU and delays at age 2 are very different scenarios. For the parent who prefers that the NICU team attempt resuscitation, these combined outcomes do not offer a sense of what happens if the baby does survive. Second, presenting NICU outcomes data as combined outcomes obscures

an important fact of neurologic morbidity in extremely preterm infants: the incidence of neurologic morbidity in NICU survivors is not very different when comparing infants at 23, 24, 25, and 26 weeks' gestation. The essential epidemiologic difference for infants born in this gestational range appears to be whether the baby will survive at all. Once the baby leaves the NICU, the risk of severe morbidity is largely the same. This is true in single-center and multicenter studies, in the United Kingdom, Canada, Europe, and the United States.[9,15]

It has been traditionally, and inaccurately, perceived that mortality follows morbidity, meaning that because infants born at 22 to 24 weeks' gestation have the highest rate of mortality, survivors have the highest rates of neurologic morbidity. This belief is supported by the lack of parsing out outcomes of mortality versus morbidity among survivors. It could contribute to an inaccurately pessimistic view of outcomes. A fascinating insight has been offered by Janvier et al., who have done extensive surveys comparing responses to requests for resuscitation of sick micro-preemies with resuscitation of comparably sick patients at other ages (from term infants to 80-year-olds).[16] Consistently, it appears that micro-preemies are devalued. For comparable likelihood of survival and comparable likelihood of neurologic morbidity in survivors, more respondents would not offer resuscitation or would allow a micro-preemie to die.

Finally, there is difficulty in assigning value to morbidity in surviving infants in the NICU. Traditional outcomes studies for survivors of NICU care are generally limited to Bayley scores at 18 to 26 months of age. This is problematic for multiple reasons. First, NICU success is often viewed as "all or none." In most neonatal follow-up literature, a Bayley Mental Developmental Index (MDI) or Psychomotor Developmental Index (PDI) greater than 70 is classified as *normal*, whereas an MDI or PDI less than 70 is classified as an *adverse outcome*. A continuum of scores may be better interpreted as a range of strengths and areas that require additional attention, rather than as a single measure of success or failure. Whether individual clinicians think of developmental delays along a continuum or not, presenting outcomes data as pass/fail in the literature may contribute to the overpessimistic attitudes toward survivors of preterm birth prevalent in the medical community.

Furthermore, studies of former preterm infants have shown limited correlation between Bayley scores at 2 years of age and school readiness by 5 years of age.[17] This has been interpreted in the neonatal community as a poor reflection on the Bayley assessment itself, yet in studies of infants with congenital heart disease there is a much tighter correlation between 2-year and later outcomes.[18] It seems more likely that the problem with assigning value to morbidity is not the Bayley assessment itself but that former preterm infants have significant capacity to continue improving their developmental progress after 2 years of age. It also may be that Bayley scores at age 2 do not yet reflect the higher-order organizational and executive functioning issues that can become problematic by school age in survivors of prematurity. Verrips et al. have attempted to assess the effects of permanent residual disability for NICU survivors and their immediate families; they have demonstrated consistently that children with disabilities and their parents place a much higher value on their lives, and the quality of those lives, than do either physicians or NICU nurses.[19] The vast majority of infants who survive the NICU, even those with significant permanent neurologic compromise, have "lives worth living," as judged by the people most affected by those lives.

Policy Implications of Limited Data

Despite the wealth of available data on outcomes of sick infants after the NICU, we are still unable to predict future neurologic morbidity with the amount of detail needed for many clinicians and parents to make decisions regarding intervention. This, along with changes in the recognition of disability as a social construct as well as a medical one, has led to legal challenges with important implications for clinical care.

Public Policy: The Baby Doe Case

In the 1980s, the federal government attempted to change the rules for neonatal decisions about babies with congenital anomalies.

In 1982, a baby with Down syndrome and esophageal atresia was born in Bloomington, Indiana (USA). Baby Doe's parents refused to consent to surgery and chose palliative care instead. The court sided with the parents. The doctor and hospital appealed. The Indiana Supreme Court refused to hear the appeal, and the baby died after 8 days.[20]

This led to a national controversy that eventually resulted in amendments to the federal Child Abuse and Treatment Act.[21,22] While this law has limited authority in regulating clinical decisions, it symbolically endorses the idea that life-sustaining treatment should not be withheld only on the basis of anticipated disability.

There is still controversy when treatments enable survival but have a high likelihood, or certainty, that survival will be accompanied by severe neurologic impairment. As a result, two questions must be asked: How severe will the neurologic impairment be? What is the likelihood that the child will have the most severe possible impairment?

The shift in moral standards regarding babies with Down syndrome was not related to technology but rather sociology.[23] The capacity to repair Arnold-Chiari malformation and duodenal atresia existed long before it was applied to children with myelomeningocele and Down syndrome. What has changed is a growing recognition that disability is as much a social construct as a medical construct, although it is always both and not one or the other.

Malpractice Cases Against Neonatologists

There are also malpractice cases against neonatologists that have shaped the decision-making climate in the NICU. In *Miller versus Hospital Corporation of America Inc.*, the doctors were sued for resuscitating a tiny premature infant over the objections of the parents. The case focused on events that had occurred in 1990, when Mrs. Miller came to a Hospital Corporation of America hospital in Texas in labor at 23 weeks' gestation. The fetus was estimated to weigh 500 to 600 g. No baby born that size had ever survived at that hospital. Mrs. Miller, her husband, and the attending physicians agreed that the baby was previable and that no intervention was indicated. The baby was born, but a different physician performed resuscitation, and the infant survived with brain damage. As a result, the Millers sued the hospital for a breach of informed consent and were awarded $50 million by a trial jury. The case wound its way to the Texas Supreme Court, which dismissed the verdict and articulated an "emergency exception" for physicians—that is, if a Texas physician finds himself or herself in the emergency position of needing to resuscitate a patient to prevent immediate death, the physician can try to perform resuscitation without being obligated to obtain consent from anyone.

Whether it would be acceptable for a physician not to perform resuscitation in an emergency was left unarticulated by the Texas court.[24]

In Wisconsin, the case of *Montalvo versus Borkovec* took the legal obligations of neonatologists and parents to a different place. The case derived from the resuscitation of a male infant born between 23 and 24 weeks' gestation, weighing 679 g. The parents claimed a violation of informed consent, arguing that the decision to use "extraordinary measures" should have been relegated to the parents. The Wisconsin Appellate Court disagreed, holding that "in the absence of a persistent vegetative state, the right of a parent to withhold life-sustaining treatment from a child does not exist." Because virtually no infant is born in a persistent vegetative state, this decision would apparently eliminate the ethical possibility in Wisconsin of a "gray zone" of parental discretion. No other jurisdiction in the United States has adopted this position. The Wisconsin Appellate Court, like the Texas Supreme Court, was silent on whether physicians have discretion not to resuscitate. However, in Texas and Wisconsin, physicians are apparently not liable if they choose to do so.[25]

A number of other state courts have addressed issues of treatment or nontreatment. In general, the courts are permissive of physicians who resuscitate infants. If courts are asked to sanction decisions to allow infants to die, most will do so only if there is consensus among physicians and parents and occasionally ethics committees. Courts are not eager to punish physicians who treat infants over parental objections or to empower physicians to stop treatment when parents want it to continue.

In 2020, President Trump issued an Executive Order entitled "Protecting Vulnerable Infant and Newborn Children." After alleging that "some hospitals refuse the required medical screening examination and stabilizing treatment or otherwise do not provide potentially lifesaving medical treatment to extremely premature or disabled infants, even when parents plead for such treatment," the Executive Order went on to summarize the legal requirements that are already in place as a result of the Baby Doe controversy, the Emergency Medical Treatment and Labor Act, and the Born-Alive Infant Protection Act. Most experts believe that the Executive Order did not add any legal requirements for treatment beyond those that were already in place.

Relationship Between Policy, Practice, and Outcomes

Many countries' professional societies have policies about resuscitation for babies born at the borderline of viability. In 2015, Guillén et al. reported results of an international study of such policies.[26] They found that, among guidelines in 31 countries, 21 (68%) supported comfort care at 22 weeks' gestation and 20 (65%) supported active care at 25 weeks' gestation. Between 23 and 24 weeks' gestation, there was wide variation from country to country. Wilkinson and colleagues also reviewed international differences in resuscitation policies and the effect of those policies on clinical practices.[27] They found differences in the thresholds for resuscitation in three European countries—Sweden, the United Kingdom, and the Netherlands. In Sweden, most neonatologists set the threshold for resuscitation at 22 weeks. In the United Kingdom, it was 23 weeks. In the Netherlands, it was 24 weeks. These practices mirrored national guidelines that endorse shifts in management on the basis of gestational age. These authors also noted some differences within countries, writing "Among Dutch respondents, 29% would withhold resuscitation at a maximum of 25 + 6/7 weeks' gestation, whereas 33% would withhold resuscitation at up to 26 + 0/7 weeks' gestation; a similar division was evident in Swedish respondents (21% indicating a maximum of up to 22 + 6/7 weeks' gestation and 29% indicating a maximum of up to 23 + 0/7 weeks' gestation) and UK respondents (29% indicating a maximum of up to 23 + 6/7 weeks' gestation and 29% indicating a maximum of up to 24 + 0/7 weeks' gestation)."

Resuscitation policies clearly shape outcome statistics. Myrhaug and colleagues showed how powerful this effect can be.[28] They conducted a meta analysis of outcomes studies of babies born between 22 and 25 weeks of gestation in 19 different countries over the past 20 years. They showed that overall survival rates for infants born at 22 weeks were near zero percent because in most studies such infants were not offered intensive care. For babies who were admitted to a NICU, by contrast, the survival rate was 24.1%.

Both national policies and practices appear to be in flux. Recent reports in some national databases (but not yet in peer-reviewed publications) suggest that resuscitation is more common than it used to be for babies born at 22 weeks of gestation (see, e.g., https://www.npeu.ox.ac.uk/assets/downloads/mbrrace-uk/reports/perinatal-surveillance-report-2018/MBRRACE-UK_Perinatal_Surveillance_Report_2018_-_Tables_and_Figures_v3.pdf; http://www.canadianneonatalnetwork.org/portal/Portals/0/Annual%20Reports/2019%20CNN%20report%20final_links.pdf).

Neonatal Resuscitation and Generational Conflict

Finally, the concept of generational conflict must be considered. Many doctors appear to be quite comfortable calling delivery-room resuscitation of 24-weekers "optional," based on gestational age alone.[29] Given what we know about the factors other than gestational age that influence outcomes, this seems to be a curiously oversimplified approach to clinical decisions. It is not an approach that is used in other areas of medicine. For example, professional societies do not recommend the allocation of intensive care unit resources based on age alone. Such a practice might be called "discriminatory ageism." However, with regard to premature babies, it ought, perhaps, to be considered equally discriminatory and labeled as "gestational ageism."[30] Some suggest that the reason to limit such treatment is because it is too expensive, but studies show that it is remarkably cost-effective compared with the use of intensive care resources for elderly patients with respiratory failure.[31,32] The relative cost-effectiveness of NICUs compared with medical intensive care units is based on the natural history of illnesses that lead to intensive care unit admissions. Most babies who are admitted to the NICU either die relatively quickly or survive. Thus most of the resources in NICUs are expended on patients who survive to leave the hospital. For adults, the opposite is true. With each passing day in the intensive care unit, the chances that an elderly patient will survive to leave the hospital go down. Thus a much higher percentage of the resources expended in adult intensive care units are used by patients who will not leave the hospital alive.

Implications of Increasingly Available Data

Recent developments in perinatal care have led to new types of data available for decision-making. These developments may change the way we think about ethical issues in the newborn period. Examples include the rise of fetal medicine and expanded genomic screening of newborns.

Fetal Medicine Centers

Many children's hospitals have fetal medicine centers. The goal of these centers is to identify fetuses at risk—particularly those with congenital anomalies—and to care for those fetuses and their mothers in centers with expertise in fetal diagnosis, therapy, and neonatal care. The hope is that such centers will allow timely, effective intervention for babies with congenital heart disease, congenital diaphragmatic hernia, or other anomalies.

The effectiveness of fetal medicine centers depends on two factors. First, they depend on effective fetal screening and diagnosis. That can only happen if all pregnant women have access to high-quality prenatal care. The existence of these centers should create an expectation and a demand for better prenatal care and fetal screening. Such screening is likely to include better imaging and screening tests; both will lead to earlier diagnosis of fetal anomalies. These diagnoses will create more complex dilemmas for perinatologists and parents who will need to decide whether to terminate the pregnancy, offer fetal therapy, or offer either palliative care or interventions after birth. Ironically, better fetal diagnosis may increase the likelihood of pregnancy termination, even when postnatal treatment is possible, because better fetal diagnosis allows more time to consider all available options.

Second, the effectiveness of fetal centers will depend on the effectiveness of fetal interventions. To date, fetal interventions have been effective in relatively few conditions. Vascular ablation for twin-to-twin transfusion syndromes clearly improves outcomes in these conditions. In utero surgery for myelomeningocele also leads to better outcomes for babies born with this condition. However, the vast majority of prenatally diagnosed conditions cannot be effectively treated in utero. The real hope of fetal medicine centers today is that they will improve outcomes by allowing better planning for interventions at the time of delivery and immediately after. In some cases, that may lead to changing the timing, mode, or location of delivery. In other cases, it may mean that a team of pediatric subspecialists will be prepared and immediately available to treat the baby in the minutes after birth. These efforts may improve outcomes.

Expanded Newborn Screening

In recent years, the number of diseases and conditions that can be diagnosed through newborn screening has expanded dramatically. Decisions about which conditions to include on the newborn screening panel are complex for at least three reasons. First, even the most accurate test has false-positives. The rarer the condition, the more likely that a positive test will be a false-positive. As more rare conditions are added to a newborn screening panel, there will be more false-positives. False-positives are not harmless. They are associated with considerable parental anxiety and can lead to potentially dangerous and unnecessary diagnostic procedures or treatments.

A second concern about expanded newborn screening is cost. Most of the cost is not for testing itself. Instead, it is for the follow-up counseling and testing after positive screening tests. Such follow-up is essential or the screening programs will not work. The Centers for Disease Control and Prevention (CDC) (2008) has expressed concern about these costs.

Finally, there is the potential for discrimination against patients for whom documented heterozygous carrier status conveys no recognized medical infirmity, but social or psychological stigma may be real. There is little funding available to assist or counsel these patients.

Many centers have started to offer whole-genome sequencing (WGS) for particular groups of newborns. Like newborn screening, such testing offers the tantalizing prospect of better diagnosis leading to better treatment for some newborns whose conditions have been difficult to diagnose using more conventional methods. However, of concern is the fact that WGS generates not one result but tens of thousands of results. Those results are more difficult to interpret than traditional newborn screening because every baby—and every adult—has many genetic variations. Some are diagnostic of rare diseases, some are known to be benign, and many are of unknown significance.

Variants can only be interpreted after a good clinical history, family history, and physical examination have been performed. Data from these preliminary steps allow physicians to assess whether there are similar or related phenotypes in other family members; if so, the inheritance pattern can then be evaluated and assessed. Physical examination findings allow physicians to begin a search for potentially relevant genes. Mode of inheritance and a comprehensive phenotype can then be used to classify the patient's genomic variants. WGS may lead to the discovery of a known pathogenic variant, a novel pathogenic variant that is likely to be disease-causing, or a variant of unknown clinical significance in a gene known to cause human disease. The issue of fetal WGS testing has emerged as an added controversial aspect of screening. Fetal centers looking to offer improved diagnostics and planning can benefit from this added information prior to delivery, because it may alter the interventions offered; however, even with excellent fetal imaging, the inability to perform a physical exam limits the interpretability of the testing.

Clinical validity is a complicated and challenging aspect of WGS. Evidence is required to prove that a specific rare variant in a particular gene, detected by WGS, is indeed pathogenic and responsible for a particular clinical phenotype.[33]

Summary

In modern neonatal care, we are fortunate to have data to inform many of our decisions. An ethical approach to using data to inform the care of sick babies means we need to understand both the opportunities and limitations of increasingly available data.

Acknowledgment

The authors gratefully acknowledge William L. Meadow, MD, PhD, previous edition author, mentor, collaborator, and friend.

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3 Development, Function, and Pathology of the Placenta

EMIN MALTEPE AND ANNA A. PENN

KEY POINTS

- The placenta is the first organ to form in mammals and is required for establishment of a maternal–fetal vascular interface capable of supplying the bioenergetic needs of the developing conceptus.
- Multiple placental cell types engage in highly varied functions, from attachment, invasion, and vascular remodeling to cell fusion, hormone production, and nutrient transport.
- Multiple mechanisms allow transport of waste and nutrients across the placenta, including diffusion, transporter protein-mediated (facilitated diffusion and active transporters), and receptor-mediated mechanisms.
- The placenta is not an inert transport interface. It consumes 40% to 60% of the oxygen and glucose delivered to the uterus at term. Thus conditions that alter placental metabolism can indirectly affect nutrient transport to the fetus.
- In the United States, iatrogenic delivery is responsible for almost half the births that occur between 28 and 37 weeks of gestation, primarily caused by placental pathologies such as preeclampsia or intrauterine growth restriction.
- Efforts to standardize placental examination after delivery are in progress so that connections between specific placental problems and poor outcomes can be better defined. In parallel, new advanced imaging techniques and biomarkers for placental function are being developed.

The placenta is a remarkable organ. Its brief existence enables the mammalian fetus to survive and thrive within the otherwise inhospitable confines of the intrauterine environment. To accomplish this, the placenta plays a range of roles, from anchoring the conceptus and preventing its rejection by the maternal immune system to enabling the transport of nutrients and wastes between the mother and the embryo/fetus. As with all organs, multiple specialized cell types derived from lineage-committed precursors are responsible for these functions. Genetic, epigenetic, and physiologic cues direct placental development across gestational stages.[1] Impairments in these processes due to intrinsic or extrinsic insults can lead to placental dysfunction and result in long-lasting increases in disease susceptibility, a process known as fetal programming.[2,3] Both preterm and term infants are at risk from poor placental function, particularly those that have extremely low birth weight (<1 kg). Preterm infants in particular may suffer from placental dysfunction in utero followed by early loss of placental support, including nutrition, hormones, and immune protection. Preterm delivery rates continue to rise while the survival of preterm infants has increased due to numerous advances in medical management and technology.[4] This convergence has generated an expanded population of patients admitted to and graduating from intensive care nurseries. Not only are these infants more likely to develop complications ssuch as bronchopulmonary dysplasia, failure to thrive, pulmonary hypertension, cerebral palsy, and blindness, but they are also more likely to develop chronic adult ailments such as diabetes and heart disease. Although further improvements in neonatal care are critical for diminishing the long-term consequences of prematurity, prevention or delay of preterm delivery will have the greatest healthcare impact for this at-risk population. A better understanding of the most common placental pathologies is therefore critically important for advancing maternal, fetal, and adult medicine.

Development of the Placenta

Trophoblast Lineage Allocation

The placenta is the first organ to form in mammals. This is because it is required for establishment of a functional maternal–fetal vascular interface capable of supplying the bioenergetic needs of the developing conceptus.[1] The fertilized embryo undergoes a series of cell divisions before implantation to produce up to eight seemingly identical cells called blastomeres. Three further sets of divisions generate the blastocyst, consisting of two distinct cell populations. Surrounding the blastocyst is the trophectoderm (TE), which gives rise to the placenta. The inner cell mass (ICM), located inside the blastocyst, gives rise to the embryo and visceral endoderm.[5,6] In mice, each blastomere is able to generate either ICM or TE derivatives and is thus totipotent. This also occurs in humans.[7] Once TE or ICM commitment occurs, however, it is considered largely irreversible. Importantly, however, individual blastomeres are found to harbor intrinsic biases regarding which lineage they adopt as early as the four-cell stage[8,9] and which appear to depend on positional cues.[5]

Multiple factors govern lineage allocation. One major determinant includes differences in polarity and adhesion between inner and outer cells of the blastocyst that are associated with differential activation of the Hippo signaling cascade.[5,10] Hippo helps restrict expression of key lineage regulatory genes such as *Cdx2* that are stochastically expressed as early as the eight-cell stage in

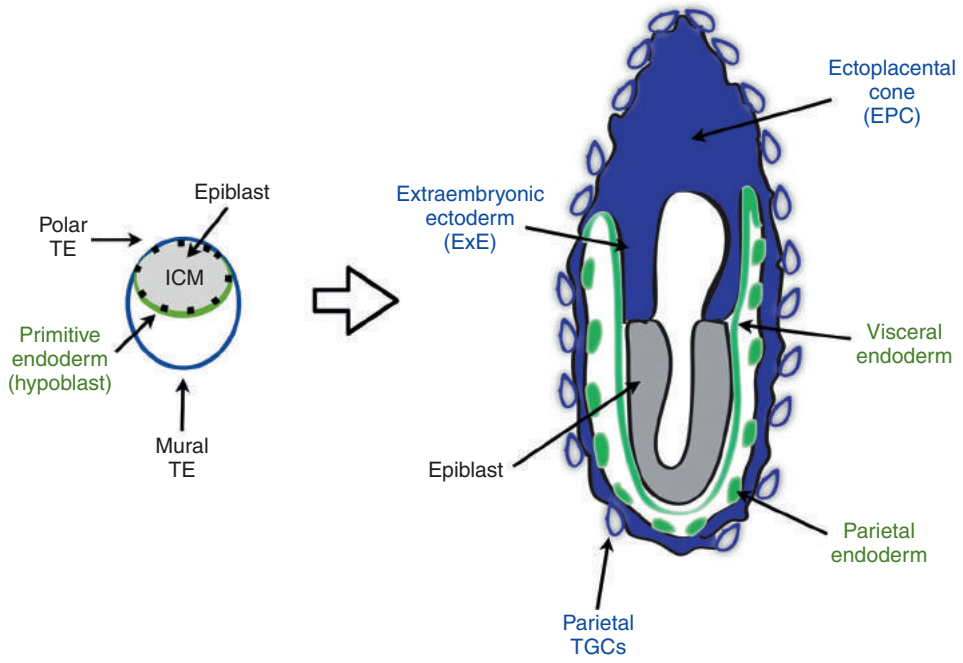
mice but restricted thereafter to the TE.[11] Notch signaling also acts in parallel with Hippo to promote *Cdx2* expression in this process.[12] Positional cues governed by E-cadherin expression help regulate Hippo signaling. Cell–cell contact within inside cells of the ICM activates Hippo and suppresses nuclear YAP activity. In mice, CDX2 helps repress genes critical for ICM identity, such as *Oct4* and *Nanog*. Its absence in mouse embryos results in the lack of TE differentiation, and all cells of the blastocyst stage embryo express the typically ICM-restricted OCT4 protein.[13] Amazingly, in mice its expression is sufficient to convert embryonic stem cells (ESCs) into trophoblast stem cells (TSCs) that can contribute to all lineages found within the placenta.[13,14] *Cdx2* expression is further maintained in mice via a positive feedback loop driven by the combinatorial activities of the transcription factors Eomesodermin (EOMES), ETS-related transcription factor 5 (ELF5), ETS proto-oncogene 2 (ETS2), and transcription factor AP-2, gamma (TCFAP2c) that help maintain the TE lineage.[15–22] Interestingly, ELF5, CDX2, and EOMES can collaborate to regulate hundreds of TSC genes by binding to enhancer elements that harbor endogenous retrovirus-derived sequences, indicating that these serve as trophoblast-specific enhancer elements in mice.[23] This opens the exciting possibility that differences in the incorporation of these foreign viral elements across different placental mammals may have contributed to the diversity of placental structures seen across the animal kingdom.

Uterine evolution paralleled placental evolution in eutherian mammals. This was recently found to involve large-scale and rapid changes in endometrial gene regulatory networks mediated by ancient transposable elements. These modulate responses to pregnancy hormones as well as other pathways to ensure pregnancy success.[24,25] Thus, a set of genetic tricks coupled with host–virus interactions enabled the rapid evolution of the placenta–uterus axis in mammals and helped contribute to the diversity of mammalian life forms and modes of procreation observed today.

Trophoblast Differentiation

The placenta is comprised of multiple different cell types that engage in highly varied functions, ranging from attachment, invasion, and vascular remodeling to cell fusion, hormone production, and nutrient transport.[1] Thus trophoblast-specific progenitors need to enact a complex set of lineage restriction decisions to help form a functioning placenta.

In the mouse, the extraembryonic ectoderm differentiates into cells that comprise the chorion and labyrinth, which perform the transport functions of the placenta; whereas the ectoplacental cone, located nearer to the uterine implantation site, differentiates into the spongiotrophoblast layer as well as glycogen trophoblasts and trophoblast giant cells (TGCs) (Fig. 3.1). To function as a transport organ, the placenta must establish an extensive vascular interface between the maternal and fetal circulatory systems. Humans and rodents have a hemochorial placenta, which means that the maternal vascular space comes in direct contact with differentiated trophoblasts, not endothelial cells.[26] In humans, trophoblasts



• **Fig. 3.1** Embryonic Development in the Mouse Conceptus. At the blastocyst stage, the conceptus is comprised of an inner cell mass (*ICM*) that gives rise to the embryo proper (*epiblast*), overlaid by a layer of primitive endoderm (*hypoblast*) that gives rise to the visceral and parietal endoderm. The outer cells comprise the trophectoderm (*TE*) that gives rise to the placenta. TE cells near the ICM are referred to as polar TE, while TE cells not in contact with the ICM are referred to as mural TE. The mural TE gives rise directly to parietal trophoblast giant cells in the initial wave of trophoblast differentiation. These cells aid the initial attachment and invasion process. The polar TE differentiates into the ectoplacental cone and extraembryonic ectoderm. The ectoplacental cone resides closest to the implantation site and also gives rise to lineages that further help incorporate the conceptus into the receptive uterus. The extraembryonic ectoderm resides closest to the developing epiblast and gives rise to lineages comprising the placental transport interface, such as the syncytiotrophoblast. *TGCs*, Trophoblast giant cells.

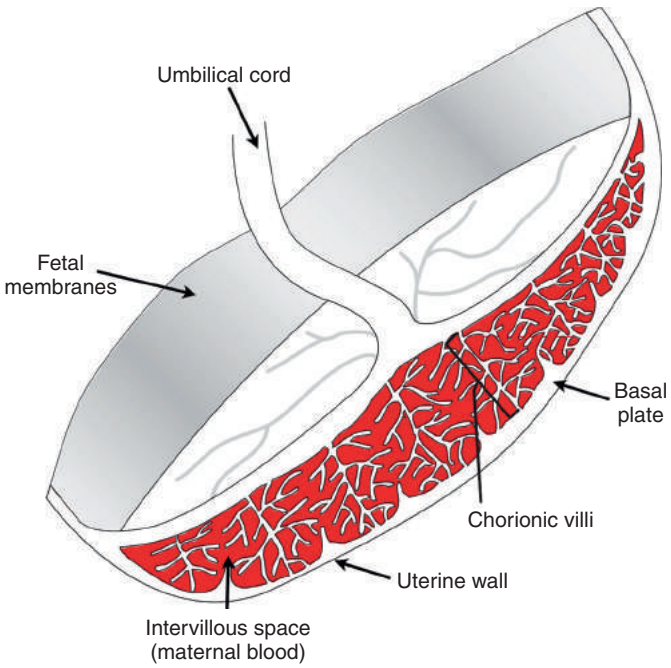
lining maternal arteriolar spaces are relatively well characterized,[27] but very little is known about the cells associated with the draining vascular bed, for example. In mice, there appear to be at least five distinct populations of TGCs that lie at various positions within these maternal vascular spaces and are defined by their location and lineage-specific gene expression.[28] These arise from various sources (Fig. 3.1). Their "giant" size is, in part, a reflection of their DNA content, which continuously replicates without engaging in cell division via a process called endoreplication.[29] Endocycles are thought to be used by cell types that need to be very large or that are highly metabolically active. Consistent with this, TGCs in the mouse placenta are responsible for the bulk of placental hormone production.[30] In humans, placental hormones are produced by the syncytiotrophoblast (SynT) layer, potentially accounting for the reduced ploidy of invasive trophoblast subtypes in this species compared with rodents, although they still appear to exhibit a significant amount of aneuploidy.[31]

Trophoblast Invasion

Primary TGCs differentiate from the mural TE, and this represents the first terminally differentiated cell type in mice to aid in implantation. In humans, the initial wave of invasion following implantation is thought to occur via formation of a primitive syncytium, through which invasive CTBs push following approximately day 13 or 14 of gestation.[32] This early wave of syncytialization does not occur in rodents. In mice, the remaining secondary TGCs differentiate either from trophoblast-specific protein alpha (Tpbpa)/4311+ outer ectoplacental cone cells or from Tpbpa/4311−chorionic progenitors.[28] Secondary TGCs come in various forms that have differing locations as well as characteristics. As the name implies, spiral artery (SpA) TGCs invade the spiral arteries and displace the smooth muscle and endothelial cells to remodel them, canal TGCs line the large vascular spaces delivering maternal blood to the base of the labyrinth, sinusoidal TGCs sit within the small vascular spaces of the labyrinth, and parietal TGCs surround large pools of deoxygenated blood that ultimately drain into the maternal uterine veins.

Trophoblasts that invade and line blood vessels appear to do so via two different mechanisms: (1) vascular invasion with endothelial mimicry and (2) vasculogenic mimicry.[33] In the former, trophoblasts invade and displace maternal endothelial cells from within maternal arterioles and include SpA-TGCs in mice or endovascular trophoblasts (EVTs, also known as extravillous trophoblasts) in humans. During vasculogenic mimicry, however, trophoblasts undergo morphogenesis to create vascular tubes de novo. Sinusoidal TGCs perform this function in mice. Whether this also occurs in human placentation is not clear. Transplanting trophoblasts subcutaneously in mice or culturing them as 3-dimensional trophospheres in vitro allows one to visualize them from de novo vascular spaces, where they generate tumors harboring large blood sinuses surrounded by trophoblasts, as opposed to host endothelium.[33,34] Many pathways known to regulate endothelial development also drive trophoblast differentiation and formation of the maternal–fetal vascular interface.[33] Furthermore, the endothelium and trophoblast are primary regulators of hemostasis in the adult and fetal circulation. Trophoblasts can regulate the coagulation cascade like endothelial cells and produce such molecules as thrombomodulin, tissue factor, tissue factor pathway inhibitor, annexin V, and endothelial protein C receptor.[35–37] These factors are critical for preventing thrombotic or hemorrhagic events from occurring in the developing placenta. Thus, mammalian placentas have solved the problem of hemochorial placentation by having trophoblasts take over functions typically performed by endothelial cells.

Remodeling of uterine vasculature is critical for successful pregnancy in humans and mice.[1,33,38,39] The equivalents of TGCs in humans, invasive EVTs, are derived from column CTB progenitors located at the tips of anchoring villi (Fig. 3.2). They migrate through the uterine parenchyma via interstitial invasion, in search of maternal spiral arterioles and veins. This invasion peaks early in pregnancy, around 9 to 12 weeks of gestation.[40] EVTs then breach the spiral arterioles via a process termed endovascular invasion and replace resident endothelial and smooth muscle cells.[41] This results in these high-resistance vessels being remodeled into low-resistance/high-capacitance conduits necessary for proper fetal perfusion as well as modulation of maternal hemodynamics.[27] While EVT interactions with veins are largely confined to the inner surface of the uterus, they migrate along much of the intrauterine course of maternal arterioles. Although endovascular invasion begins quite early and typically begins within the center of the placental bed, uterine arterial blood only begins to flow into the intervillous space by the end of the first trimester. Before this point, EVTs paradoxically plug these vessels, preventing blood flow to the placenta. As a result, all of first trimester placental development occurs in a highly hypoxic environment with the bulk of placental nutrients being provided by endometrial secretions (i.e., histiotrophic nutrition).[42] Only about one-third of the



• **Fig. 3.2** Human Placenta at Midgestation. The human (and mouse) placenta is disc-shaped (discoid) and hemochorial (i.e., fetal trophoblasts are in direct contact with maternal blood). In contrast to the labyrinthine placenta in mice, the human placenta is comprised of chorionic villi that project into the intervillous space bathed by maternal blood. Fetal blood vessels coursing through the umbilical cord branch when they reach the placenta and dive into the chorionic villi where gas, nutrient, and waste exchange occurs. Tips of anchoring villi attach to the uterine wall (basal plate) and send out waves of invasive cytotrophoblasts (also known as extravillous trophoblasts) to both anchor the placenta and establish blood flow to it. (Adapted from Hunkapiller NM, Fisher SJ. Placental remodeling of the uterine vasculature. *Methods Enzymol*. 2008;445:281–302.)

uterine SpAs are actually invaded by 18 weeks' gestational age,[43] indicating that the more lateral arteries are only invaded throughout the second and third trimesters in a progressive manner[44,45] because most are completely remodeled when examined following delivery at term.

Following unplugging of these vessels, maternal blood begins to bathe floating chorionic villi that are covered by a layer of multinucleated SynTs. SynTs form as a result of the fusion of lineage-committed progenitors. The need for multinucleated syncytium formation is not clear but may have been driven evolutionarily by a response to viral infections that may help minimize pathogen transmission to the fetus.[46] A combination of fusogenic protein expression, particularly syncytins,[47–54] and dramatic cytoskeletal rearrangement appears to be essential for this trophoblast fusion. Additionally, caspase 8 activity—frequently implicated in apoptosis—aids this process during human SynT formation.[55] These cytoskeletal changes are frequently accompanied by another apoptosis-associated process—externalization of phosphatidylserine to the outer leaflet of the plasma membrane. Typically acting as an "eat me" signal for the clearance of apoptotic cells, phosphatidylserine externalization is associated with SynT fusion.[56,57] Apoptosis is not completed during SynT formation, however, and the syncytium is maintained in this "preapoptotic" state until being sloughed off into the maternal circulation. As a result of the dramatic cytoskeletal changes required for cell fusion, in addition to changes in the composition of the membrane lipid bilayer, the biophysical properties of the SynTs change to become much more rigid, possibly aiding the infection barrier properties of the placenta.[58]

Placental Functions

Transport

The transport functions of the placenta are performed by the multinucleated SynTs that sit at the maternal–fetal interface. Multiple mechanisms allow transport of waste and nutrients across the placenta.[59] The simplest is diffusion. The high surface area of the placental transport interface, along with the hemochorial nature of the rodent and human placentas, enables efficient diffusion across the placenta. The rate of diffusion depends on the molecular properties and concentrations of the solute, however, in addition to the composition of the exchange barrier.[60] In the human placenta at term, a single SynT layer separates maternal blood from fetal capillary endothelium, whereas in the mouse, two SynT layers as well as an sinusoidal (S)-TGC layer, surprisingly, separate the vascular spaces.[61] These layers are progressively thinned out to minimize their barrier properties and increase the surface area for exchange.[50] Oxygen is transported across the placenta via passive diffusion, aided by the high affinity of fetal hemoglobin and the concentration differential across the maternal–fetal vascular beds. The orientation of the maternal and fetal vascular blood spaces produces a countercurrent exchange mechanism in mice. This maximizes transport efficiency in rodents, whereas the human placenta has a less efficient multivillous arrangement that necessitates a larger placental size relative to the mouse.[26]

Hydrophilic molecules do not readily cross plasma membranes. Transporter protein-mediated mechanisms are typically required for transporting hydrophilic molecules. Classic transporter proteins include facilitated diffusion transporters (i.e., the glucose transporter [GLUT] family[62]) as well as active transporters (i.e., transporters associated with calcium transport[63] and the amino acid transporters[64]). Transport can occur down a concentration gradient, as is the case with GLUT1-mediated glucose transport,[64] or against a concentration gradient, as is the case with calcium[65] and amino acid transport.[66] Interestingly, nearly all amino acids in the fetal circulation are found at higher levels than in the maternal circulation, indicating active uptake and/or synthesis of these nutrients via the placenta or fetus.

The fetal–placental unit is both physically and metabolically interconnected with each other and the maternal circulation. Ultimately, all fetal–placental metabolism is constrained by the nutrients delivered from the maternal circulation. However, the placenta and fetal liver are both capable of producing and metabolizing various nutrients that impact their levels in the fetal–placental circulation largely independent of placental transport mechanisms.[67] This has been well described in ovine species, wherein the fetal liver of sheep in utero actively produces large quantities of serine and glutamate that are consumed by its placenta.[68,69] In the placenta, serine is converted to glycine via a process that contributes to one-carbon metabolism-dependent DNA methylation pathways that play important roles in cell fate regulation and growth mechanisms.[70] Additionally, nonglucose carbohydrates such as fructose, mannose, inositol, and sorbitol are also either transported by or synthesized in the placenta[66,71] and play important roles in regulating fetal growth as well as in redox regulation.

Finally, antibody-mediated immunity is transferred from mother to fetus across the placenta via receptor-mediated mechanisms.[72,73] Immunoglobulin G (IgG) transport across the human placenta, for example, begins at approximately 16 weeks' gestation, and fetal serum IgG levels reach maternal levels by 26 weeks' gestational age. This process is highly efficient, enabling fetal concentrations to exceed maternal values at term.

Metabolism

The placenta is not an inert transport interface. It consumes 40% to 60% of the oxygen and glucose delivered to the uterus at term, despite only comprising approximately 10% to 20% of the total mass of the uterus at that time.[74,75] Changes in this metabolism can regulate placental biology. Mitochondrial fusion, a process that enables greater mitochondrial bioenergetic capacity, is critical for invasive TGC formation in mice, triggering placental failure when compromised.[76,77] Interestingly, mitochondrial fusion can promote cardiomyocyte differentiation as well, highlighting conserved mechanisms between cellular metabolism and cell fate determination.[78] Thus, alterations of placental metabolic function can impact oxygen and nutrient delivery to the fetus, both by altering placental metabolic demand intrinsically as well as by impacting cell fate regulatory pathways. Primary culture of human CTBs indicates that they exhibit high rates of aerobic glycolysis when compared with other terminally differentiated adult cells,[79] much like rapidly proliferating cancer cells that rely on high glycolytic flux rates to augment biosynthetic precursor production. Aerobic lactate production (i.e., the Warburg effect), also allows the placenta to produce and transfer large amounts of lactate to the fetus, which can readily oxidize it. Interestingly, the placenta also appears able to metabolize lactate during midgestation but loses this ability by term.[80] These studies additionally suggest that glycogen breakdown (glycogenolysis) helps supply the high rates of glucose consumption in proliferating trophoblasts before differentiation into terminally differentiated SynTs. Interestingly, excess glycogen accumulation has been noted within the SynT layer of

some human preeclampsia (PE) placentas,[81] consistent with their altered turnover and suggesting a potential link to altered glucose metabolism in the setting of this pregnancy complication. Importantly, epidemiologic studies confirm that derangements in glucose and fatty acid metabolism may drive pregnancy complications. For example, maternal gestational diabetes mellitus (GDM) and obesity are independently and additively associated with elevated rates of PE[82] as well as spontaneous preterm birth.[83] There may be shared mechanisms involving impaired trophoblast invasion contributing to PE and preterm labor (PTL) pathogenesis. For example, up to 30% of patients with spontaneous PTL have placental lesions consistent with impaired SpA remodeling typically observed during PE.[84–86] Thus, improving our understanding of the links between placental metabolism and placental development may shed light on the growing epidemic of preterm birth.

Metabolic stressors such as hypoxia at high altitude or placental underperfusion associated with intrauterine growth restriction (IUGR) alter placental metabolism in particular ways. During isolated hypoxia induced at high altitude, for example, the human placenta appears to decrease its consumption of oxygen in favor of glycolysis to maintain its bioenergetic needs,[87] which preserves placental growth. While preserving fetal oxygen (O_2) delivery, this comes at the expense of glucose, however.[88] Given that fetal hypoglycemia is strongly associated with fetal growth restriction, this limits fetal growth. With maternal undernutrition, however, where uterine O_2 delivery is relatively spared, glucose delivery to the fetus is compromised[89,90] in a manner associated with restricted placental growth. Given that the placenta is a complex organ with multiple different cell types,[28,50] it is likely that changes in placental cellular composition due to alterations in cell fate regulatory pathways play important roles in the reallocation of placental metabolic flux patterns. Consistent with this, maternal calorie restriction leads to a loss of glycogen trophoblasts in the mouse.[90] Importantly, isolated hypoxia results in increased hypoxia-inducible factor-1 (HIF-1) levels and target gene expression in the human placenta,[91–93] and HIF activity regulates trophoblast cell fate decisions, suggesting a potential contribution to these pathologic changes.[94–97] HIF activity can mediate metabolic adaptation to hypoxia via numerous ways,[98] including repressing mitochondrial O_2 consumption while increasing glycolysis and modulating glucose transport as well as amino acid metabolism.

Endocrine Function

In addition to performing the essential transport functions of the placenta in humans, SynTs secrete numerous pregnancy-related hormones.[1] Mammalian placenta produces a greater diversity of hormones in greater quantity than any other single endocrine tissue. Near term, steroid hormones (primarily estrogens and progestins) are being made at the rate of 0.5 g/day, and protein hormones (lactogens, growth factors, and other hormones similar to those of the hypothalamic–pituitary–adrenal [HPA] axis) are being made at more than twice this rate. Many of these hormones are species specific, but the categories of hormones (i.e., steroids, pituitary-like, hypothalamic-like etc.) and their endocrine, paracrine, and autocrine functions in pregnancy are frequently conserved (Table 3.1).

Steroid Hormones

The placenta is an "incomplete" steroidogenic organ and does not express a complete set of enzymes for de novo production of estrogens and progestins. Steroid hormone synthesis in the placenta is dependent on precursors from mother and fetus, leading to the concept of an integrated *maternal–fetal–placental unit*.[99]

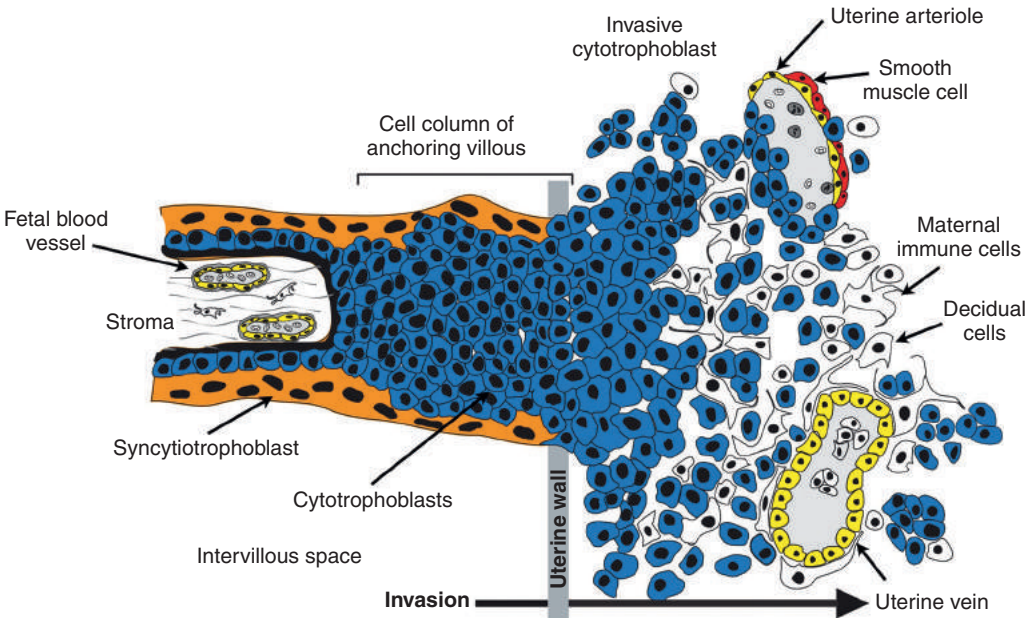
TABLE 3.1 Placental Classification (Incorporating the 2014 Amsterdam Placental Workshop Group Criteria)

1. Placental Vascular Processes
   a. Maternal stromal-vascular lesions
      Developmental
         Superficial implantation/decidual arteriopathy
         Increased immature extravillous trophoblast
      Malperfusion
         Global/partial
         Early: distal villous hypoplasia
         Late: accelerated villous maturation
         Segmental/complete
         Villous infarct(s)
      Loss of integrity
         Abruptio placenta (atrial)
         Marginal abruption (venous)
         Acute
         Chronic
   b. Fetal stromal-vascular lesion
      Developmental
         Villous capillary lesions
         Delayed villous maturation (maturation defect)
         Dysmorphic villi
      Malperfusion
         Global/partial
         Obstructive lesions of umbilical cord
         Recent intramural fibrin in large fetoplacental vessels
         Small foci of avascular or karyorrhectic villi
      Segmental/complete
         Chorionic plate or stem villous thrombi
         Large foci of avascular or karyorrhectic villi
      Loss of integrity
         Large vessel rupture (fetal hemorrhage)
         Small vessel rupture (fetomaternal hemorrhage)
         Villous edema

2. Placental Inflammatory Immune Processes
   a. Infectious inflammatory lesions
      Acute
         Maternal inflammatory response: chorioamnionitis, subchorionitis
         Fetal inflammatory response: chorionic/umbilical vasculitis
      Chronic
         Villitis (CMV, others)
         Intervillositis (malaria, others)
   b. Immune/idiopathic inflammatory lesions
      Villitis of unknown etiology and related/associated lesions
         Chronic villitis
         Chronic chorioamnionitis
         Lymphoplasmacytic deciduitis
         Eosinophil T-cell fetal vasculitis
         Chronic histiocytic intervillositis

3. Other Placental Processes
   Massive perivillous fibrin(oid) deposition (maternal floor infarction)
   Abnormal placental shape or umbilical insertion site
   Morbidly adherent placentas (accreta)
   Meconium-associated changes
   Increased circulating nucleated red blood cells

CMV, Cytomegalovirus.
From Redline RW. Classification of placental lesions. *Am J Obstet Gynecol.* 2015;213(4 Suppl):S21-8.



• **Fig. 3.3** Basal Plate of the Human Placenta at Midgestation. A multinucleated layer of syncytiotrophoblasts (SynTs) covers the finger-like projections of chorionic villi. SynTs perform all of the transport functions of the placenta. A single cell layer of cytotrophoblasts (CTBs) sits just below the SynT layer and is referred to as villous CTB. These cells fuse with each other as they differentiate into SynTs. At the tips of anchoring villi sits a column of CTBs that are proliferative and put out waves of invasive CTBs (extravillous trophoblasts) that help anchor the placenta to the uterus. Importantly, these also invade maternal spiral arterioles where they replace the endothelial lining, induce the apoptotic death of surrounding smooth muscle cells, and transdifferentiate into an endothelialized cell type. This invasion typically courses through the extent of the uterine myometrium. CTBs can also invade veins but do not appear to remodel them. Additionally, maternal uterine immune cells, such as natural killer cells, appear to play an important role in enabling CTBs to invade the uterus and its vasculature. Finally, establishment of a receptive deciduum is also of critical importance for proper placental development and pregnancy success. (Adapted from Genbacev O, Krtolica A, Kaelin W, et al. Human cytotrophoblast expression of the von Hippel-Lindau protein is downregulated during uterine invasion in situ and upregulated by hypoxia in vitro. *Dev Biol*. 2001;233(2):526–536.)

Fig. 3.3 diagrams the tissues and enzymes that participate in the biosynthesis of progestins and estrogens.[100] The concentration of steroid hormones in the maternal circulation increases dramatically throughout gestation.[101]

Progesterone

Maternal cholesterol, derived from low-density lipoprotein, is transported to the placenta and bound to low-density lipoprotein receptors on SynTs, where it is incorporated by endocytosis and hydrolyzed to free cholesterol in lysosomes.[102] There is no significant 3-hydroxy-3-methylglutaryl coenzyme A activity in human placenta, and thus maternal cholesterol must be used for production of pregnenolone—the first step in steroid synthesis. Cholesterol is converted to pregnenolone in the mitochondria by cytochrome P450 cholesterol side-chain cleavage enzyme. After transfer to the cytosol, progesterone is produced from pregnenolone by type-1 3β-hydroxysteroid dehydrogenase.[103,104] Before the ovarian–placental shift, the corpus luteum of pregnancy is the primary source of progesterone, but by 35 to 47 days postovulation the placenta produces enough progesterone to maintain pregnancy.[105] The majority (>90%) of progesterone goes to the mother and the rest to the fetus. A limited amount of pregnenolone is also released into the circulation. The fetus has the enzyme activity needed for pregnenolone synthesis but has minimal ability to produce progesterone. High levels of circulating fetal progesterone are of placental origin so that circulating progesterone levels thus reflect placental function, not fetal well-being.

Progesterone can be metabolized to 17-hydroxyprogesterone (17-OHP), but relative efficiency of the enzymes favors progesterone production. 17-OHP levels do rise in the third trimester as progesterone levels peak. Additional progesterone metabolites, particularly 5-dihydroprogesterone (5-DHP) and its metabolite allopregnanolone, are also produced in the SynT at increased levels during gestation.[106] These steroids have been hypothesized to play an endocrine role in fetal brain development and provide neuroprotection in the face of hypoxia.[107,108]

Progesterone[109] or a synthetic form of 17-OHP[110] is used therapeutically in gestation as an adjunct for pregnancy maintenance after in vitro fertilization or in the second half of gestation for prevention of preterm delivery in women with a prior history of preterm birth.[111] Progesterone is required for the maintenance of pregnancy in part by means of its suppressant effect on uterine contractions.[100,105,112] Progesterone inhibits genes that promote contractility[113] and has immunosuppressive activity that may promote uterine quiescence.[112,114] Progesterone also counteracts uterine estrogen effects. Unlike the drop in progesterone levels prior to labor seen in most mammals, there is no progesterone withdrawal per se that occurs before labor in women; however, modulation of progesterone receptor expression in combination with a shift in the progesterone to estrogen balance is presumed to play the same

biological role. The relationship of therapeutic response to normal physiologic mechanisms at work in the maternal–fetal–placental unit is not yet understood.

Estrogens

Unlike the requirement for maternal precursors for progesterone production, estrogen production relies on fetal precursors. In pregnancy, estrogens are synthesized from C19 steroids,[115] primarily from dehydroepiandrosterone sulfate (DHEA-S) made in the fetal adrenals. The fetal adrenals rapidly inactivate steroids through sulfatization. Pregnenolone is sulfated and converted to DHEA-S,[116] which then may be hydroxylated in the fetal liver. These biologically inactive androgens are then transferred back to the placenta. Placental sulfatases rapidly cleave the sulfate, and placental 3β-hydroxysteroid dehydrogenase converts DHEA or hydroxylated DHEA to androstenedione or hydroxylated androstenediones, respectively. These androgens are then aromatized to estrone (E1), 16α-OH estrone, or 15α-OH estrone and then converted to estradiol (E2), estriol (E3), or estetrol (E4) respectively by placental 17β-hydroxylation.[117–119] E3 is the major estrogen of pregnancy with the majority secreted into the maternal compartment; E1 is the only estrogen preferentially secreted into the fetal compartment. Although maternal DHEA-S serves as 40% of the precursor for E2 synthesis, E3 and E4 are formed predominantly from fetal precursors because the maternal liver has limited 5α-hydroxylation or 16α-hydroxylation capabilities.[120] E3 and E4 are thus indicators of fetal function,[101] although neither is a clinically useful marker because of rapid shifts in circulating levels. The primary function of high E3 levels remains unclear, but it does increase uteroplacental blood flow.[121]

Estrogens influence uterine growth, blood flow, contractility, metabolism, and breast development.[122] However, high estrogen levels are not apparently needed for pregnancy. Parturition can proceed in the absence of fetal and placental sulfatase[123] or aromatase,[124] although in the latter case both fetus and mother are virilized. In such pregnancies, there is still circulating estradiol. There are no reports of pregnancy without detectable estrogen levels, suggesting that a basal level of estrogen is likely required. Before parturition, an increase in the estrogen to progesterone ratio occurs within the intrauterine tissues and may increase prostaglandin (PG) and oxytocin (OT) activity. Steroid hormone production is altered by trophic hormones and other factors, including hypothalamic-like releasing or inhibiting hormones. In turn, estrogens affect other endocrine systems (i.e., renin–angiotensin system)[125] and support organ maturation, such as surfactant production in the lung.[126]

Glucocorticoids

In addition to the sex steroids, circulating levels of glucocorticoids and mineralocorticoids are increased in pregnancy.[127] The placenta has the ability to produce cortisol and to convert it to inactive cortisone via 11β-hydroxysteroid dehydrogenase type 2. This enzyme also converts maternal cortisol to cortisone at the placental interface. The primary role of this system appears to be to protect the fetus from elevated cortisol exposure, which may play a role in long-term reprogramming of the fetal HPA axis.[128] Dual oxidative and reductive enzymatic activity regulates the balance between cortisol and cortisone.[129] In the placenta, oxidation of cortisol to cortisone predominates, whereas in the decidua the reverse reaction dominates, potentially providing localized hormone exposure.

Pituitary-Like Hormones

A combination of pituitary-like growth hormones is required to support fetal growth while maintaining maternal metabolic homeostasis.

Human Chorionic Gonadotropin

Human chorionic gonadotropin (hCG) is one of the first hormones of pregnancy, produced by trophoblasts even before placenta formation,[130] and is unique to human pregnancy.[131] After placentation, hCG is synthesized primarily by the SynT[132] and passes into the maternal circulation via secretion into the intravillous space. hCG is a glycoprotein heterodimer (36 to 40 kDa) composed of α and β subunits encoded by genes on chromosome 6 and 19, respectively.[102,133] The α subunit is homologous to pituitary thyroid-stimulating hormone (TSH), luteinizing hormone (LH), and follicle-stimulating hormone (FSH), while the β subunit is homologous to LH. Intact hCG (i.e., having both α and β subunits) is required for hCG endocrine activities. Since it shares a receptor with LH, the LH chorionic gonadotrophin receptor (LHCGR), hCG mimics the function of LH, but the functions of LH and hCG are quantitatively different due to the longer half-life of hCG and its relative stability compared with the pulsatile release of pituitary LH.[134]

hCG maintains corpus luteal progesterone production until this function shifts to the maturing placenta. hCG peaks approximately 2 weeks after the shift of progesterone production from ovary to placenta, potentially minimizing the chance of loss of the progestational environment. hCG can be detected in human serum or urine within a week of conception and is the most frequently used biochemical marker for pregnancy. hCG doubling time may be used in early gestation to predict general pregnancy outcome. After hCG can first be detected, it increases with a doubling time averaging 2.11 days. It reaches peak levels of approximately 50 international units (IU)/mL at 9 to 10 weeks from the date of the last menstrual period, declining to 1 IU/mL by midgestation.[104] An abnormally slow doubling time of hCG is considered to be a sign of a poor prognosis for pregnancy outcome, while rising hCG without detection of an intrauterine embryo suggests an ectopic pregnancy.[135]

In addition to corpus luteum maintenance, hCG has multiple additional activities that regulate placental structure and function. hCG acts as an autocrine signal in trophoblasts expressing LHCGR promoting the differentiation of SynTs, thus amplifying its own production since it is made primarily by these cells.[119] Phosphorylation of the receptors via this pathway also decreases LHCGR expression in differentiating SynTs, thus completing a feedback loop.[136] hCG may also have roles in endometrial angiogenesis, uterine quiescence, and immunotolerance to the fetus.[104,133,135] In addition, hCG can alter maternal TSH levels, elevating free thyroxine (T4), although this increase does not appear to cause maternal hyperthyroidism.[137]

Glycosylation state and subunit availability regulate hCG activity. A hyperglycosylated form (hCG-H) has been detected in early pregnancy as well as in choriocarcinoma cells. hCG-H appears to enhance trophoblast invasion[133] and thus may be a very early biomarker of placental invasion of the endometrium. A decreased level of hCG glycosylation in very early pregnancy has been correlated with early pregnancy loss.[103,133,138] Isoform production may also regulate activity. β-Subunit production exceeds α-subunit production in early pregnancy, but this ratio rapidly shifts to

α-subunit excess, increasing as gestation progresses; circulating hCG is mostly intact hCG or free α-hCG. It has been proposed that ratios of hCG isoforms (intact hCG, independent subunits, and nicked breakdown products) present in maternal blood and urine might be useful for detection of pregnancy-related disorders since only intact hCG is fully active.[139]

Local and systemic factors influence hCG production. Locally, its expression is regulated by a releasing factor—gonadotropin-releasing hormone (GnRH) 1 and 2.[140–142] Neurotransmitters,[119] cyclic adenosine monophosphate,[117] epidermal growth factor (EGF),[118] activing,[143] cytokine,[144] and PGs[145] regulate hCG production, as does hCG itself as noted previously. Each of these factors is produced by the placenta as well as by other extraembryonic tissues. hCG alters placental steroidogenesis by stimulating both progesterone and estrogen formation. Estrogens inhibit GnRH stimulation of hCG,[122] thereby completing a feedback axis in the placenta.

Human Chorionic Somatomammotropin

Human chorionic somatomammotropin (hCS), originally known as human placental lactogen,[146,147] has both growth hormone-like and lactogenic activity. hCS is detectable in extraembryonic tissues within 10 days of conception and in maternal serum by the third to fourth week of gestation. It is a single 191 amino acid nonglycosylated peptide chain with considerable homology to growth hormone (GH) (96%) and PRL (67%); it is transcribed from a gene cluster on chromosome 17 containing two genes for hCS, one hCS pseudogene and two GH genes.[148,149] SynTs produce hCS at a constant rate during gestation; thus, hCS levels reflect total placental mass and gross placental function. By term, hCS is made at 1 g/day, representing 10% of total placental protein synthesis.

hCS is considered one of the major diabetogenic factors of pregnancy, along with placental steroids, placental GH variant (hGH-V), and maternal cortisol. It is almost exclusively found in maternal rather than fetal circulation. This has led to the hypothesis that the primary role of hCS is to ensure adequate fetal nutrition because in maternal circulation it induces metabolic changes such as mobilization of fatty acids, insulin resistance, decreased utilization of glucose, and increased availability of amino acids through decreased maternal use of protein.[150] Circulating maternal glucose and free fatty acids are thus increased. While glucose readily crosses the placenta, fatty acids cross slowly, thus biasing glucose delivery toward the fetus and use of fatty acids for maternal energy, especially during maternal fasting. Within the placenta, hCS may regulate insulin-like growth factor (IGF-1)[151] and alter fetal growth through direct action on placental nutrient transport systems. In addition to its metabolic activity, the lactogenic activity of hCS may prepare the breast for lactation, working synergistically with PRL and steroids.[152] Most recently, a role for hCS as a placental angiogenic factor has been suggested.[153]

Placental Growth Hormone Variant

hGH-V is encoded in the same chromosome 17 gene cluster as hCS and pituitary GH. Two transcripts are generated from the hGH-V gene, a major form and an alternatively spliced version. Secreted hGH-V is translated from the major version and is produced in a highly bioactive 22 kD nonglycosylated form and to a lesser degree in a 25 kD glycosylated form.[152] Early in pregnancy, maternal pituitary GH is produced, but from 15 to 20 weeks' gestation to term hGH-V secretion increases, suppressing maternal GH. hGH-V peaks about a month before term delivery and disappears from maternal circulation immediately after delivery.[154] hGH-V is not detected in the fetal circulation. Much like hCS, hGH-V modifies maternal metabolism to meet fetal needs. hGH-V primarily appears to control maternal IGF-1 production.[154] In mice overexpressing hGH-V (not normally found in rodents), body weight was increased, IGF-1 levels were elevated, and insulin resistance developed, suggesting that hGH-V strongly contributes to the insulin resistance of pregnancy[155] and increases the risk of gestational diabetes and other pregnancy-related pathologies. This risk is counterbalanced by the placental lactogens hCS and PRL, which induce increased insulin secretion by pancreatic β-cell expansion. hGH-V secretion is tonic, in contrast to pulsatile pituitary GH, and is not regulated by hypothalamic-releasing factors.[154] Secretion is inhibited by elevated glucose and mildly increased by hypoglycemia, creating a feedback loop that may ensure constant delivery of nutrients to the developing fetus.

Insulin-Like Growth Factors

IGF1 and IGF2 are the primary somatotrophs in gestation. IGFs are highly homologous single chain polypeptides with similarities to pro-insulin made in human placental tissues.[156] The majority of the components of the insulin/IGF system are found in the placenta (IGF1, IGF2, and the IGF-binding proteins [IGFBP] 1–6)[157] except insulin itself, although maternal insulin has profound indirect effects on fetal growth and wellbeing. hGH-V levels regulate placental IGF levels,[151] and IGFBPs are carrier proteins expressed in the human placenta that prevent IGF from degradation while blocking bioactivity.[156,158,159] IGFBP1 is also produced by the decidua in large amounts. IGFBPs are themselves regulated by protease activity and through posttranslational modifications, adding a further layer of regulatory complexity.

IGF1 is expressed predominantly in SynT throughout gestation with some CTB expression, while IGF2 is expressed only in CTB with a declining level across gestation.[154,156–158] At physiologic concentrations, both IGF1 and IGF2 bind to the IGF1 receptor (IGF1R). The localization of IGF1R shifts during gestation; initially it is predominantly expressed on SynTs (closer to the maternal circulation), and by term it is mainly expressed on the fetal CTB side, reflecting the shifting activity from maternal to fetal growth control.[157] The IGF2 receptor (IGF2R; also known as the cation-independent mannose-6-phosphate receptor) controls extracellular IGF2 concentrations by mediating the endocytosis and degradation of IGF2, rather than by direct signaling via the receptor.[160] An additional receptor, possibly a variant of the insulin receptor, may mediate some of the fetal growth effects of IGF2.[161]

Information on the role of IGFs in fetal growth comes from genetic manipulation in mouse models as well as examination of human tissues, especially from fetal growth restricted pregnancies.[160] Disruption of mouse insulin-like growth factor (*Igf1*), *Igf2*, or insulin-like growth factor 1 receptor (*Igf1r*) genes retards fetal growth,[161] while disruption of Igf2r or overexpression of IGF2 enhances fetal growth.[162] In humans, mutations in the *IGF1* or *IGF1R* genes are extremely rare, and no *IGF2* gene deletions have been reported.[160] However, *IGF2* is an imprinted gene normally expressed exclusively from the paternal allele in placenta and fetal tissues. Changes in *IGF2* expression because of abnormal imprinting have been linked to both overgrowth (Beckwith–Wiedemann syndrome) and growth retardation (Russel–Silver syndrome).[160] Whether placentally derived IGFs—versus fetal IGFs—directly contribute to these fetal growth changes is uncertain since these

factors also have paracrine effects in the placenta that determine nutrient transport and placental growth.

IGF1 can promote SynT differentiation, while IGF2 does not appear to have this function despite its very early placental expression. In vitro experiments suggest that placental mass is regulated directly by placental IGFs[159]; in vivo, loss of IGF2 reduces the placental surface area available for gas and nutrient exchange more than IGF1 loss. Both IGFs increase nutrient transport, especially of amino acids, which may be reflected in elevated fetal amino acids associated with gestational diabetes.[159,163] IGFs may alter fetal growth through additional mechanisms since they potentiate EGF activity,[164] increase prolactin and progesterone production,[165,166] and inhibit placental thromboxane production.[167]

Other Secreted Growth Factors

Platelet-derived growth factor A, transforming growth factor (TGF)-α, and TGF-β[168] expression in blastocysts appear to be involved in implantation. Other growth factors, including EGF, basic FGF, nerve growth factor, granulocyte colony-stimulating factor, and hepatocyte growth factor as well as growth factor receptors are expressed by the placenta and membranes at later gestation stages.[169–172] The actions of many of these growth factors may be nonclassical autocrine actions. For example, EGF is made in SynTs, and EGF receptors on SynTs correlate with trophoblast differentiation rather than proliferation.[173–175] Additional growth factor actions on placental development are under intensive investigation.

Inhibin and Activin

Inhibin and activin, an antagonist and agonist of pituitary FSH, respectively, are expressed by CTBs and fetal membranes[176,177] while activin receptors are expressed in SynT.[178] Inhibin inhibits hCG and reduces progesterone production,[177] while activin has the opposite effect.[179] Inhibin elevation is associated with fetal trisomy 21, while elevated activin is associated with PE and gestational diabetes.[178] Thus, during pregnancy these hormones may serve as potential biomarkers of placental pathologies.

Proopiomelanocortin Hormones

Pituitary-like peptides derived from proopiomelanocortin (POMC), including adrenocorticotropic hormone (ACTH), melanocyte-stimulating hormone, β-endorphins, and β-lipoproteins, as well as full length POMC itself, are found in the human placenta.[180,181] The processing of POMC in the placenta is different from in the pituitary; POMC is released largely intact from the placenta while it is cleaved into several peptide hormones in the nonpregnant state. While pituitary POMC-derived peptides respond to and regulate physiologic stress, placental POMC is not inhibited by glucocorticoids nor do circulating levels correlate with ACTH or cortisol levels, although they do correlate with corticotrophin-releasing hormone (CRH) levels.[181] Chorionic CRH is produced by the placenta and stimulates the release of chorionic ACTH (see later).[182] The physiologic role of chorionic ACTH has not been defined, but it may affect placental cortisol production or maternal resistance of ACTH suppression by glucocorticoids.

Hypothalamic-Like Hormones

Every known hypothalamic-releasing or inhibiting hormone has a placental analogue.[140–142,183] These hormones act in placental paracrine–autocrine regulatory networks that control release of placental endocrine hormones.

Gonadotropin-Releasing Hormone

In the placenta, chorionic GnRH, which regulates the paracrine axis, is important for early pregnancy maintenance as well as regulating gonadal steroid production through stimulation of pituitary LH and FSH.[184] Two isoforms of GnRH (GnRH1 and GnRH2) are produced.[142,185,186] GnRH1 is encoded on chromosome 8 as a precursor protein that includes a signal sequence, the GnRH decapeptide, a processing sequence, and a GnRH-associated peptide.[187] GnRH2 is encoded on chromosome 20 and has 70% homology to GnRH1. GnRH1 and GnRH2 signal through the same G protein-coupled receptor, GnRHR1, expressed in SynTs, but may activate different intracellular signaling pathways.[185,188,189] Blocking GnRHs or GnRHR1 activity can lead to pregnancy failure,[190–193] possibly through alteration of hCG and placental steroids, whose production and release they modulate.[194] The release of placental GnRH1 is affected by cyclic adenosine monophosphate, PGs, epinephrine,[195] and inhibin,[146] while the expression of GnRHR1 is regulated by GnRH1, activin, and inhibin, creating a feedback loop.[185]

Corticotrophin-Releasing Hormone and Urocortins

Chorionic CRH and CRH receptors are expressed in placenta and fetal membranes.[196–199] Urocortins, members of the CRH-hormone family, are also produced and bind to CRH receptors as well.[200] Early in gestation, CRH family members may promote immune tolerance.[201] As gestation progresses, CRH and urocortin levels rise, peaking at term with delivery.[200] These hormones stimulate POMC-derived hormones, including ACTH and β-endorphinins[200,202] as well as PG release, suggesting roles in parturition.[203] CRH can also stimulate fetal adrenal estrogen and glucocorticoid production,[204] which may contribute to the timing of parturition. Glucocorticoids can increase placental CRH expression,[205] in contrast to glucocorticoid inhibition of CRH in the hypothalamus, creating a positive feedback loop that amplifies CRH activity.[206] Because of its tight association with delivery timing, CRH is often viewed as a *placental clock*[207] and may be biomarker of pregnancy pathology. In pregnancies complicated by hypertension, the maternal circulating levels of CRH are already elevated by 28 weeks of pregnancy, whereas local urocortin levels may be decreased.[200,208] CRH has been proposed as a predictor for preterm delivery,[207,209] but significant clinical utility has not yet been demonstrated.[210,211]

Thyrotropin-Releasing Hormone

Chorionic thyrotropin-releasing hormone[212] is made by the placenta and fetal membranes, but a clear role for either the mother or the fetus has not been identified. Pituitary TSH does not cross the placenta, nor does the placenta make thyroid hormone itself, but maternal T4 and triiodothyronine (T3) cross the placenta carried by placentally produced transthyretin.[213–215] The placental role in thyroid metabolism has been of considerable recent interest since thyroid disease is common in women of childbearing age and impacts pregnancy outcomes.[216] Early maternal hypothyroidism appears to be associated with lower intelligence quotient in offspring, but conflicting reports exist on the impact of maternal hypothyroidism after onset of fetal thyroid function in midgestation.[216,217] Maternal T4 continues to cross from maternal to fetal circulation in the second and third trimesters; even fetuses with complete thyroid dysgenesis have 30%–50% normal T4 levels in cord blood.[218] Placenta regulation of thyroid hormone transport and metabolism may play a critical role in fetal well-being, but the regulatory pathways remain to be defined.

Growth Hormone-Releasing Hormone, Somatostatin, and Ghrelin

Additional releasing factors are made in the CTB, including growth hormone-releasing hormone,[219] somatostatin,[220] and ghrelin[221] and may regulate hGH-V production as well as placental differentiation.[222]

Leptin

Leptin is normally secreted by adipocytes and decreases food intake through hypothalamic actions, but in pregnancy, the placenta is the primary leptin source.[223,224] The precise roles of leptin in the placenta, the mother, or the fetus are not known but may differ significantly from the nonpregnant state, as leptin levels in pregnancy do not correlate with body mass nor produce satiety.[225,226] Increased leptin levels are seen in PE and gestational diabetes.[227,228]

Oxytocin

OT is another hypothalamic hormone produced in the placenta and membranes.[229] OT is a potent uterotonic hormone used clinically to induce or speed labor. However, neither circulating maternal OT nor locally produced OT appears to increase markedly before labor; rather, uterine response to OT is increased through increases in OT receptor (OTR) expression and function.[230,231] Progesterone suppresses OTR signaling during gestation,[229] but a decline in progesterone activity at term (although not absolute progesterone levels in humans) increases OTR expression, making the uterus more responsive to OT.

Additional Placental Secreted Factors

Vasoactive Peptides

The angiotensin–renin system has been described in the placenta and is thought to be a factor in the regulation of vascular tone in the placental bed. Multiple vasoactive peptides—VEGF, endothelin, angiotensin, arginine vasopressin, and atrial natriuretic peptide—and their receptors are placentally expressed.[232–236] A balance of these factors is likely required for appropriate fetoplacental perfusion. For example, atrial natriuretic peptide inhibits the vasoconstrictive action of endothelin and angiotensin and induces vasodilatation in the uterus and the placenta.

Endogenous Opioid Peptides

Opioid peptides, enkephalins[237] and dynorphin,[238] and their receptors are expressed in placenta, with an increase in placental receptors at term.

Cytokines

Cytokines—interferons, tumor necrosis factor-α (TNF-α), leukemia inhibitory factor, and interleukins[239–243]—and their receptors are produced by the placenta as well as by uterine endothelial cells and invading macrophages.[242,244–246] Successful implantation requires a proinflammatory cytokine environment,[247,248] while pregnancy maintenance requires cytokine expression that suppresses the maternal immune response.[137,249] Before parturition, this balance again shifts back to proinflammatory cytokines.[244–246,249] The balance of cytokines and related factors, either proinflammatory or antiinflammatory, may be a key trigger for preterm labor caused by intrauterine infection or other types of inflammation.[137,245] Cytokine expression also regulates trophoblastic and vascular placental function.[250] Cytokines affect these activities by regulation of other cytokines, growth factors, hormones, and prostanoid production.[251–253]

Eicosanoids

Eicosanoids, such as thromboxanes (TXAs), PGs, and leukotrienes are inflammatory mediators expressed in placenta that are derived from arachidonic acid.[254,255] Human term placentas convert arachidonic acid primarily to TXAs and the PGs PGE2, PGF2α, and PGD2.[254,256] Much like cytokines, they play a role in trophoblast implantation[257] and in parturition.[258,259] After implantation, these factors, particularly prostacyclin (PGI2) and PGE2, appear to be vasoregulators of the fetal–placental unit.[259–261] PGI2 is a potent vasodilator in placental vessels, an inhibitor of platelet aggregation, and a uterine relaxing factor; its loss has been implicated in PE. TXA2 opposes PGI2, and production is increased in PE; low-dose aspirin preferentially inhibits TXAs in the placenta and may decrease development of PE.

Immunologic Function

Throughout pregnancy the risk of fetal infection must be balanced against fetal rejection. This balance is maintained on both the maternal and fetal sides of the placenta. Unique features of the cells at the placental interface are required to allow the genetically distinct fetal "graft" to inhabit the maternal host. Placental trophoblast cells directly encounter maternal immune cells: SynTs covering the placental villi are bathed in maternal blood and the invading trophoblasts exposed to the maternal decidua. Different strategies appear to be used at these sites to prevent destruction by cytotoxic maternal immune cells. For example, neither SynTs nor invading trophoblasts express classic human leukocyte antigen (HLA)-A or HLA-B class Ia major histocompatibility complex antigens nor HLA class II antigens. However, invading trophoblasts do express nonclassic HLA-G and HLA-C — HLA types that can actually suppress immune responses, especially through leukocyte inhibitory receptors on uterine natural killer (NK) cells and macrophages. A balance of innate immunity and modulation of adaptive immune responses is required, and this balance shifts throughout gestation.[262,263]

The decidua is replete with innate immune cells including T cells, regulatory T cells, macrophages, dendritic cells, and uterine NK (uNK) cells. The best-studied subtype is the NK population. NK cells peak and constitute the largest leukocyte population in the early pregnant uterus, accounting for 60% to 70% of total lymphocytes. These cells diminish in proportion as pregnancy proceeds. Despite being replete with cytotoxic perforin, granzymes A and B, and the natural cytotoxicity receptors (NKp30, NKp44, NKp46, NKG2D, NGK2B4, and LFA-1), these NK cells are tolerant cytokine-producing cells at the maternal–fetal interface.[264] The temporal occurrence around the SpAs and timed amplification of these specialized NK cells observed during the first trimester implicate their role in SpA remodeling. NK cell-deficient mice display abnormalities in decidual artery remodeling and trophoblast invasion, possibly because of a lack of uNK cell-derived interferon γ.[265] Other studies have shown that uNK cells are a major source of VEGF-C, angiopoietins 1 and 2, and TGF-β1 within the placental bed that decrease with gestational age.[266] These observations implicate uNK cells in promoting angiogenesis. Recent studies suggest that VEGF-C may induce the non-cytotoxic activity in maternal immune cells as well.[267] Additional molecules expressed on trophoblasts, such as members of the B7

family that alter lymphocyte activity and FasL, which interacts with Fas leukocyte receptors, may also modulate cytotoxicity in the placenta.

Both maternal macrophages and Hofbauer cells (macrophages in the villi that are derived from the fetus) are present in the placenta during pregnancy. These cells may prevent uterine infections or facilitate vascular remodeling and immune suppression.[268] Much like NK populations, alterations in macrophage activation, both maternal and fetal, have been linked to pregnancy complications such as IUGR, preterm birth, and PE.[269]

Maternal tolerance to fetal alloantigens was initially explored in the context of T-helper cells (Th)1/Th2 balance in mice, with Th2 cells and cytokines proposed to predominate over Th1 cellular immune response under normal pregnancy. In human pregnancy, the role of specialized T lymphocytes—termed *regulatory T cells* (Tregs)—in producing immune tolerance has emerged. Tregs are potent suppressors of T cell–mediated inflammatory immune responses and prevent autoimmunity and allograft rejection. $CD4^+CD25^+$ Tregs are found in the decidua throughout pregnancy. Fetal-specific Tregs persist between pregnancies, and they accumulate and reexpand their population rapidly in subsequent pregnancies, potentially providing a persistent protective regulatory memory to fetal antigen.[270] The specific role of these Tregs in human pregnancy loss remains to be defined.

Immunosuppressive immune modulators are also highly expressed at the placental interface. Many of the endocrine factors produced by the placenta (progesterone, PGE2, and interleukins) as described above appear critical to maternal immune modulation. For example, the antiinflammatory cytokine interleukin (IL)-10 is expressed by human trophoblasts and Tregs, increasing across the first two trimesters and then declining before delivery. Low IL-10 expression has been linked to pregnancy loss and preterm delivery as well as PE. However, how IL-10 protects the fetus is poorly understood. IL-$10^{-/-}$ mice are fertile if maintained pathogen-free but are highly susceptible to complications from infection, suggesting that IL-10 deficiency plus a "second hit" such as infection, environmental factors, or hormonal dysregulation may contribute to poor pregnancy outcomes.[271]

There is no generalized immunosuppression in pregnant women. Rather, there is a balance struck between specific types of immune suppression and activation. Indeed, cytokine production capacity is higher in pregnant than in nonpregnant women. It is the balance of proinflammatory to antiinflammatory cytokines that may determine outcome. When this balance is altered early in gestation, implantation could be affected, while immune alterations in late gestation may contribute to susceptibility to preterm birth, particularly in the face of an immune challenge. Similar shifts in the fetal immune response from tolerance to activation are being investigated across normal gestation.[272] The role of placental immune activation in poor neonatal outcome, particularly in neurologic complications, has become an area of active study in the past decade.[273,274] Defining and manipulating placental immune responses are key components of current efforts to improve pregnancy outcomes.

Regulation of Placental Function

Understanding placental dysfunction and disease during pregnancy is critical to improving neonatal and adult outcomes.[275,276] Healthy development of the placenta requires efficient metabolic, immune, hormonal, and vascular adaptation by the maternal system as well as the fetus. Abnormal placentation and placental infections can lead to preeclampsia, growth retardation, or preterm birth, which can have a lifelong bearing on health. Most major obstetric syndromes originate in early gestation because of abnormal trophoblast invasion or immune dysregulation but present clinically in late gestation. Maternal factors such as ascending infections, obesity, hypertension, diabetes, and environmental exposures also contribute to placental dysfunction.

Evaluation of Placental Dysfunction

Until recently, placental assessment was performed almost exclusively after delivery, using traditional anatomic pathology techniques. While significant correlations have been described between specific pathologic lesions and neonatal outcomes, our understanding of how and when these lesions develop during gestation and ultimately lead to poor outcomes remains limited. There has been a recent resurgence of interest in developing advanced tools to investigate placental function during gestation, particularly following the launch of the Human Placenta Project by the Eunice Kennedy Shriver National Institutes of Child Health and Disease.[277,278] In addition to promoting the development of new advanced imaging techniques[279,280] and biomarker methods for measuring placental function,[281] there has been a renewed interest in standardizing placental histopathologic classification and diagnosis. In 2014 an international group of placental pathologists met in Amsterdam to establish consensus guidelines for placental examination and classification of lesions;[282] these are summarized in Table 3.1.

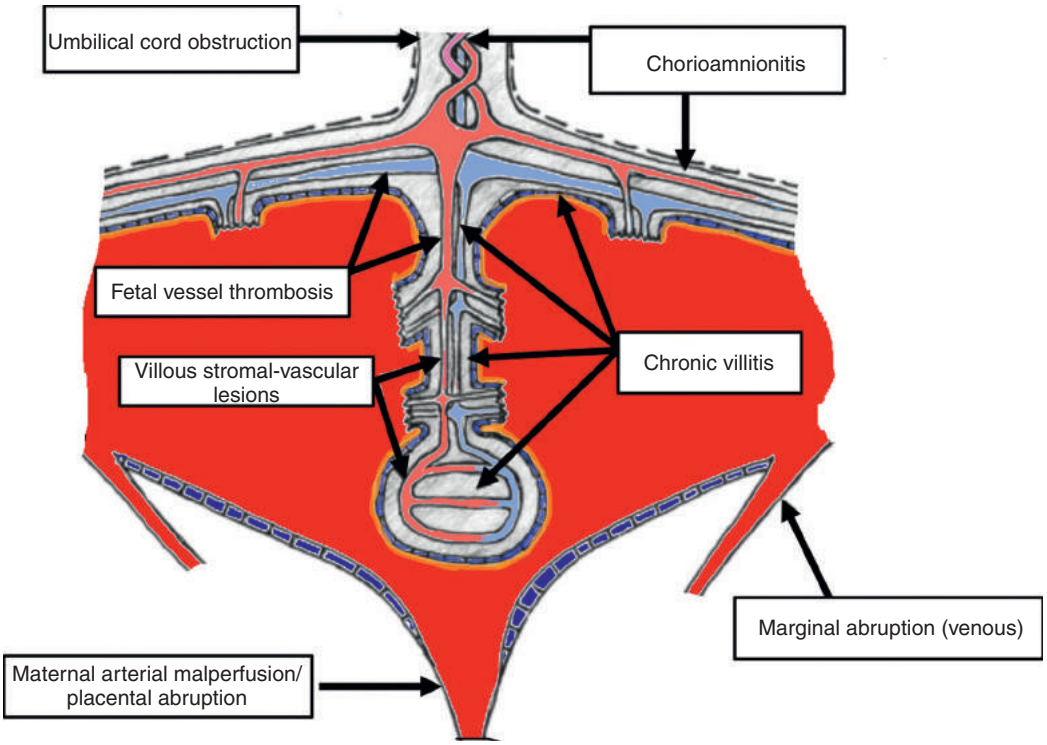
Placental Histopathology

Direct examination of the placenta after birth can give some clues to the timing and extent of important adverse prenatal or neonatal events. Some disorders are readily apparent in the delivery room (listeria lesions, placental abruption associated with large clots, abnormal cord insertion), and others require more detailed gross and microscopic examination. In a high-risk delivery service, approximately 50% of placentas qualify for pathologic examination.[283]

The majority of placental lesions described by pathologists involve either vascular or immunologic/infectious processes (Table 3.1). Vascular processes can be further localized as maternal or fetal lesions (Fig. 3.4 villous maternal stromal-vascular lesions versus fetal vascular lesions), potentially providing clues to both the underlying etiology of the lesion and its implications for maternal or fetal heath. Immune processes likewise can be subdivided into chronic or acute infections and also distinguished from inflammatory processes associated with immune activation without infection (see Fig. 3.4, chronic villitis). Links have been made between each major pathologic lesion type and different pregnancy complications (Table 3.2).

Placental Imaging

Ultrasound (US) imaging remains the standard imaging method used for placental evaluation during pregnancy because of its availability, safety, and relatively low cost. However, US evaluation of placental anatomy can be limited by placental implantation site, maternal body habitus, and amniotic fluid volume, and US-detected lesions correlate poorly with postnatal histopathology.[284] As a primary screening tool, placental US has proven very useful, but its use for functional placental assessment that can predict which pregnancies are at risk of later placental compromise is limited.



• **Fig. 3.4** Histopathologic Sites of Action of Major Placental Disease Processes. (Modified from Redline RW. The clinical implications of placental diagnoses. *Semin Perinatol.* 2015;39:2–8.)

TABLE 3.2 Common Placental Causes of Specific Adverse Pregnancy Outcomes

1. **Fetal Growth Restriction**
   Maternal stromal-vascular lesions: global/partial vascular malperfusion with accelerated villous maturation
   Fetal stromal-vascular lesions: developmental lesions (superficial implantation), global/partial vascular malperfusion (fetal thrombotic vasculopathy)
   Villitis of unknown etiology
2. **Spontaneous Preterm Birth**
   Maternal inflammatory response: acute chorioamnionitis
   Maternal vascular lesions: mild global/partial vascular malperfusion with accelerated villous maturation
   Acute marginal placental abruption
3. **Preterm Fetal Death**
   Maternal stromal-vascular lesions: global/partial vascular malperfusion with accelerated villous maturation
   Fetal global/partial vascular malperfusion (cord accidents/obstruction)
   Placental abruption
4. **Term Fetal Death**
   Fetal stromal-vascular lesions: developmental lesions (delayed villous maturation), global/partial vascular malperfusion (cord accidents/obstruction)
   Placental abruption
   Fetomaternal hemorrhage
5. **Term Neurologic Injury**
   Fetal stromal-vascular lesions: global/partial vascular malperfusion (fetal thrombotic vasculopathy or cord occlusion)
   Chronic villitis of unknown etiology with obliterative fetal vasculopathy
   Acute chorioamnionitis with severe fetal inflammatory response
   Presence of multiple placental lesions

Modified from Redline RW. Classification of placental lesions. *Am J Obstet Gynecol.* 2015; 213(4 Suppl):S21-8.

The placenta may be visible on US as early as 5 weeks' gestation.[285] It becomes readily visible by 15 weeks' gestation by US and undergoes progressive increases in thickness and diameter as well as changes in echogenicity and shape.[284] Both increased and decreased placental size are associated with abnormal development and risk of fetal complications. Many placental lesions can be seen by US as pregnancy progresses. Some, such as placental lakes, which are anechoic regions of low maternal blood flow, are very common but appear to be of limited clinical significance. Others, including echogenic areas of infarct, may be significant when large or centrally located. Placental calcifications increase across gestation. There appears to be an association between early development of calcifications and poor placental function, but use of early, high-grade calcification to predict pregnancy outcome has proved unreliable.[284] Doppler velocimetry is used in conjunction with anatomic US to functionally assess placental blood flow.[286] Fetal villous vascular damage results in high resistance in the umbilical artery (UA) circulation, and chronic fetal hypoxia decreases umbilical venous flow. Loss of UA end-diastolic flow is associated with severe IUGR and indicative of significant fetal compromise. Monitoring of placental blood flow allows detection of high resistance and poor circulation within the placenta but is usually apparent only when significant fetal compromise has already occurred. Additional US measures that may be more predictive of placental compromise, such as 3-dimensional placental volumes and vascularization indices[284] as well as new elastography and higher resolution US techniques, are under investigation.

Magnetic resonance imaging (MRI) is increasingly being used for anatomic placental evaluation, and advanced functional techniques may yield information on oxygenation, vascularization, and metabolism.[279,280] MRI benefits from having multiplanar images in a wider field of view as well as having higher spatial and

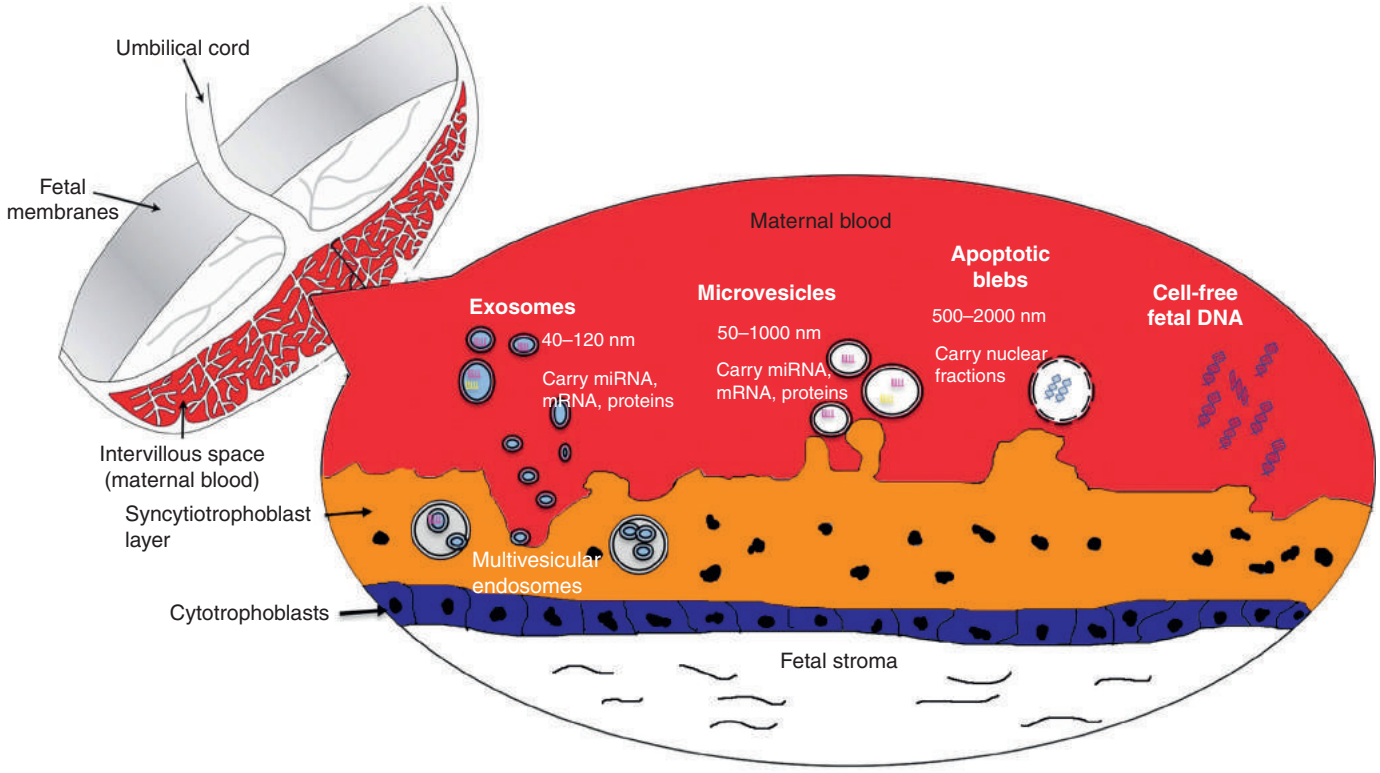
temporal resolution when compared with US. It is currently used primarily to assess fetal structural anomalies and, more recently, for improved detection of placenta accreta and other invasive placental anomalies.[287] Precise quantitation of placental volume is more readily performed using MRI than US and may allow earlier prediction of fetal growth retardation based on small placental size.[280,288] Use of standard MRI gadolinium-based contrast agents has been limited due to fetal safety concerns, but new agents are being developed. Placental application of noncontrast functional MRI methods, including diffusion-weighted imaging and diffusion tensor imaging, are being investigated along with methods that rely on endogenous contrast agents, such as hemoglobin-based detection of oxygen level changes measured by blood oxygen level-dependent MRI.[279,280] Use of placental MRI for real-time assessments is likely to provide new information about placental vascular development and function as well as new diagnostic tools for use in high-risk pregnancies.

Serum Biomarkers of Placental Disease

A major goal of pregnancy screening is to identify women early in gestation who will go on to develop placenta-mediated complications that threaten either fetal or maternal health so that targeted early therapies can be provided. Detection of factors released by the placenta into maternal circulation that predict disease is a longstanding area of investigation. In addition to physiologic secretion of placental factors into maternal circulation, cellular stress (i.e., oxidative, hypoxic, or inflammatory stress) can lead to increased villous trophoblast turnover with release of placental vesicles and cellular debris into the circulation.[281] Maternal serum analytes, circulating cell-free DNA, and extracellular vesicle contents derived from the placenta are under investigation as potential biomarkers of placental dysfunction.

Serum Analytes

Maternal serum screening has been applied successfully in the identification of fetuses at increased risk of aneuploidy or structural anomalies (open neural tube defects, abdominal wall defects). The association is less clear between pregnancies at risk of placental dysfunction and abnormal values for the most common first and second trimester serum screening markers: alpha fetoprotein, hCG, unconjugated estriol (uE_3), inhibin-A, and pregnancy-associated plasma protein-A (PAPP-A). Both single-serum analyte abnormalities and combinations have been assessed for their value as biomarkers of specific pregnancy complications with limited success.[289] For example, higher levels of second trimester inhibin-A levels are associated with PE, although no predictive cutoff level has been identified. Elevated inhibin-A in combination with UA Doppler abnormality, however, may be strongly predictive of



• **Fig. 3.5** Placental Products Circulate in Maternal Blood During Gestation. The placental syncytiotrophoblasts produce and secrete extracellular vesicles of various sizes, shapes, and functions into the maternal circulation in both healthy and compromised pregnancies. The function and cargo of these vesicles is not yet completely defined but likely plays a role in maternal–fetal cross-talk between cells during pregnancy. Circulating placental products may also provide biomarkers for placental function and pathologies. Exosomes are constitutively secreted products of the endosomal pathway that carry proteins, micro-ribonucleic acid (RNA), and messenger RNA that can modulate maternal cell functions, including immune and endothelial responses. Microvesicles and apoptotic bodies are larger vesicles produced by direct budding of the plasma membrane following alterations in cellular conditions (oxygen tension, glucose, or calcium level changes) or as part of apoptosis, respectively. These vesicles may carry unsorted protein and nucleic acid cargo picked up from the cell cytosol. They may have a proinflammatory role in pregnancy. Circulatory cell-free DNA can be up to 13% of cell-free DNA in maternal circulation. Circulatory cell-free DNA is fragmented and significantly smaller than maternal fragments, allowing fragment size and sequence to be used to distinguish fetal from maternal origin. *mRNA*, Messenger ribonucleic acid; *miRNA*, micro RNA; *nm*, nanometers.

PE.[290] Likewise, extensive studies of low PPAP-A and UA Doppler changes suggest an association with fetal growth restriction and PE.[291] Some alterations of these maternal serum markers have been associated with specific pathologies linked to poor placental function, but the strongest associations are serum marker abnormalities and a generalized increased risk of third trimester fetal death.[289] Increased surveillance or treatment of these pregnancies has not yet shown clinical benefit.

Additional maternal serum biomarkers of placental dysfunction, particularly in PE, include elevated circulating sFlt-1 and reduced PlGF. Syncytial stress leads to placental secretion of these and other angiogenic factors.[281] A recent meta-analysis suggested that the diagnostic accuracy of maternal sFlt-1/PlGF for early onset PE is high, but false positives and false negatives are both greater than 15%, limiting the utility of this ratio as a broad clinical screening tool.[292]

Circulating Cell-Free Fetal DNA

Direct measurement of DNA found in maternal serum has become possible in the past decade. Continuous turnover of placental villous trophoblasts releases placental microparticles and freely circulating nucleic acids (Fig. 3.5). These fragments are called fetal but actually originate from the placenta.[293] Noninvasive prenatal screening for aneuploidies and genetic mutations using circulating cell-free fetal DNA (cffDNA) screening from maternal serum has rapidly gained popularity in the past 5 years. Confined placental mosaicism with a normal fetal karyotype can confound these screening results, but the high sensitivity and specificity of these tests combined with their limited risk compared with chorionic villous sampling or amniocentesis have led to their rapid clinical adoption. Total cffDNA levels, rather than specific cffDNAs, may also be useful biomarkers for placental health and function. cffDNA is increased in PE both before and during the development of clinical symptoms, likely because of increased trophoblast apoptosis associated with oxidative stress.[294] High concentrations of cffDNA are also associated with increased preterm birth risk.[295] Cell-free RNA and miRNAs have also been found in maternal circulation, and their utility as biomarkers is actively being assessed.

Extracellular Vesicles

Multiple types and sizes of vesicles are shed by the placenta in both normal and compromised pregnancies (Fig. 3.5), but the amounts and contents vary with placental health.[281] Exosomes are a subtype of extracellular vesicle derived from endosomes that carry proteins and RNAs and that are released by exocytosis into the extracellular space. Exosomes play a significant role in intercellular signaling in multiple systems, and their role in pregnancy has garnered intense interest in the past few years.[296] Placentally derived exosomes carry SynT-specific proteins, including placental alkaline phosphatase and the miRNAs from H19 described previously, allowing their identification as placental vesicles in maternal circulation. Their release is regulated by multiple environmental factors including oxygen tension and glucose concentrations, making them particularly appealing as reporters of placental function. Exosomes mediate communication between the placenta and maternal immune cells, and widespread placental–maternal cellular communication using this mechanism has been proposed. There is a general increase in placental exosomes in maternal circulation across gestation, and these levels may vary with placental pathology; the vesicle contents may also reflect pathology, such as decreased cell fusion proteins in exosomes from preeclamptic placentas.[296] As placental exosome biology becomes better defined, use of exosomes as biomarkers of placental function is an exciting possibility.

Summary

The placenta is a complex organ that develops from many cell types to form a sophisticated interface between mother and fetus that integrates intrinsic and extrinsic signals to optimize fetal development. The consequences of impaired placental function have lifelong impact not only on individual offspring but potentially on multiple generations, effected by epigenetic changes. Understanding and optimizing placental health is critical, not just to newborns but also to improving health outcomes for the entire population.

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4

Abnormalities of Fetal Growth

REBECCA A. SIMMONS

KEY POINTS

- Normal fetal growth is determined by a number of factors.
- The pattern of normal fetal growth involves rapid increases in fetal weight, length, and head circumference during the last half of gestation.
- Year-over-year increases in birth weight for gestational age are attributed to improvements in living conditions and maternal nutrition as well as changes in obstetric management.
- Variations in fetal growth have been identified in diverse populations and are associated with geographic location.
- There is no universal agreement on the classification of an SGA infant.

Definitions

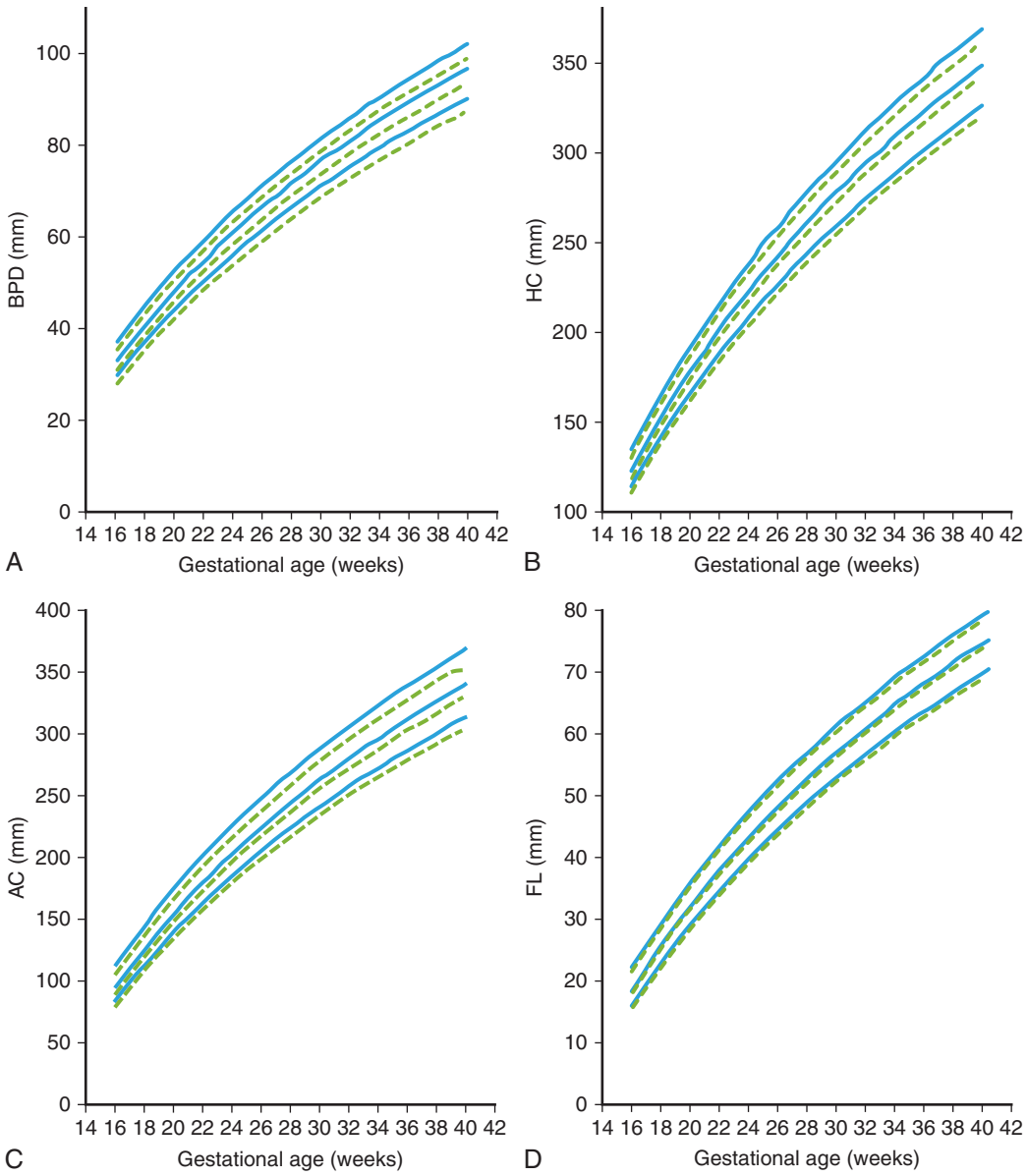
The duration of pregnancy is an integral component of prenatal growth assessment, and all currently prevailing definitions of fetal growth are gestational age specific. Assessing the gestational age accurately, however, can be challenging, and any error in dating will lead to misclassification of the infant, which can have significant clinical implications. In many instances, the method of gestational age determination has contributed to variations in the gestational age specific reference growth curves. For example, some nomograms are based on approximating the gestational age to the nearest week whereas others use completed weeks. The birth weight charts are also affected by other variables that may limit their reliability. Many of these, such as fetal sex, race, parity, birth order, parental size, and altitude, contribute to the normal biologic variations in human fetal growth. There is continuing controversy on whether the reference growth charts should be customized by multiple variables or developed from the whole population. The customized approach predicts the optimal growth in an individual pregnancy and therefore specifically defines suboptimal growth for that pregnancy. However, it has been argued that such an approach may lead to a profusion of standards and may not contribute to improving the outcome of small for gestational age (SGA) infants. In recognition of the utility of a national standard, a population-based reference chart for fetal growth was developed from all the singleton births (over 3 million) in the United States in 1991.[1] More recently, a similar national population-based fetal growth chart, which is also sex specific, has been developed in Italy. In a multicenter cross-sectional study, 8070 ultrasonographic examinations from low-risk singleton pregnancies between 16 and 40 weeks of gestation were used to develop growth curves: biparietal diameter (BPD), head circumference (HC), abdominal circumference (AC), and femur length (FL). Quantile regression was used to examine the impact of fetal sex across the biometric percentiles of the fetal measurements considered together with parents' height, weight, parity, and race[2] (Fig. 4.1).

There is no universal agreement on the classification of an SGA infant. Various definitions appear in the medical literature, making comparisons between studies difficult. Additionally, investigators have shown that the prevalence of fetal growth restriction varies according to the fetal growth curve used.[1] The most common definition of SGA refers to a weight below the 10th percentile for gestational age or birth weight less than 2 standard deviations (SD) from the mean. Some investigators also use measurements below the third percentile to define SGA. However, these definitions do not make a distinction between infants who are constitutionally small, growth-restricted and small, and those that are not small but growth-restricted relative to their potential. For example, as many as 70% of fetuses who weigh below the 10th percentile for gestational age at birth are small simply because of constitutional factors such as female sex or maternal ethnicity, parity, or body mass index; they are not at high risk of perinatal mortality or morbidity. In contrast, true intrauterine growth restriction (IUGR) is associated with numerous perinatal morbidities. This has clinical relevance to perinatologists and neonatologists, as many of the tiniest premature neonates in the neonatal intensive care units are probably growth restricted.

Increasing intervention for fetal growth restriction (FGR) is changing the definition of SGA as defined by population-derived birthweight centiles.[3] This was recently shown in Victoria, Australia, where improved detection of FGR reduced the proportion of babies with birthweight <3rd centile from 3.1% to 1.9%.[4] Over time, it is projected that this will cause the birthweight cutoff that defines the 3rd centile to increase. In that same Australian population, it has been projected that the birthweight defining the 3rd centile at 40 weeks' gestation will increase by 150 g over 35 years.[3] The authors of these studies proposed using the INTERGROWTH-21st birthweight charts that are derived from healthy populations specifically selected to demonstrate ideal fetal growth applicable across all countries.[5] This is an active and important area of research, and it is clear that there is not consensus around the world as to which growth charts should be utilized.

Patterns of Altered Growth

Neonates with intrauterine growth retardation can be classified as demonstrating either symmetrical or asymmetrical growth. Infants with symmetric IUGR have reduced weight, length, and



• **Fig. 4.1** Comparison of the 5th, 50th, and 95th centiles of the biometric measurements between male *(blue line)* and female sex *(green dashed line)* in a fetus with a paternal height of 180 cm, maternal height of 160 cm, maternal weight of 60 kg, mother nulliparous, and of white race. (A) Biparietal diameter. (B) Head circumference. (C) Abdominal circumference. (D) Femur length.

head circumference at birth. Weight (and then length) of infants with asymmetric growth retardation is affected, with a relatively normal or "head-sparing" growth pattern. Factors intrinsic to the fetus in general cause symmetrical growth restriction, whereas asymmetric IUGR is often associated with maternal medical conditions such as preeclampsia, chronic hypertension, and uterine anomalies. Asymmetrical patterns generally develop during the third trimester, a period of rapid fetal growth. However, now that fetal surveillance is more common, asymmetrical growth restriction is often diagnosed in the second trimester.

Factors that are well-recognized to limit the growth of both the fetal brain and body include chromosomal anomalies (e.g., trisomies), congenital infections (toxoplasmosis, rubella, cytomegalovirus, and herpes simplex [TORCH], malaria, human immunodeficiency virus [HIV], and parvovirus), dwarf syndromes, and some inborn errors of metabolism. Cardiac and renal structural anomalies are common conditions associated with SGA. These conditions retard fetal growth primarily by impaired cell proliferation. Recognized causes of IUGR are listed in Table 4.1.

Fetal Causes of Growth Restriction

Fetal factors affecting growth include fetal gender, familial genetic inheritance, and chromosomal abnormalities or dysmorphic syndromes. In one large population-based study, the frequency of IUGR among infants with congenital malformations was 22%. The majority of the infants affected had genetic abnormalities including chromosomal, submicroscopic single gene disorders and imprinting defects, such as Russell Silver.[6] Triploidies are the most common anomaly in fetuses below 26 weeks, and trisomy 18 above 26 weeks. Sometimes, the chromosomal anomaly can be confined to the placenta, and cause growth restriction in

TABLE 4.1 Causes of Intrauterine Growth Restriction

Genetic	Inheritance, chromosomal abnormalities, fetal gender
Maternal constitutional effects	Low maternal prepregnancy weight, low pregnancy weight gain, ethnicity, socioeconomic status, history of intrauterine growth restriction
Nutrition	Low prepregnancy weight (body mass index), low pregnancy weight gain, malnutrition (macronutrients, micronutrients), maternal anemia
Infections	TORCH infections (toxoplasmosis, rubella, cytomegalovirus, herpes simplex)
Decreased O_2-carrying capacity	High altitude, maternal congenital heart disease, hemoglobinopathies, chronic anemia, maternal asthma
Uterine/placental anatomy	Abnormal uterine anatomy, uterine fibroid, vascular abnormalities (single umbilical artery, velamentous umbilical cord insertion, twin-twin transfusion), placenta previa, placental abruption
Uterine/placental function	Maternal vasculitis (system lupus erythematosus), decreased uteroplacental perfusion, maternal illness (preeclampsia, chronic hypertension, diabetes, renal disease)
Toxins	Tobacco, ethanol, lead, arsenic

a chromosomally normal fetus. Mosaicism is defined as the presence of two or more different chromosomal complements in the fetoplacental unit developed from a single zygote. It is caused by a viable somatic postmitotic error occurring in an initially normal conceptus, or a meiotic error resulting in trisomy with subsequent postzygotic trisomic rescue.

Fetal gender also influences size, with male infants showing greater intrauterine growth than female infants.[7–9]

Placental Causes of Growth Restriction

In mammals, the major determinant of intrauterine growth is the placental supply of nutrients to the fetus.[10] Indeed, in many species, fetal weight near term is positively correlated to placental weight as a proxy measure of the surface area for materno-fetal transport of nutrients. The nutrient transfer capacity of the placenta depends on its size, morphology, blood flow, and transporter abundance.[10] In addition, placental synthesis and metabolism of key nutrients and hormones influences the rate of fetal growth.[11] Changes in any of these placental factors can therefore affect intrauterine growth. However, the fetus is not just a passive recipient of nutrients from the placenta. The fetal genome exerts a significant acquisitive drive for maternal nutrients through adaptations in the placenta, particularly when the potential for fetoplacental growth is compromised.

Placental maturation at the end of pregnancy is associated with an increase in substrate transfer, a slowing (but not cessation) of placental growth, and a plateau in fetal growth near term.[12] Fetal size and placental growth are directly related, and placentas from pregnancies yielding growth-restricted infants demonstrate a higher incidence of smallness and abnormality than those from pregnancies with appropriately grown infants. The difference in size is seen even in a comparison of placentas associated with growth-restricted infants and those associated with appropriate for gestational age (AGA) infants of the same birth weight.[13] Clinical conditions associated with reduced placental size (and subsequent reduced fetal weight) include maternal vascular disease (preeclampsia, eclampsia, and chronic maternal hypertension), uterine anomalies (fibroids, abnormal uterine anatomy), placental infarctions, unusual cord insertions, and abnormalities of placentation.

Multiple gestations are associated with greater risk for fetal growth restriction. The higher risk stems from crowding and abnormalities with placentation, vascular communications, and umbilical cord insertions. Divergence in fetal growth appears from about 30 to 32 weeks in twin gestation compared with singleton pregnancies, although this may occur earlier in gestation.[14] Abnormalities in placentation are also more common with multiple gestations.[12] Monochorionic twins can share placental vascular communication (twin-twin transfusion), leading to fetal growth restriction during gestation. Fetal "competition" for placental transfer of nutrients raises the incidence of growth restriction and discordance in growth between fetuses. The rate of birth weights less than the fifth percentile is higher in monochorionic twins. Placental growth is restricted in utero because of limitation in space, leading to a higher incidence of placenta previa in multiple-gestation pregnancies. Additionally, abnormalities in cord insertions (marginal and velamentous cord insertions) and occurrence of a single umbilical artery are more frequently found in multiple gestations.

Investigators have shown an effect of altitude on placenta function, thereby impairing fetal growth, with infants born at high altitudes having lower birth weights.[15,16] Differences in fetal growth are detected from about 25 weeks with pregnancies at 4000 m.[17] In these high-altitude pregnancies, the abdominal circumference is most affected.[18] Interestingly, investigators have shown that adaptation to high altitude during pregnancy is also possible. Tibetan infants have higher birth weights than infants of more recent immigrants of ethnic Chinese origin living at the same high-altitude (2700 to 4700 m) region of Tibet.[19] Tibetan infants also have less IUGR than infants born to more recent immigrants to the area.

Maternal Causes of Growth Restriction

Maternal health conditions associated with chronic decreases in uteroplacental blood flow (maternal vascular diseases, preeclampsia, hypertension, maternal smoking) are associated with poor fetal growth and nutrition. Infants born to women with preeclampsia are at substantial risk for IUGR.[20] Investigators have shown that the extent of growth restriction correlates with the severity and the onset during pregnancy of the preeclampsia.[21] Ødegård et al. showed that fetuses exposed to preeclampsia from early in pregnancy had the most serious growth restriction, and more than half of these infants were born SGA.[21] Chronic maternal diseases (cardiac, renal) may

decrease the normal uteroplacental blood flow to the fetus, and thus may also be associated with poor fetal growth.[22]

Maternal constitutional factors have a significant effect on fetal growth. Maternal weight (prepregnancy), maternal stature, and maternal weight gain during pregnancy are directly associated with maternal nutrition, and correlate with fetal growth.[23–25] Numerous studies show that these findings are often confounded by highly associated cultural and socioeconomic factors. The woman with a previous SGA infant has a higher risk of a subsequent small infant.[26] Investigators have shown a higher incidence of SGA infants to be associated with lower levels of maternal education.[27] Parity of the mother also affects fetal size, nulliparous women having a higher incidence of SGA infants.[28] A large population-based study in Sweden found that women who were older than 30 years and were nulliparous or had hypertensive disease were at increased risk of preterm and term growth-restricted infants.

Studies have shown differential fetal growth for women of diverse ethnicities, with Latina and white women having higher rates of large for gestational age (LGA) infants, and African-American and South Asian women having a higher incidence of small for gestational age (SGA) infants.[29–31] These gender and ethnic differences in birth weight become pronounced after 30 weeks of gestation.[32] Investigators in California have shown that US-born Black women have higher rates of prematurity and LBW infants than foreign-born Black women. Other researchers have found that even among women with very low risk of LBW infants (married, age 20 to 34 years, 13 or more years of education, adequate prenatal care, and absence of maternal health risk factors and tobacco or alcohol use), the risk of delivering an SGA infant is still higher for African-American women than for white women.[29,30] It is unclear whether these differences in fetal growth are due to inherent differences or differential exposure to environmental factors, including stress.

Maternal nutrition significantly impacts fetal growth, primarily in developing countries.[26,33–36] Although numerous factors interact with and affect fetal development, maternal malnutrition is assumed to be a major cause of IUGR in developing countries.

Teen pregnancy represents a special condition in which fetal weight is highly influenced by maternal nutrition. Teen mothers (<15 years) have been shown to have a higher risk for delivering a growth-restricted infant.[37] Teen pregnancies are complicated by the additional nutritional needs of a pregnant mother who is still actively growing, as well as by the socioeconomic status of pregnant teens in developed countries.[38]

The effects of micronutrients on pregnancy outcomes and fetal growth have been less well studied. Maternal intake of certain micronutrients has also been found to affect fetal growth. Zinc deficiency has been associated with fetal growth restriction as well as other abnormalities, such as infertility and spontaneous abortion.[39] Additionally, dietary intake of vitamin C during early pregnancy has been shown to be associated with an increase in birth weight.[40] Others have shown strong associations between maternal intake of folate and iron and infant and placental weights.[33] In developing countries, the effects of nutritional deficiencies during pregnancy are more prevalent and easier to detect. Rao and colleagues estimated that one third of infants in India are born weighing less than 2500 g, mainly because of maternal malnutrition.[41] These investigators have shown significant associations between infant birth weight and maternal intake of milk, leafy greens, fruits, and folate during pregnancy. However, many of these studies have not been replicated, and thus, the possible role of nutrient supplementation on fetal growth remains to be determined.

Although toxins such as cigarette smoke and alcohol have a direct effect on placental function, they may also affect fetal growth through an associated compromise in maternal nutrition. Other environmental toxins (lead, arsenic, mercury) are associated with IUGR and believed to affect fetal growth by entering the food chain and depleting body stores of iron, vitamin C, and possibly other nutrients.[42,43]

Numerous studies have shown associations between birth weight and maternal intake of macronutrients and micronutrients, but the effects of nutritional supplements used during pregnancy on fetal growth are equivocal.[44–47] This is underscored by the results of a large double-blind, randomized controlled trial including 1426 pregnancies that was carried out in rural Burkina Faso.[48] Pregnant women were randomly assigned to receive either iron and folic acid (IFA) or the United Nations International Children's Emergency Fund (UNICEF)/World Health Organization (WHO)/United Nations University (UNU) international multiple micronutrient preparation (UNIMMAP) daily until 3 months after delivery. Birthweight was only increased by 52 g and birth length by 3.6 mm. Unexpectedly, the risk of perinatal death was marginally but significantly increased in the UNIMMAP group (OR: 1.78; 95% CI: 0.95, 3.32; $P = .07$). However, another more recent study found that the effects of iron-containing supplements on birth weight depended on baseline hemoglobin concentrations. The iron-containing supplements improved birth weight in women with very high hemoglobin levels before 20 weeks of gestation in a large Chinese cohort.[49]

Maternal socioeconomic status and ethnicity have also been identified as risk factors for IUGR and poor health outcomes in infants in both developing and developed countries.[50] In the United States, low levels of maternal and paternal education, certain maternal and paternal occupations, and low family income are associated with lower birth weights in children of African-American, Hispanic, and white women.[51–53] In large population-based studies from Sweden, Brazil, France, and Denmark, investigators have similarly shown a higher incidence of fetal growth restriction in association with low maternal education.[27,54–56] The incidence of IUGR is also higher in women without medical insurance.[9,57,58] Interestingly, Mexican-born immigrants in California have better perinatal outcomes (including birth weight) than both African-Americans and US-born women of Mexican descent.[31] The reasons for this apparent paradox are unclear, but one postulate is the tendency of recent immigrants to maintain the favorable nutritional and behavioral characteristics of their country of origin.[59] These studies support the speculation that the differences in fetal growth between groups do not reflect inherent differences in fetal growth but rather stem from inequalities in nutrition, health care, and other environmental factors.[60,61]

Smoking

Maternal smoking during pregnancy is associated with a reduction in birth weight of approximately 250 g. In developed countries, cigarette smoking is the single most important cause of poor fetal growth.[62,63] However, the literature describing associations between maternal smoking and reduced fetal measurements is inconsistent. For example, maternal smoking is associated with reduced second trimester growth in some studies[64,65] but not all.[66] Abdominal and proximal muscle growth restriction has been linked to maternal smoking in one population[67] but to peripheral fetal growth (i.e., femur length) in others.[64,65] To address some of these inconsistencies, a recent meta-analysis was conducted of 16

studies from 8 populations.[68] They found that maternal smoking during pregnancy was associated with reduced fetal measurements after the first trimester, particularly reduced head size and femur length.[68] These effects were attenuated if mothers quit or reduced cigarette consumption during pregnancy. Kataoka and associates report that cigarette smoking also appears to have a dose-dependent effect on the incidence of IUGR, with this effect being seen especially with heavy smoking and smoking during the third trimester.[69] These investigators have shown that if women stop smoking during the third trimester, their infants' birth weights are indistinguishable from those of infants born to the normal population. Other researchers have shown that even a reduction in smoking is associated with improved fetal growth.[70,71] Numerous potential causes of the effects of smoking on fetal growth have been suggested, including direct effects of nicotine on placental vasoconstriction, decreased uterine blood flow, higher levels of fetal carboxyhemoglobin, fetal hypoxia, adverse maternal nutritional intake, and altered maternal and placental metabolism.[72,73]

Short-Term Outcomes

IUGR alters many physiologic and metabolic functions in the fetus and neonate that result in a number of morbidities. A large cohort study of 37,377 pregnancies found a five- to sixfold greater risk of perinatal death for both preterm and term fetuses that had IUGR.[74] Predictive factors for perinatal mortality in preterm IUGR fetuses reveal that of all antenatal factors examined, only oligohydramnios and abnormal umbilical artery Dopplers with absent or reversed diastolic flow were predictive of perinatal mortality.[75] Although the growth-restricted fetus may show symmetric or asymmetric growth at birth, it is unclear whether the proportionality of the fetus with IUGR truly affects outcomes or is related to the timing or the severity of the insult. Multiple studies have found that symmetric IUGR resulted in higher levels of prematurity and higher rates of neonatal morbidity.[76] In contrast, Villar and associates have shown that infants with asymmetric IUGR have higher morbidity rates at birth.[77] They found that infants with low ponderal index measurements (which they defined as weight/length^3) had higher risk for low Apgar scores, long hospitalization, hypoglycemia, and asphyxia at birth than infants with symmetric IUGR. Other investigators propose that IUGR represents a continuum, with asymmetric IUGR occurring as the severity of the growth restriction increases. Data also suggest that the more severe the growth restriction, the worse the neonatal outcomes, including risk of stillbirth, fetal distress, neonatal hypoglycemia, hypocalcemia, polycythemia, low Apgar scores, and mortality.[63,78]

Fetal growth restriction is associated with intrauterine demise. Almost 40% of term stillbirths and 63% of preterm stillbirths are SGA, and perinatal mortality for intrauterine SGA infants is higher overall than that for appropriately grown term and preterm infants.[25,76] Overall, intrauterine death, perinatal asphyxia, and congenital anomalies are the main contributing factors to the higher mortality rate in SGA infants. The effects of acute fetal hypoxia may be superimposed on chronic fetal hypoxia, and placental insufficiency may be an important etiologic factor in these outcomes. Investigators have described higher incidences of low Apgar scores, umbilical artery acidosis, need for intubation at delivery, seizures on the first day of life, and sepsis in SGA infants.[77,79]

Preterm infants with growth abnormalities have a much higher risk of adverse outcomes. Preterm SGA infants have a higher incidence of a number of complications, including sepsis, severe intraventricular hemorrhage, respiratory distress syndrome, necrotizing enterocolitis, and death, than normally grown preterm infants.[79–81] Additionally, SGA infants have a higher incidence of chronic lung disease at corrected gestational ages of 28 days and 36 weeks.

Neonatal hypoglycemia and hypothermia occur more frequently in growth-restricted infants.[82] These metabolic abnormalities presumably occur from decreased glycogen stores, inadequate lipid stores, impaired gluconeogenesis, and altered β-cell sensitivity to glucose in the growth-restricted neonate. Growth-restricted neonates have inadequate fuel stores and are at increased risk for hypoglycemia during fasting, and these risks are increased in preterm SGA infants. Infants with IUGR also have a higher incidence of hypocalcemia, the incidence correlating strongly with the severity of the growth restriction.[78]

Developmental Outcomes: Early Childhood

Neurologic outcomes, including intellectual and neurologic function, are affected by growth restriction. Overall, neurologic morbidity is higher for SGA infants than AGA infants. Even without identified perinatal events, SGA infants have a higher incidence of long-term neurologic or developmental handicaps. SGA infants born at term appear to have double or triple the risk for cerebral palsy: between 1 to 2 per 1000 live births and 2 to 6 per 1000 live births.[83] The rate of cerebral palsy is also higher in preterm growth-restricted infants than in preterm infants with appropriate fetal growth.[84] At 7 years of age, children whose birth was associated with hypoxia-related factors had a higher risk for adverse neurologic outcomes. Infants with symmetric IUGR (or perhaps more severe restriction) were at higher risk than infants with asymmetric IUGR. Other researchers have shown higher rates of learning deficits, lower IQ scores, and increased behavioral problems in children with a history of fetal growth restriction, even at 9 to 11 years of age.[85]

Long-Term Consequences: The Developmental Origins of Adult Disease

Programming

The period from conception to birth is a time of rapid growth, cellular replication and differentiation, and functional maturation of organ systems. These processes are very sensitive to alterations in the intrauterine milieu. *Programming* describes the mechanisms whereby a stimulus or insult at a critical period of development has lasting or lifelong effects. The "thrifty phenotype" hypothesis proposes that the fetus adapts to an adverse intrauterine milieu by optimizing the use of a reduced nutrient supply to ensure survival, but because this adaptation favors the development of certain organs over that of others, it leads to persistent alterations in the growth and function of developing tissues.[86] Also, although the adaptations may aid in survival of the fetus, they become a liability in situations of nutritional abundance.

Epidemiology

It has been recognized for nearly 70 years that the early environment in which a child grows and develops can have long-term effects on subsequent health and survival.[87] The landmark cohort study of 300,000 men by Ravelli and colleagues showed that men who were exposed in utero to the effects of the Dutch famine of 1944 and 1945 during the first half of gestation had significantly

higher obesity rates at age 19 years.[88] Subsequent studies demonstrated a relation between low birth weight and the later development of cardiovascular disease and impaired glucose tolerance in men in England.[89,90] Those men who were smallest at birth (2500 g) were nearly seven times more likely to have impaired glucose tolerance or type 2 diabetes than those who were largest at birth. Barker and colleagues also found a similar relationship between lower birth weight and higher systolic blood pressure and triglyceride levels.[91]

Valdez and associates observed a similar association between birth weight and subsequent glucose intolerance, hypertension, and hyperlipidemia in a study of young adult Mexican-American and non-Hispanic white men and women participants in the San Antonio Heart Study.[92] Normotensive, nondiabetic individuals whose birth weights were in the lowest tertile had significantly higher rates of insulin resistance, obesity, and hypertension than subjects whose birth weights were normal. In the Pima Indians, a population with extraordinarily high rates of type 2 diabetes, McCance and coworkers found that the development of diabetes in the offspring was related to both extremes of birth weight.[93] In their study, the prevalence of diabetes in subjects 20 to 39 years old was 30% for those weighing less than 2500 g at birth, 17% for those weighing 2500 to 4499 g, and 32% for those weighing 4500 g or more. The risk for development of type 2 diabetes was nearly fourfold higher for those whose birth weight was less than 2500 grams.[93] Other studies of populations in the United States,[94] Sweden,[95,96] France,[97,98] Norway,[99] and Finland[100] have all demonstrated a significant correlation between low birth weight and the later development of adult diseases.

Studies controlling for the confounding factors of socioeconomic status and lifestyle have further strengthened the association between low birth weight and higher risk of coronary heart disease, stroke, and type 2 diabetes. In 1976, the Nurses' Health Study was initiated and a large cohort of American women born from 1921 to 1946 established. The association between low birth weight and increased risks of coronary heart disease, stroke, and type 2 diabetes remained strong even after adjustment for lifestyle factors, such as smoking, physical activity, occupation, income, dietary habits, and childhood socioeconomic status.[101]

The Role of Catch-Up Growth

Many studies have suggested that the associations between birth size and later disease can be modified by body mass index (BMI) in childhood. The highest risk for the development of type 2 diabetes is among adults who were born small and become overweight during childhood.[102] Insulin resistance is most prominent in Indian children who were SGA at birth but had a high fat mass at 8 years of age.[103] Similar findings were reported in 10-year-old children in the United Kingdom.[104] In a Finnish cohort, adult hypertension was associated with both lower birth weight and accelerated growth in the first 7 years of life. A recent study from the UK of 259 children born SGA showed that catch-up weight gain in early childhood was associated with higher abdominal fat mass, blood pressure, and glycemia at age 6. Interestingly, in these children, less height gain was associated with reduced limb lean and fat mass.[105]

Interpretation of the findings of these studies is complicated by the vague definitions of *catch-up growth*. The term, which can encompass either the first 6 to 12 months only or as much as the first 2 years after birth, usually refers to realignment of one's genetic growth potential after IUGR. This definition allows for fetal growth retardation at any birth weight; even large fetuses can be growth retarded in relation to their genetic potential. However, postnatal factors can obviously affect infant growth in the first few months of life. For example, breast-feeding appears to protect against obesity later in childhood, yet breast-fed infants usually exhibit higher body mass during the first year than formula-fed infants. Although it is likely that accelerated growth confers an additional risk to the growth-retarded fetus, these conflicting results demonstrate the need for additional, carefully designed studies to determine just how childhood growth rates affect the later development of cardiovascular disease and type 2 diabetes.

Size at Birth, Insulin Secretion, and Insulin Action

The mechanisms underlying the association between size at birth and impaired glucose tolerance or type 2 diabetes are unclear. A number of studies of children and adults have shown that nondiabetic or prediabetic subjects with low birth weight are insulin resistant and thus are predisposed to development of type 2 diabetes.[23,95–97,103,106–110] IUGR is known to alter the fetal development of adipose tissue, which is closely linked to the development of insulin resistance.[111] In a well-designed case-control study of 25-year-old adults, Jaquet and colleagues demonstrated that individuals who were born SGA at 37 weeks or later had a significantly higher percentage of body fat in adulthood (15%).[98] Insulin sensitivity, even after adjustment for BMI or total fat mass, was markedly impaired in these SGA subjects. There were no significant differences between the SGA and control groups with respect to parental history of type 2 diabetes, cardiovascular disease, hypertension, or dyslipidemia. Of importance to generalization of the findings to other populations, the causes of IUGR in these subjects were gestational hypertension (50%), smoking (30%), maternal short stature (7%), congenital anomalies (7%), and unknown (6%).

The adverse effect of IUGR on glucose homeostasis was originally thought to be mediated through programming of the fetal endocrine pancreas. Growth-restricted fetuses and newborns have been shown to have a reduced population of pancreatic β-cells.[112] Jensen and colleagues measured insulin secretion and insulin sensitivity in a well-matched population of 19-year-old glucose-tolerant white men whose birth weights were either below the 10th percentile (SGA) or between the 50th and 75th percentiles (controls).[113] To eliminate the major confounding factors, such as "diabetes genes," the researchers ensured that none of the participants had a family history of diabetes, hypertension, or ischemic heart disease. They found no differences between the groups with regard to current weight, BMI, body composition, and lipid profile. When data were controlled for insulin sensitivity, insulin secretion was found to be lower by 30%. However, insulin sensitivity was normal in the SGA subjects. These investigators hypothesized that defects in insulin secretion may precede defects in insulin action and that once SGA individuals accumulate body fat, they demonstrate insulin resistance.

Epidemiologic Challenges

The data described in the preceding section suggest that low birth weight is associated with glucose intolerance, type 2 diabetes, and cardiovascular disease. However, the question remains whether these associations reflect fetal nutrition or other factors that contribute to birth weight and the observed glucose intolerance.

Because of the retrospective nature of the cohort identification, many confounding variables were not always recorded, such as lifestyle, socioeconomic status, education, maternal age, parental build, birth order, obstetric complications, smoking, and maternal health. Maternal nutritional status, either directly in the form of diet histories or indirectly in the form of BMI, height, and pregnancy weight gain, were usually not recorded. Instead, birth anthropometric measures were used as proxies for presumed undernutrition in pregnancy.

Size at Birth Cannot Be Used as a Proxy for Fetal Growth

Birth weight is determined by the sum of multiple known and unknown factors, including gestational age, maternal age, birth order, genetics, maternal prepregnancy BMI, and pregnancy weight gain plus multiple environmental factors, such as smoking, drug use, infection, and maternal hypertension. Some of these determinants may be related to susceptibility to adult disease and others may not. Conversely, some prenatal determinants of adult outcomes may not be related to fetal growth. A good example of how size at birth may potentially be a proxy for an underlying causal pathway is the hypothesis that essential hypertension in the adult is due to a congenital nephron deficit.[114] More recent studies have tended to support the association between low nephron number and increased susceptibility to hypertension.[115–117]

Genetics versus Environment

Several epidemiologic and metabolic studies of twins and first-degree relatives of patients with type 2 diabetes have demonstrated an important genetic component of diabetes.[118] The association between low birth weight and risk of type 2 diabetes in some studies could theoretically be explained by a genetically determined reduced fetal growth rate. In other words, the genotype responsible for type 2 diabetes may itself restrict fetal growth. This possibility forms the basis for the *fetal insulin hypothesis*, which suggests that genetically determined insulin resistance could result in insulin-mediated low growth rate in utero as well as insulin resistance in childhood and adulthood.[119] Insulin is one of the major growth factors in fetal life, and monogenic disorders that affect the fetus's insulin secretion or insulin resistance also affect its growth.[120–123] Mutations in the gene encoding glucokinase have been identified that result in low birth weight and maturity-onset diabetes of the young. However, such mutations are rare, and no analogous common allelic variation has yet been discovered, but it is likely that some variations exist that, once identified, will explain a proportion of the cases of diabetes in LBW subjects.

There is obviously a close relationship between genes and the environment. Not only can maternal gene expression alter the fetal environment, but the maternal intrauterine environment also affects fetal gene expression. An adverse intrauterine milieu is likely to have profound long-term effects on the developing organism that may not be reflected in birth weight.

Cellular Mechanisms

Fetal malnutrition has two main causes: poor maternal nutrition and placental insufficiency. In the extensive literature about the fetal origins hypothesis, these two concepts have not been clearly discerned. Such a distinction is necessary, however, because maternal nutrition has probably been adequate in the majority of populations in which the hypothesis has been tested. Only extreme maternal undernutrition, such as occurred in the Dutch famine, reduces the birth weight to an extent that could be expected to raise the risk of adult disease.[124] Thus it is reasonable to assume that placental insufficiency has been a main cause of low birth weight in these populations. The oxygen and nutrients that support fetal growth and development rely on the entire nutrient supply line, beginning with maternal consumption and body size but extending also to uterine perfusion, placental function, and fetal metabolism. Interruptions of the supply line at any point could result in programming of the fetus for the future risk of adult diseases.

The intrauterine environment influences development of the fetus by modifying gene expression in both pluripotential cells and terminally differentiated, poorly replicating cells. The long-range effects on the offspring (into adulthood) are determined by which cells are undergoing differentiation, proliferation, or functional maturation at the time of the disturbance in maternal fuel economy. The fetus also adapts to an inadequate supply of substrates (such as glucose, amino acids, fatty acids, and oxygen) through metabolic changes, redistribution of blood flow, and changes in the production of fetal and placental hormones that control growth.

The fetus's immediate metabolic response to placental insufficiency is catabolism, consuming its own substrates to provide energy. A more prolonged reduction in availability of substrates leads to a slowing in growth. This enhances the fetus's ability to survive by reducing the use of substrates and lowering the metabolic rate. Slowing of growth in late gestation leads to disproportion in organ size because the organs and tissues that are growing rapidly at the time are affected the most. Placental insufficiency in late gestation may, for example, lead to reduced growth of the kidney, which is developing rapidly at that time. Reduced replication of kidney cells may permanently reduce cell numbers because there seems to be no capacity for renal cell division to catch up after birth.

Substrate availability has profound effects on fetal hormones and on the hormonal and metabolic interactions between the fetus, placenta, and mother. These effects are most apparent in the fetus of the mother with diabetes. Higher maternal concentrations of glucose and amino acids stimulate the fetal pancreas to secrete exaggerated amounts of insulin, and the fetal liver to produce higher levels of insulin-like growth factors (IGFs). Fetal hyperinsulinism stimulates the growth of adipose tissue and other insulin-responsive tissues in the fetus, often leading to macrosomia. However, many offspring of diabetic mothers with fetal hyperinsulinism are not overgrown by usual standards, and many with later obesity and glucose intolerance were not macrosomic at birth.[125,126] These observations suggest that birth weight is not a good indicator of intrauterine nutrition.

Molecular Mechanisms: Epigenetics

Epigenetic programming occurs during early development at two critical time points that involve preimplantation embryos and primordial germ cell development. These modifications influence lineage and tissue-specific gene expression, are biologically stable, and are generally maintained throughout the lifetime of the organism. However, some of these modifications have a potential

to be influenced by extrinsic (environmental factors, such as diet, physical activity, in utero environment, chemical exposure, and stress) and intrinsic (genetically determined) factors and hence can be reprogrammed by being erroneously erased, reestablished, maintained, or reversed. These abrupt epigenetic changes theoretically could increase disease susceptibility later in the life of the organism.

Epigenetic modification is typically described as heritable changes in gene function that occur without a change in the nucleotide sequence.[127] Epigenetic regulation includes DNA methylation, histone modifications, and noncoding RNAs. DNA methylation, specifically cytosine methylation of palindromic CpG sequences, is the most studied epigenetic mark so far and occurs mainly at the fifth position of the cytosine ring. DNA methylation is necessary for cell-specific gene expression, plays an important role during embryonic development, and establishes imprinting and X chromosome inactivation.[128–130] DNA methylation in promoter regions has been associated with transcriptional silencing. However, emerging data show that the effect of DNA methylation depends on the genomic location, and it may also affect alternative splicing, genomic stability, transcriptional elongation, and transcription of noncoding RNAs. Thus, DNA methylation may also be associated with increased gene expression.

Most CpG islands remain unmethylated in normal cells; however, under some circumstances, such as cancer and oxidative stress, they can become methylated de novo.[131] It is not known why particular CpGs are susceptible to aberrant methylation. A study by Feltus et al. suggests that there is a "sequence signature associated with aberrant methylation."[132]

The NH_2-terminal tail of histones can be covalently modified by histone methyltransferases, histone deacetylases (HDACs), and histone acetylases. Histone modifications alter chromatin compaction, and influence the recruitment of transcriptional regulators, which modulate gene expression.[133] Multiple histone modifications have been described, including acetylation, methylation, phosphorylation, ubiquitination, and SUMOylation of amino acid residues (e.g., lysine or arginine).[134] Many of these modifications act in concert to regulate gene expression.

The metabolic or nutritional state of the organism directly influences epigenetic modifications as essentially all known epigenetic modifications rely upon substrates derived from intermediary metabolism, such as S-adenosyl methionine (SAM), acetyl CoA, a-ketoglutarate, and nicotinamide adenine dinucleotide (NAD^+).[135]

There are numerous studies in humans examining the relationship between fetal nutrient availability and epigenetic modifications in the offspring.[136] Many of these are confounded by small sample size, cellular heterogeneity of tissues examined, and lack of validation. Moreover, most DNA methylation assays are performed in total peripheral blood monocytes, where the unique methylation profiles of the various cellular lineages complicate interpretation of the data. Furthermore, most changes appear to be stochastic and not reproducible in other cohorts. Despite these issues, multiple studies in diverse populations repeatedly show changes in DNA methylation associated with low birth weight and/or altered nutrient availability. Thus, it is likely that an adverse in-utero milieu does indeed induce epigenetic modifications in the offspring, but whether these modifications have biological relevance remains to be determined. The field of "epigenetic epidemiology" remains an active and growing field of investigation.

Macrosomia

Excessive fetal growth (macrosomia, being large for gestational age) is found in 9% to 13% of all deliveries and can lead to significant complications in the perinatal period.[137] Maternal factors associated with macrosomia during pregnancy include increasing parity, higher maternal age, and maternal height. Additionally, the previous delivery of a macrosomic infant, prolonged pregnancy, maternal glucose intolerance, high pre-pregnancy weight or obesity, and large pregnancy weight gain have all been found to raise the risk of delivering a macrosomic infant.[138]

Maternal complications of macrosomia include morbidities related to labor and delivery, including prolonged labor, arrest of labor, and higher rates of cesarean section and instrumentation during labor. Also, the risks of maternal lacerations and trauma, delayed placental detachment, and postpartum hemorrhage are higher for the woman delivering a macrosomic infant.[137] Complications of labor are more pronounced in primiparous women than in multiparous women.[138] The neonatal complications of macrosomia include traumatic events, such as shoulder dystocia, brachial nerve palsy, birth trauma, and associated perinatal asphyxia. Other complications for the neonate are elevated insulin levels and metabolic derangements, such as hypoglycemia and hypocalcemia.[139] In a large population-based study in the United States, macrosomia (defined as birth weight greater than 4000 g) was detected in 13% of births. Of these, shoulder dystocia was noted in 11%.[137]

Macrosomia is often not detected during pregnancy and labor. The clinical estimation of fetal size is difficult and has significant false-positive and false-negative rates. Ultrasonography estimates of fetal weight are not always accurate, and the literature reports a wide range of sensitivity estimates for the ultrasound detection of macrosomia. Additionally, there is controversy in how to define macrosomia and which ultrasound measurement is most sensitive in predicting macrosomia. Stirnemann and colleagues have demonstrated a linear relation between abdominal circumference and birth weight.[140] They showed that the equations commonly used for estimated fetal weight (EFW) have a median error rate of 7%, with greater errors seen with larger infants. Using receiver operating characteristics (ROC) curves to measure the diagnostic accuracy of ultrasound, O'Reilly-Green and Divon reported sensitivity and specificity rates of 85% and 72%, respectively, for estimation of birth weight exceeding 4000 g.[141] In their study, the positive predictive value (i.e., a positive test result representing a truly macrosomic infant) is about 49%. Chauhan and associates found lower sensitivity for the use of ultrasound measurement of abdominal and head circumference and femur length (72% sensitivity), similar to the sensitivity of using clinical measurements alone (73%).[142] Other investigators have shown that clinical estimation of fetal weight (43% sensitivity) had higher sensitivity and specificity than ultrasound evaluation in predicting macrosomia.[143] In a retrospective study, Jazayeri and coworkers showed that ultrasound measurement of abdominal circumference of greater than 35 cm predicts macrosomia in 93% of cases and is superior to measurements of biparietal diameter or femur.[144] Other researchers have reported an abdominal circumference of either more than 37 cm or more than 38 cm to be a better predictor.[145,146]

Numerous investigators have also questioned whether antenatal diagnosis improves birth outcomes in macrosomic infants. Investigators point to the low rates of specificity of antenatal tests

resulting in high rates of false-positive results.[141,147] Antenatal identification of macrosomia (or possible macrosomia) may lead to a higher rate of cesarean section performed for infants with normal birth weights.[138,143,148] Macrosomia is a risk factor for shoulder dystocia, but the majority of cases of shoulder dystocia and birth trauma occur in non-macrosomic infants.[143] A retrospective study of infants weighing more than 4200 g at birth showed a cesarean section rate of 52% in infants predicted antenatally to have macrosomia, compared with 30% in infants without such a antenatal prediction. The antenatal prediction of fetal macrosomia is also associated with a higher incidence of failed induction of labor and no reduction in the rate of shoulder dystocia.[149] Using retrospective data from a 12-year period, Bryant and colleagues estimated that a policy of routine cesarean section for all infants with estimated fetal weight greater than 4500 g would require between 155 and 588 cesarean sections to prevent a single case of permanent brachial nerve palsy.[147]

Summary

There are many identified biologic and genetic factors associated with fetal growth and abnormalities of fetal growth. Physicians are limited in their ability to identify a causative agent in every case. Modification of fetal growth is possible and occurs from such diverse influences as socioeconomic status, maternal nutrition, and maternal constitutional factors. Abnormal fetal growth influences not only acute perinatal outcomes but also health during infancy, childhood, and, intriguingly, adulthood. In schools of public health, students are taught to search "upriver" for solutions to health problems. Solutions for ill health in adulthood may lie in the identification of methods to improve the health of the fetus.

Suggested Readings

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5

Multiple Gestations and Assisted Reproductive Technology

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KEY POINTS

- The multiple birth rate has increased by 76% in the past three decades; however, it has recently started to stabilize or even decline.
- Increased utilization of assisted reproductive technologies and women delaying childbearing are the main contributors to the high rate of multiples.
- Multiple gestations are at high risk for both maternal and fetal morbidity and mortality, which increase as fetal number increases.
- Most neonatal complications in multiple gestations are sequelae of prematurity, including low birthweight, respiratory distress syndrome, neonatal intensive care unit (NICU) admission, intraventricular hemorrhage, and necrotizing enterocolitis.
- Up to 80% of women with multiple gestations experience antepartum complications, which include preterm labor, preterm premature rupture of membranes (PPROM), and placental abruption.
- Strategies for decreasing the rate of multiples resulting from assisted reproductive technology (ART) include increasing the number of single embryo transfers performed in in vitro fertilization (IVF) and using "low and slow" protocols for superovulation cycles with gonadotropins.

Epidemiology of Multiples

There are multiple factors influencing the increase in multiple births in the United States in the past half-century. The incidence of multiples was stable through the 1970s at 2% of all births.[1] We then began to see an "epidemic of multiples," with the twin birth rate increasing by 76% from 1980 to 2009.[1] The overall proportion of total national births that were multiples increased from 1.8% in 1971 to 3.30% in 2019.[2–4] Recently, twin birth has started to decline, and from 2014 to 2019, the twin birth rate declined 5%.[4] In 2019, the lowest number of twin births was reported since 2002.[4]

Twins continue to account for the vast majority of multiple pregnancies, comprising 97% in 2019.[4] The growing use of assisted reproductive technology (ART), in addition to delayed childbearing until age-related fertility issues become apparent, has contributed greatly to multiple birth rates. The rate of women delaying childbearing has dramatically increased, with women over the age of 30 accounting for 20% of births in 1980, compared with more than 45% in 2019.[1,4] Increased maternal age is associated with increased rates of twinning, and compared to mothers under 20, mothers aged 30 to 39 are twice as likely and mothers aged 40 and over are three times as likely to have a twin birth.[5] Between 1980 and 2009, the twin birth rate increased by 100% in women 30 to 35 years old and by more than 200% in women aged 40 and older.[1] On the other hand, recent declines in twin births have been limited to mothers aged 30 and older, and mothers aged 40 and over had a 23% decline in twinning from 2014 to 2018.[5] Despite the decline in twinning rate, in 2018, more than 5% of births to women aged 40 or older were multiple births.[2] Spontaneous twinning is more common as women age, perhaps related to higher follicle-stimulating hormone (FSH) levels in the follicular phase leading to ovulation of more than one oocyte, with a peak at age 37.[6] However, the increasing age of childbearing women is estimated to account for only one-third of the rise in twinning, with ART responsible for the remainder.[1] Since the birth of the first in vitro fertilization (IVF) baby in 1978, the numbers of IVF clinics, ovarian stimulation cycles, and live births from ART have all steadily increased. In 2017, ART contributed to the births of 1.9% of total infants, 14.7% of multiple birth infants, and 17.3% of triplet and higher order multiple (HOM–four or more) infants.[7] About 3.4% of all US births are multiples, yet in 2017, the ART twin birth rate was 26%, and the ART HOM birth rate was 0.9%.[7]

It is important to note that since 1997, IVF has *not* been the leading contributor to the multiple birth rate, contributing to under 15% of multiple births in 2017.[7,8] Non-IVF treatment options, including ovulation induction with oral medications and superovulation with injectable gonadotropins, were responsible for 19% of twins and 45% of triplets or HOMs in 2011.[3] These modalities involve less monitoring and are subject to less control than IVF and thus less susceptible to efforts to decrease the multiples rate. The proportion of triplet or HOM births arising from medically assisted conceptions has been declining in recent years: from 84% in 1998 to 77% in 2011.[3] The incidence of triplet and HOM births increased by a factor of 6.7 from 1971 to 1998 but has since decreased by 29% from 1998 to 2013.[3] This is largely due to a decrease in the number of embryos transferred per cycle of IVF and to the reticence of providers to use superovulation with injectable gonadotropins.

Given the maternal, perinatal, and neonatal complications associated with multiples, the goal of infertility treatment is one healthy child. Multifetal pregnancies drastically affect individuals, families,

and public health systems. Of particular importance in both maternal and fetal outcomes are fetal number and placentation.

Diagnosing Zygosity and Chorionicity

Zygosity refers to the number of oocytes and spermatozoa involved in conception while chorionicity refers to the type of placentation. Determining zygosity and chorionicity is important medically, genetically, and psychosocially for the individual and family. The chorion–amnion arrangement is crucial to antepartum management, as it determines risks of complications such as twin-twin transfusion syndrome (TTTS), intrauterine growth restriction (IUGR), growth discordance, congenital anomalies, and cord accidents. Chorionicity also guides next steps in cases of one fetal demise or desired selective reduction. Dizygotic twins result from fertilization of two separate ova by two spermatozoa, and these comprise 67% of spontaneous twins.[9] Monozygotic twins (MZTs) are identical and result from fertilization of a single ovum with one spermatozoon and subsequent postzygotic division.[10] The timing of this division-splitting determines the number of fetuses, chorionic plates, and amniotic sacs.

To determine zygosity, we can use prenatal determination of fetal gender and, more recently, single-nucleotide polymorphism-based noninvasive prenatal testing has been used to evaluate fetal genome components at as early as 9 weeks' gestation.[11] In the postnatal period, fetal blood typing and DNA analysis can be used to determine zygosity.[6,12] Diagnosing chorionicity is possible using ultrasound markers, including the number of placental sites, thickness of the dividing membrane, and the lambda sign. The lambda sign indicates dichorionicity and is a triangular projection of tissue that extends beyond the chorionic surface of the placenta.[13]

A first trimester screening ultrasound is essential in diagnosing multiple pregnancy. In a large randomized trial, in the cohort of women that did not have a screening ultrasound, 37% were not diagnosed as having a twin pregnancy until 26 weeks, and 13% were not diagnosed until the time of delivery.[14] Current ultrasonographic technology is very effective at diagnosing chorionicity, and amnionicity and first trimester ultrasounds are most accurate. In one study, the reported sensitivity, specificity, and positive and negative predictive values for ultrasonography at less than 14 weeks gestation for chorionicity were 89.8%, 99.5%, 97.8%, and 97.5%, respectively.[15] Overall, chorionicity was correctly diagnosed antenatally in 95% of cases.[15]

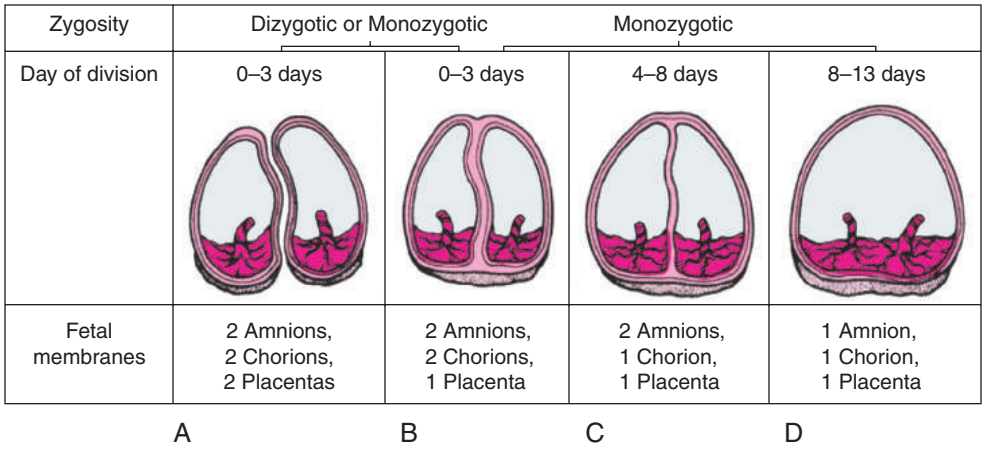
The Effect of Chorionicity

Chorionicity and placentation greatly affect fetal morbidity and mortality in multifetal pregnancies. Dizygotic twins (DZTs), with few exceptions, lead to a dichorionic, diamniotic (DCDA) arrangement in which the placenta can be separate or fused. Rare cases of dizygotic, monochorionic, diamniotic (MCDA) twins have been reported.[16] Theories as to the etiology of this include fusion of two genetically distinct zygotes or fertilization of a binovular follicle.[10]

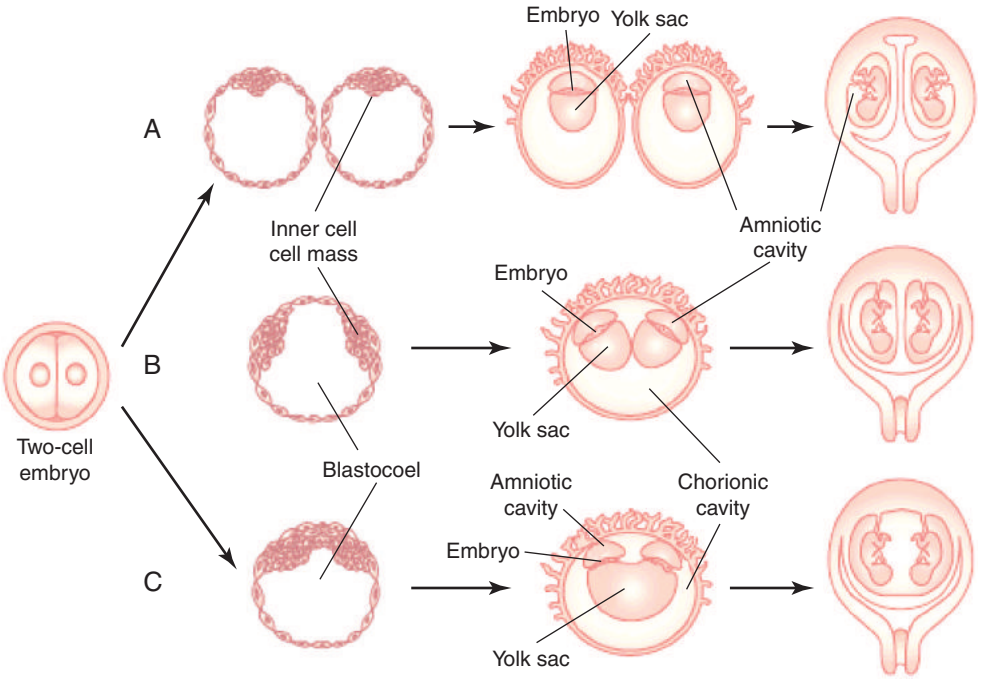
The highest rates of spontaneous twinning are in Nigeria, where 1 out of every 12 persons is a member of a twin pair, and the lowest twinning rates are seen in China, where 1 in every 70 persons is a member of a twin pair. North American and European countries are considered to have an intermediate prevalence of spontaneous dizygotic twinning.[17] Risk factors for DZT include advancing maternal age, increased parity, female relatives with DZT, taller height, and higher body mass index.[17,18] Historically, a seasonal trend in DZT has been seen, with higher rates among conceptions in the summer and autumn months.[17]

The true incidence of MZTs is difficult to ascertain because of its rarity, inaccuracies in diagnosis, and lack of confirmatory studies at birth, but spontaneous MZT rates are estimated to occur in 0.3% to 0.5% of all pregnancies and in less than 30% of all twins.[6,18,19] This rate was geographically constant prior to the advent of ART,[6] Unlike DZT, it is unclear whether MZT is related to genetics or environment.[17,20] Familial association has been seen but is very rare.[6,21]

Chorionicity in monozygotic gestations is determined by the timing of the embryonic division (Figs. 5.1 and 5.2). In 18% to 36% of MZTs, the zygote divides within 72 hours of fertilization, resulting in a DCDA gestation (the placenta can



• **Fig. 5.1** Placentation and Membranes Based on Timing of Embryonic Division. (A) Two amnions, two chorions, and separate placentas from the division of either a dizygotic or monozygotic embryo within 3 days of fertilization.[1] (B) Two amnions, two chorions, and one fused placenta from the division of either a dizygotic or monozygotic embryo within 3 days of fertilization.[2] (C) Two amnions, one chorion, and one placenta from monozygotic embryonic cleavage, days 4 to 8 after fertilization. (D) One amnion, one chorion, and one placenta from a monozygotic embryo splitting days 8 to 13 after fertilization. (Modified from Gibbs R, Karlan B, Haney A, et al. *Danforth's Obstetrics & Gynecology*. 10th ed. Philadelphia: Lippincott, Williams & Wilkins, 2008.)



• **Fig. 5.2** Types of Monozygotic Placentation. (A) Dichorionic diamniotic pregnancy.[1] (B) Monochorionic diamniotic pregnancy.[2] (C) Monochorionic monoamniotic pregnancy. (Adapted from Hall JG. Twinning. Lancet. 2003;362:735–743; and Benirschke K, Kim CK. Multiple pregnancy. *N Engl J Med*. 1973;288:1276–1284.)

be separate or fused); 60% to 75% split between days 4 and 8, leading to an MCDA unit; and 1% to 2% separate between days 8 and 13, leading to a monochorionic, monoamniotic (MCMA) pregnancy.[6,9,10,22] Embryonic division after day 13 results in conjoined twins that are MCMA.[22]

There are no naturally occurring animal models of MZT with the exception of armadillos. Models of MZT can be induced in laboratory animals by exposure to toxins or manipulation of the zona pellucida.[6] The cause of spontaneous monozygotic twinning in humans is unknown. An uneven sex ratio has been noted, with 0.484 male:female pairs in all monozygotic twins and 0.231 in monoamniotic twins, leading to the theory that skewed X inactivation may play a role.[6,23] The development of two inner cell masses (ICM) at the blastocyst stage can lead to MZT, either from damage or through immune-mediated cell-to-cell crosstalk.[6]

Although the majority of ART MZTs are MCDA, any of the three monozygotic, placental arrangements can transpire after ART, implying that the timing and mechanism of embryonic splitting are variable.[24,25]

Increase in Monozygotic Twins With Assisted Reproductive Technology

The first reported association between ART and MZT preceded numerous accounts of similar findings.[26] The majority (>90%) of ART twins are dizygotic secondary to transferring multiple embryos; however, the rate of MZTs per pregnancy after fertility treatment is higher (0.7% to 2%) versus the general population (0.3% to 0.5%).[9,24,27–31] A recent population-based study found a 60% higher risk of MZT for ART conceptions compared to natural conceptions.[32] It is also suspected that the incidence of MZT in ART may be underestimated because DCDA gestations after transfer of more than one embryo are often assumed to be dizygotic, and genetic analysis is rarely performed postnatally.[25] Several theories to explain the mechanism responsible for elevated MZTs with ART have been proposed. There follows a discussion of those theories.

Age

As mentioned previously, spontaneous dizygotic twinning increases with advancing maternal age, but the connection between age and MZTs is controversial.[18–20] Some studies report trends toward elevated MZT rates in older women, whereas others found no association with increasing maternal age and MZT.[19,20,33] A recent meta-analysis found that the MZT risk following IVF is significantly higher in women younger than 35 years, while other studies have found no association between MZT risk and age.[34,35] Overall, the correlation between age and MZT in ART remains unclear.

Zona Pellucida Manipulation

The zona pellucida (ZP), an acellular protein surrounding the ovum, provides a species-specific sperm barrier and decreases polyploidy by inhibiting penetration by multiple sperm.[36] It has been shown that the thickness and hardness of the ZP can vary in relation to stimulation protocol, elevated FSH or estradiol (E2) levels, and culture conditions. There is debate as to whether ZP manipulations performed during IVF affect MZT risk. Manipulation of the ZP in IVF occurs via both intracytoplasmic sperm injection (ICSI) and assisted hatching.[27] The injection of one sperm into a mature oocyte (i.e., ICSI) is most commonly performed for male factor infertility. AH is achieved with an artificial breach in the ZP by laser, chemical,

or mechanical methods and has been shown to increase clinical pregnancy rates, although not live birth rates, in patients with a poor prognosis.[37] A Cochrane review demonstrated that AH is associated with an increase in multiple pregnancy, with an odds ratio of 1.39.[38] However, the increase in MZT with AH was not statistically significant, and this was confirmed in other large studies.[27,31,38,39] However, the data are conflicting, with a retrospective review of over 35,000 IVF cycles showing a threefold risk of MZT with AH, a finding also demonstrated in a recent meta-analysis.[35,40] Similarly, there are mixed data regarding the association between ICSI and the risk of MZT. One study noted ICSI to be an independent predictor of MZT with an odds ratio of 2.42, with an even higher risk of MZT when ICSI was combined with day 5 embryo transfer.[41] However, more recent data have called this into question, with a recent study examining twinning after single embryo transfer finding a decrease in rates of MZT with ICSI.[42] The defect created in the ZP for ICSI is much smaller than for AH, lending plausibility to other recent studies that have shown no association.[35,39,43]

Blastocyst Transfer

After oocyte retrieval and insemination, embryos undergo intrauterine transfer at either the cleavage stage (day 2 to 3 after retrieval) or the blastocyst stage (day 5 to 6 after retrieval). Blastocyst-stage embryo transfer yields higher live birth rates, and lowers overall multiple rates because of an increase in single embryo transfer at the blastocyst stage.[44,45] However, several studies have supported the increased incidence of MZTs with extended culture to the blastocyst stage.[31,35,39,41–43] One theory is that extended culture may alter the integrity of the ZP, leading to an increased risk of splitting of the inner cell mass.[35] Another theory is that differences in culture media modify the odds of MZT. One institution initially noted increased MZT after blastocyst transfer (5.6% vs. 2%), but a follow-up study 3 years later demonstrated similar MZT rates between blastocyst and day 3 embryos (2.3% vs. 1.8%), indicating that changes in culture media and an experienced embryology team may affect the rate of MZTs.[46,47] Another recent study noted that use of sequential media was associated with higher rate of MZT in fresh, but not frozen, embryo transfers.[48] Elevated glucose levels in extended culture and subsequent glucose-induced apoptotic remodeling of the ICM and ICM splitting in extended culture in murine and human embryos may explain the phenomenon of MZTs in blastocysts.[49,50]

Higher quality embryos are more likely to be cultured out to the blastocyst stage, which may be confounding when comparing day of transfer. Recent data also indicate that younger oocyte age, another marker of embryo quality, is related to higher incidence of MZT.[25,31,51] Another environmental factor that may affect MZTs is temperature variation in frozen-thawed embryo cycles. Although rare evidence links frozen embryo transfers and temperature fluctuations to MZTs, most studies show no difference between fresh and frozen-thawed embryos and multiple rates or MZT rates.[35,39,48,52,53]

Ovulation Induction and Superovulation

Human studies of ovarian stimulation with clomiphene citrate and gonadotropins reveal a twofold increased risk of MZTs compared with the general population. MZT incidences of 1.5% after ovulation induction with gonadotropins compared to 0.72% after IVF, and 0.87% with IVF with ICSI and AH suggest that elevated levels of steroid hormones may increase rates of MZTs regardless of ZP manipulation.[28] However, this finding may be confounded by a higher baseline risk of MZT in patients with infertility.[54]

Neonatal Complications Associated With Multiples

Multiple pregnancies account for a small percentage of overall live births but are responsible for a disproportionate amount of morbidity and mortality. This is largely attributable to complications of prematurity, as women with multiple pregnancies are six times more likely to deliver preterm and 13 times more likely to deliver before 32 weeks than those with a singleton.[55] Of twin pregnancies, 61% are delivered preterm (i.e., before 37 weeks), compared with 8.5% of singleton pregnancies, and 20% of twins are delivered before 34 weeks.[4] Triplet and higher-order gestations are at even higher risk of prematurity, with 63% of triplets and 85% of quadruplets delivered before 34 weeks.[4] The risk of low birth weight (<2500 g) and very low birth weight (<1500 g) is also increased in multiple gestations. Of twin pregnancies, 9% are very low birth weight and 55% low birth weight, compared to 1% and 7% of singletons, respectively.[4] As a consequence, multiples have a fivefold increased risk of stillbirth and a sevenfold increased risk of neonatal death.[56] The average gestational ages at delivery for twins, triplets, and quadruplets are 35.3, 31.9, and 29.5 weeks, respectively, corresponding to neonatal intensive care unit (NICU) admission rates fivefold higher in twins and 17-fold higher in triplets and HOMs.[55,57]

Neonatal and maternal complications are more common in twin pregnancies, and this risk increases with triplets and HOMs (Table 5.1). Multiples have increased risks of intraventricular hemorrhage, periventricular leukomalacia, cerebral palsy, sepsis, necrotizing enterocolitis, and respiratory distress syndrome.[58–61] Surviving infants of preterm multifetal pregnancies have higher rates of developmental handicap.[60,62] Higher fetal number correlates with increased risk of growth restriction, earlier delivery, low birth weight (LBW), NICU admission, length of stay, risk of major handicap and cerebral palsy, and death in first year of life.[61–63] A recent retrospective cohort of 82 triplet gestations from 2007 to 2014 (65 conceived with ART) revealed that 88% required antepartum admission, 65% experienced preterm labor (PTL), 10% had congenital anomalies, and 5% died in the perinatal period.[64]

It is important to note that singleton pregnancies after assisted conception have increased complications, including preterm delivery, LBW, and perinatal mortality and for mothers, higher rates of antepartum hospital admission, cesarean delivery and blood transfusion.[65–69] Similar to ART singleton versus spontaneous singleton outcomes, ART multiples may have higher morbidity compared with spontaneous multiples. Assisted-conception twins have been shown to have increased risk for LBW, preterm delivery, cesarean delivery, NICU admission, longer length of stay, respiratory distress syndrome, and birth weight discordance versus spontaneously conceived twins in several studies.[70–75] In contrast, other studies suggest comparable outcomes in assisted conception and spontaneous multiples.

TABLE 5.1 Morbidity and Mortality in Multifetal Gestations

Characteristic	Singleton	Twins	Triplets	Quadruplets
Mean birth weight	3285 g	2345 g	1680 g	1419 g
Mean gestational age	38.5 weeks	35.0 weeks	31.7 weeks	30.3 weeks
Percentage less than 34 weeks of gestation	2.1	19.5	63.1	82.6
Percentage less than 37 weeks of gestation	8.2	60.3	98.3	97.4
Rate of cerebral palsy (per 1000 live births)	1.6	7	28	—
Infant mortality rate (per 1000 live births)	5.4	23.6	52.5	96.3

From American College of Obstetricians and Gynecologists. ACOG Practice Bulletin No. 231: Multifetal gestations: twin, triplet, and high-order multifetal pregnancies. *Obstet Gynecol.* 2021;137:e145–e162.

Rates of pregnancy-induced hypertension, gestational diabetes mellitus, preterm premature rupture of membranes (PPROM), placenta previa, placental abruption, congenital malformations, and severe morbidity were similar in IVF twins versus spontaneous twins.[66,71,73,76]

A common phenomenon in ART is the "vanishing twin," an arrest of development and subsequent absorption of one or more fetuses of a multiple gestation in the first trimester.[77] Estimates of the incidence of a vanishing twin after ART range from 10% to 30% and are increased in HOMs.[10,78,79] The majority of vanishing twins (80%) occur prior to 9 weeks.[10] Demise of a twin after the first trimester is more common in monozygotic gestations.[80] Recent evidence suggests that the surviving twin is at increased risk for LBW, preterm birth, and perinatal mortality.[81–83] The risk of LBW is related to the gestational age at the time of a twin demise, with later gestations conferring more risk.[84]

Fetal Complications

Besides fetal number, another critical factor in pregnancy outcome is placental architecture. Monochorionic multiples experience higher rates of morbidity and mortality, largely because of placental factors.[22,85,86] Negative outcomes such as stillbirth, neonatal mortality, congenital malformations, necrotizing enterocolitis, and cerebral palsy are higher in monochorionic twins compared with dichorionic twins.[85–88] These complications are thought to be related to placental sharing and the vascular anastomoses that may cause unequal distribution of placental blood flow, causing TTTS.[88]

TTTS affects 10% of MCDA gestations and usually presents in the second trimester with oligohydramnios of the donor twin and polyhydramnios in the recipient twin.[55,89] Overall, TTTS accounts for half of perinatal deaths in MCDA twins, and surviving infants are at risk for long-standing adverse neurologic outcomes.[90–92] The severity of TTTS can be assessed using the Quintero staging system, which ranges from stage I to stage V. The most severe sequelae of TTTS are fetal hydrops and death of one or both twins.[93] Laser fetoscopic ablation can be utilized to treat TTTS by interrupting the vascular anastomoses and functionally dividing the placenta into two regions.[89] The incidence of progression from stage I to more severe stages of TTTS is 10% to 27%.[94–96] The optimal management strategy for stage I TTTS remains debated as there are similar survival rates of 85% to 86% for cases managed expectantly and those managed with fetoscopic laser photocoagulation.[95,97] The prognosis for advanced TTTS that is stage III or higher is poor, with a loss rate of 70% to 100% for untreated cases.[89] Even advanced cases treated with laser ablation have a 5% to 20% risk of neurologic handicap long-term and a 30% to 50% risk of perinatal mortality.[89]

MCMA twins are very rare, occurring in 1 in 10,000 pregnancies, but they suffer the highest risk of perinatal morbidity and mortality.[55] Similar to MCDA twins, MCMA twins are susceptible to TTTS, growth discordance, IUGR, preterm delivery, and congenital anomalies, but they also face the unique complication of cord entanglement.[98] These factors historically account for perinatal mortality rates of up to 80%.[55] However, lower morbidity and mortality rates (2% to 25%) reported in recent articles may be attributed to increased prenatal diagnosis and fetal surveillance.[98–101]

Maternal Complications

Approximately 80% of women pregnant with multiples experience antepartum complications versus 25% of those with singletons.[55,102] Pregnancies with multiples are at increased risk of hyperemesis gravidarum, hypertensive disorders, gestational diabetes, PTL, placental abruption, cesarean delivery, and postpartum hemorrhage.[55] The risk of severe acute maternal morbidity, including intensive care unit admission, uterine rupture, eclampsia, major obstetric hemorrhage, and peripartum hysterectomy, is elevated fourfold.[103,104] Approximately 20% of the elevated risk for maternal morbidity in twin gestations may be mediated by cesarean deliveries.[104] Mothers with two or more fetuses are at increased risk for three of the major causes of maternal mortality: postpartum hemorrhage, venous thromboembolism, and hypertensive disorders.[104–106] Hypertensive disorders occur in 12.7% of twin and 20% of triplet pregnancies compared with 6.5% of singletons.[107]

Plurality correlates with maternal morbidity, with triplets and HOMs experiencing significantly increased maternal morbidity compared to twins; one study found a maternal complication rate of 65% among triplet gestations and 71% among quadruplet gestations.[108] They have higher rates of PPROM, postpartum hemorrhage, gestational diabetes, placental abruption, cesarean delivery, and hypertensive disorders.[61,107,109] Results of a retrospective triplet cohort showed that 29% of gestations were complicated by PPROM, 26% by PTL, 19% by pre-eclampsia or HELLP (hemolysis, elevated liver enzymes, low platelet count) syndrome, and 10% by gestational diabetes.[110]

Psychosocial Factors

As would be expected, psychosocial stressors increase with pregnancy and delivery of multiples. As fetal number increases, parents report decreased quality of life, increased social stigma, increased

parenting stress, and decreased marital satisfaction.[111–114] Studies have also demonstrated an increased risk of postpartum depression in mothers of multiples with up to a threefold increase compared to mothers of singletons.[115–117] It is estimated that adequately caring for 6-month-old triplets and performing household duties would require 197.5 hours per week, clearly exceeding the 168 hours available.[118] Additionally, many multiple pregnancy infants are born preterm and have ongoing health issues, further exacerbating parental stress. Most studies have not demonstrated a psychosocial difference between naturally conceived multiples and multiples conceived with ART.[111,114,119]

Cost

Multiple gestations have a large economic impact, both for individual families and society. As previously discussed, multiples have increased risks of preterm delivery and LBW, which are the major drivers of short-term and long-term costs.[120] The average first-year medical costs for preterm infants are up to 10 times higher than for term infants.[55] According to gestational age, the mean initial hospital charge for infants born between 26 and 28 weeks gestation is approximately $240,000 compared with approximately $4800 for a term infant.[120] By birth weight, infants weighing less than 1250 g cost on average approximately $250,000 compared with infants weighing more than 2500 g, who cost on average $5800.[120] Daily NICU costs are over $3500 per infant, and many prolonged stays exceed $1,000,000.[121] Overall, the cost of a twin gestation is fourfold to fivefold that of a singleton, and this increases to 10- to 20-fold for triplets and HOMs.[122,123]

ART multiples and their associated comorbidities have a significant role in healthcare expenditures. The estimated financial cost of ART-associated preterm deliveries in the United States is $1 billion annually.[124] Women with ART pregnancies have higher rates of antenatal hospitalization with longer length of stay than women who conceived naturally, contributing to increased costs.[65] A Dutch study showed that multiples conceived with IVF were 3.3 times more costly than singletons conceived with IVF through 5 years of life.[125] If iatrogenic multiples were singletons in future, the estimated cost savings would be $6.3 billion in the first year of life.[126] Data also indicate that ART-conceived infants, especially in the case of multiples, have higher risks of hospital admission in the first 1 to 2 years of life, incurring additional costs.[76,127]

Decreasing the Risk of Multiples

Primary forms of preventing multiples include canceling ovulation induction cycles in which multifollicular development occurs, or converting those cycles to IVF, and limiting the number of embryos transferred in IVF cycles. Embryo transfer policies vary significantly on the global stage, ranging from strict legislation to recommendations from professional societies that are not binding. In some countries, single embryo transfer (SET) is mandatory with rare exceptions.[128,129] The American Society for Reproductive Medicine (ASRM) and the Society for Assisted Reproductive Technology (SART) established transfer guidelines in the United States, beginning in 1998, to assist in determining an appropriate number of embryos to transfer and help decrease the rate of multiples. Recommended limits are based on age, prognosis, and embryo stage, and further differentiate between a favorable prognosis (euploid embryos, high-quality embryos, expectation of high-quality embryos for potential cryopreservation, previous live birth after an IVF cycle, and frozen embryo transfer) and less favorable prognosis.[130]

In the most recent 2017 guidelines, ASRM highly recommends consideration of SET in patients younger than 38 with favorable prognosis.[130] In all age groups, transfer of a euploid embryo is recommended to be limited to one as prognosis is most favorable.[130] Recommendations state that the number of embryos transferred in a frozen cycle should not exceed the recommended limits in a fresh cycle.[130] These transfer guidelines are not legally binding, and they are subject to interpretation or adjustment based on clinical experience and unique patient characteristics.[130]

The rates of SET in the United States have been increasing in response to the epidemic of multiples, with the proportion of fresh autologous cycles reported to the CDC in which one embryo was transferred increasing from 9.3% in 2005 to 28.8% in 2014.[131] Among all embryo transfer procedures in the United States in 2017, 67.3% were SETs.[7] Similarly, the proportion in which three or more embryos were transferred decreased from 79% in 1998 to 24% in 2011, which corresponded to a 33% reduction in the proportion of IVF-conceived triplet and higher-order gestations.[3] The term "elective SET" (eSET) refers to couples who have more than one quality embryo available for transfer but elect to transfer only one. The rate of eSET in the United States has historically been quite low but increased from 1% of good-prognosis patients in 2005 to 15.3% in 2012, though there is significant variation by state.[3,132] These efforts have resulted in an increase in the singleton birth rate after IVF with 2005 marking the first year that the majority of IVF deliveries were singletons; in 2017, 73.6% of ART-conceived infants were singletons.[3,7] The goal outcome of ART is a non-low birth weight, term singleton, and eSET is most strongly associated with this outcome.[133]

There are many factors contributing to the increase in SET and stabilization of the multiple birth rate. These include revised guidelines from ASRM that lowered the recommended number of embryos to transfer, improved technology leading to more blastocyst transfers, and expanded insurance coverage for ART.[3] The overall number of embryos transferred each year has steadily declined since publication of the initial ASRM guidelines in 1998, and it is estimated that from 1998 to 2012, between 13,500 and 16,300 HOM deliveries were prevented, yielding a cost saving of more than $6 billion.[134,135] A cost-effectiveness model in a system with no infertility coverage incorporating neonatal and obstetrical cost estimates demonstrated that SET saves approximately $3.5 million per 250 patients.[136]

A recent retrospective cohort analysis of the SART outcomes between 2004 and 2013 showed a decline in live birth rate (LBR) of 10% to 15% for SET compared to double embryo transfer (DET).[132] For example, in autologous first cycles for women aged 35 to 37 years, the LBR for SET was 46.8% compared to 56.2% for DET.[132] Notably, the multiple birth rate (MBR) increased from approximately 2% with SET to greater than 49% with DET.[132] LBR was significantly higher for blastocyst embryo transfer compared to cleavage embryo transfer; this is reflected in the ASRM transfer guidelines.[132] A study of the national IVF outcomes data for 2013 demonstrated that clinics with higher eSET rates in women less than 38 years of age had lower MBRs with no significant difference in cumulative LBR.[137] Overall, there were very comparable pregnancy rates for younger

good-prognosis patients undergoing eSET compared to DET on day 5 to 6.[137,138] Preimplantation genetic screening may be another tool to improve embryo selection to reduce the rate of multiple gestations.[130] A randomized controlled trial found that in women under 43 years of age, transfer of a single euploid blastocyst resulted in similar ongoing pregnancy rate to transfer of two untested blastocysts, however the rate of twins was impressively 0% compared to 53%.[139]

Expanding insurance coverage for ART is another strategy that may help to decrease multiple births. As of early 2021, 17 states have enacted infertility insurance mandates that vary in coverage of infertility diagnosis and ART treatment. State-mandated IVF insurance coverage is associated with fewer embryos transferred per cycle.[140] Additionally, women with IVF insurance coverage are more likely to choose eSET, possibly because of less personal financial risk to patients with coverage.[130,133]

Patient education is a powerful tool to increase the acceptability of eSET. Many patients are undereducated as to the risks and complications associated with multiples and elect to have more than one embryo transferred because of a desire for twins. In studies of patient preference, the majority of couples with infertility reported a preference for having twins to having one child at a time.[141,142] It is incumbent upon providers to explain the multitude of reasons that one healthy liveborn infant is the goal of every IVF cycle. Educational programs targeted to improve attitudes toward SET have shown some success.[143,144]

Strategies for reducing multiples caused by non-IVF infertility therapy should also be considered. As previously discussed, ovulation induction and superovulation contribute to nearly 20% of twin births in the United States.[3] During superovulation cycles with gonadotropin stimulation, follicular growth is monitored with ultrasound, and E2 levels are serially measured in an attempt to minimize multifollicular development. Overall, the risk of multiples correlates with younger age, increasing E2 levels, and the size and number of follicles.[77,145,146] However, attempts to define thresholds at which couples are at low risk for multiples have been unsuccessful.[146–148] A strategy that has shown promise is the use of "low and slow" protocols, using low-dose gonadotropin stimulation over a longer period of time. Studies examining these protocols have documented multiple rates of 5% or less.[149–151] Lastly, some have advocated for the elimination of superovulation as an intermediary step between oral ovulation induction and IVF, given the associated high rate of multiples and HOMs, and this has been adopted as a cost-effective strategy by some health plans.[8,152]

Multifetal Pregnancy Reduction

As the rates of ART procedures, multiple pregnancies, and prematurity-related sequelae have increased, so has the use of selective reduction. Primary prevention of multiple gestations is optimal; however, in reality, multifetal pregnancies continue to occur. Multifetal pregnancy reduction (MFPR) provides an option to reduce the risk of fetal or neonatal morbidity and mortality.[55,77] First developed in the 1980s, termination of one or more fetuses is performed to reduce the final fetal number. The procedure most often involves passage of a needle transabdominally into the thorax of the fetus and subsequent injection of potassium chloride.[153] Selective termination refers to reduction of an abnormal fetus that is part of a multifetal gestation. The majority of patients choose to reduce a triplet or HOM pregnancy to twins, but the number reducing to singletons is increasing with heightened awareness of the morbidity associated with twins.[153,154] Improvements in MFPR techniques have enhanced success rates such that quadruplets or triplets reduced to twins have comparable outcomes to natural twins.[153] Studies have shown that pregnancies reduced from triplets to twins deliver at higher gestational ages and have lower rates of miscarriage, PTL, gestational diabetes, LBW, cesarean delivery, and neonatal death compared to nonreduced triplet pregnancies.[155–157] The average pregnancy loss rate currently quoted as a complication of MFPR is about 4%, down from 13% in the 1990s.[153,154] It is important to consider that MFPR is not without medical and psychological risks, and the ethical framework of each individual will guide decision making.[158] A discussion regarding the ethical and medical implications of MFPR should be undertaken with all patients prior to undergoing ART.[55]

Summary

Over the last three decades, advances in ART have helped countless infertile couples achieve parenthood. This has had the unintended consequence of causing an epidemic of multiple births, which are associated with maternal, fetal, and neonatal morbidity and mortality. The subsequent psychosocial and economic costs are significant. Births attributable to ART are likely to continue to increase with improvements in both effectiveness and accessibility, and strategies aimed at primary prevention of multiples should be a major focus going forward.

Acknowledgment

The contributions of Kerri Marquard, Kelle Moley, and Darcy Broughton to the previous edition of this chapter are gratefully acknowledged.

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6

Prematurity and Stillbirth: Causes and Prevention

JULIA JOHNSON AND MANEESH BATRA

KEY POINTS

- Preterm birth, defined as birth prior to 37 weeks gestation, is the leading cause of under-five child mortality worldwide.
- Preterm birth can be spontaneous or medically indicated, in which cesarean delivery or induction of labor is performed to avoid the risks of continued pregnancy.
- Preterm birth and stillbirth should be considered as occurring on a spectrum rather than distinct entities, due to overlapping physiology and prevention strategies.
- Significant disparities in preterm birth and stillbirth exist by race reflective of long-standing effects of structural racism.
- Preterm birth and stillbirth have been associated with multiple risk factors, including demographic, environmental, genetic, placental, nutritional, microbial, and infectious factors.
- Further study of these factors and their associated pathways may lead to discovery of more direct ways to control the biology of pregnancy and, ultimately, the prevention of preterm birth and stillbirth.
- Global efforts to reduce preterm birth and stillbirth are critical for improving perinatal and child mortality globally and will require continued sustained and coordinated efforts.

Preterm Birth and Stillbirth: Burden in the United States and Global Estimates

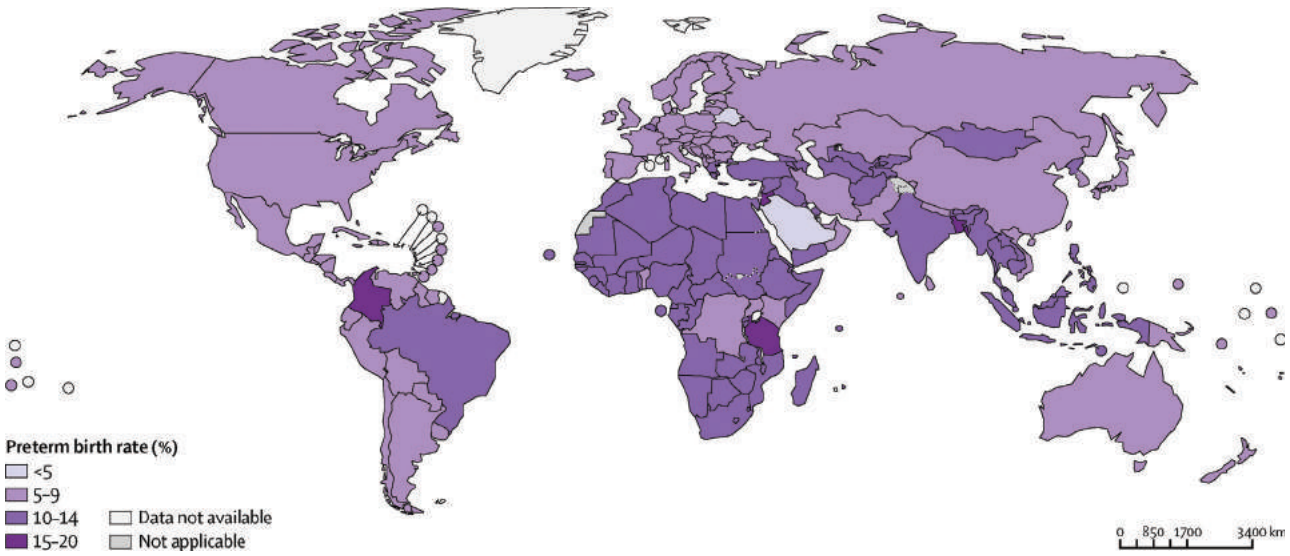
Preterm birth, defined as birth prior to 37 weeks gestation, affects 15 million newborns globally every year.[1] It is the leading cause of under-five child mortality worldwide and accounts for one million deaths annually.[2] More than 10% of newborns in the United States are born preterm, the annual cost of which exceeds $26 billion.[3,4] Globally, among survivors, preterm birth is a major cause of disability-adjusted life years due to lifelong neurologic and developmental sequelae.[5] The rate of preterm birth in the United States increased dramatically in the late 20th century from less than 7% in the 1960s to a peak of 12.8% in 2006; the preterm birth rate was 10.2% in 2019.[4] Globally, the preterm birth rate was estimated at 10.6% in 2014, though significant regional and country variation exists (Fig. 6.1).[1]

In the United States, stillbirth is typically defined as a fetal death at 20 weeks gestation or later or at a birth weight greater than or equal to 350 g. Estimates of stillbirth in the United States are complicated by inconsistent reporting criteria by state, with variable gestational age and birth weight cutoffs used. In 2019, there were 21,478 stillbirths reported in the United States via the National Vital Statistics System; annual stillbirths in the United States exceed the number of neonatal deaths.[6] Comparison of stillbirth rates across countries and regions are challenging given differential reporting mechanisms and metrics and difficulty differentiating between fresh stillbirths or fetal deaths during labor and delivery and early neonatal deaths in very low resource settings.[7,8] The World Health Organization (WHO) definition of stillbirth incorporates both early fetal deaths (500 to 999 g or 22 0/7 to 27 6/7 weeks) and late fetal deaths (≥1000 grams or ≥28 0/7 weeks; Fig. 6.2).[7,9] In 2020, the United Nations Inter-agency Group for Child Mortality Estimation released its first-ever report on the global burden of stillbirths, reporting an estimated 2 million late fetal deaths globally in 2019.[8] Approximately half of stillbirths occur in six countries: India, Pakistan, Nigeria, the Democratic Republic of the Congo, China, and Ethiopia; 27% of global stillbirths occur in low income countries, which account for only 16% of the world's live births.[8]

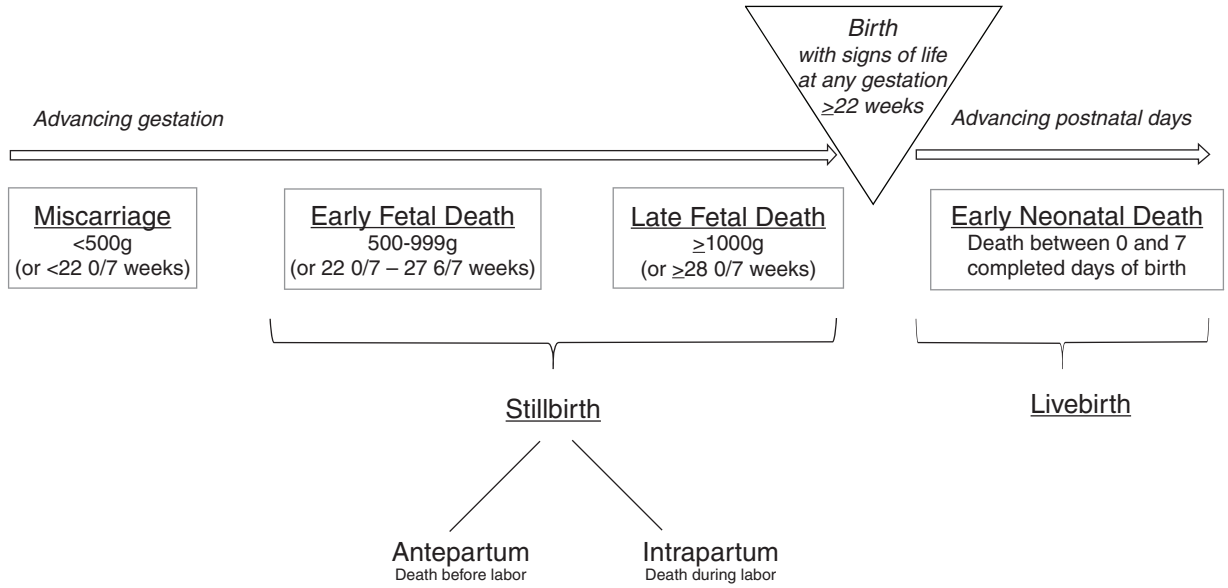
Preterm birth and stillbirth should not be considered distinct entities but rather as occurring along the same spectrum, both in terms of overlapping pathophysiology and targets for prevention. This is particularly true in the case of fresh stillbirths, a significant proportion of which could be averted with improved access to care and earlier obstetric intervention. Approximately 40% of stillbirths worldwide occur during labor (Fig. 6.3).[8]

Pathophysiology of Preterm Birth and Stillbirth

Preterm birth can be spontaneous, in which labor starts too soon, or medically indicated, in which cesarean delivery or induction of labor is performed to avoid the risks of continued pregnancy to the mother or fetus, e.g., due to preeclampsia or intrauterine growth restriction. Another subtype of spontaneous preterm birth is associated with premature preterm rupture of membranes (PPROM), in which rupture of amniotic membranes occurs both prior to 37 weeks gestation and prior to the onset of labor (Fig. 6.3).[10] Approximately 45% of preterm births result from spontaneous preterm labor, 30% from medically indicated delivery, and 25% from PPROM, though recent data suggest a rise in



• **Fig. 6.1** Map Representing Country-Level Preterm Birth Rates in 2014. (From Chawanpaiboon S, Vogel JP, Moller AB, et al. Global, regional, and national estimates of levels of preterm birth in 2014: a systematic review and modelling analysis. *Lancet Glob Health*. 2019;7[1]:e37–e46.)



• **Fig. 6.2** Classification of fetal and neonatal mortality based on timing of death, World Health Organization recommendations. (From Patterson JK, Aziz A, Bauserman MS, et al. Challenges in classification and assignment of causes of stillbirths in low- and lower middle-income countries. *Semin Perinatol*. 2019;43:308–314.)

medically indicated preterm deliveries.[10–12] The extent to which the etiologic pathways leading to each type of preterm delivery are distinct versus overlapping is uncertain. Etiologic differences also exist between early preterm birth (e.g., <28 weeks gestation) and late preterm birth, although the definition of "early" versus "late" preterm birth is somewhat arbitrary in the context of continuous gestational progression.

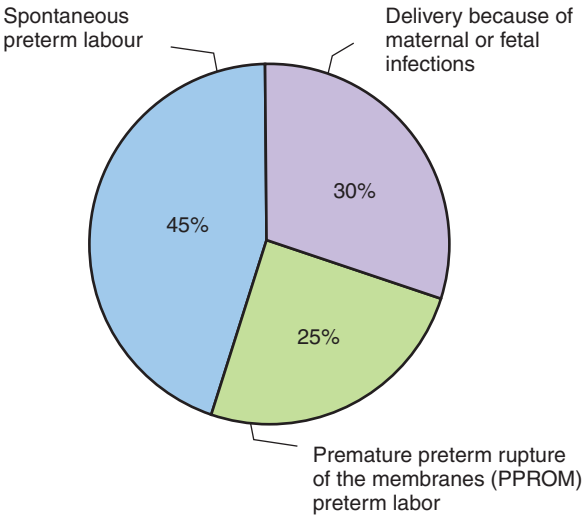
Spontaneous preterm birth is an exceedingly complex phenomenon that is not well understood. Physiologic or disease processes that induce uterine contractility, cervical dilatation, or rupture of amniotic membranes prior to 37 weeks gestation can lead to preterm birth (Fig. 6.4).[13,14] There is evidence that spontaneous preterm birth may be related to perturbations in the maternal-fetal environment, such as abnormal placental development, infection, uterine overdistension, and inflammation. While the majority of preterm births remain unexplained, the biology underlying disease states that contribute to preterm birth is beginning to be elucidated.[15–19]

Stillbirth, preterm delivery, and growth restriction exist along a spectrum, with overlapping etiologies. Fetal growth restriction is a marker of placental insufficiency, which is often implicated in both stillbirth and preterm delivery (Fig. 6.5).[20,21] Other common clinical antecedents of stillbirth and preterm delivery include spontaneous preterm labor or rupture of membranes and infection. Increasingly, inflammation is implicated as a common pathway contributing to stillbirth and preterm birth.[18,22–25] However,

approximately 40% of stillbirths are unexplained.[20] Further understanding of gestational biology and preterm birth will likely provide insight into the etiology of stillbirth. More frequent inclusion of stillbirth in studies of preterm delivery—especially early preterm delivery—will be important to fully understanding either outcome.

Demographic Factors and Disparities

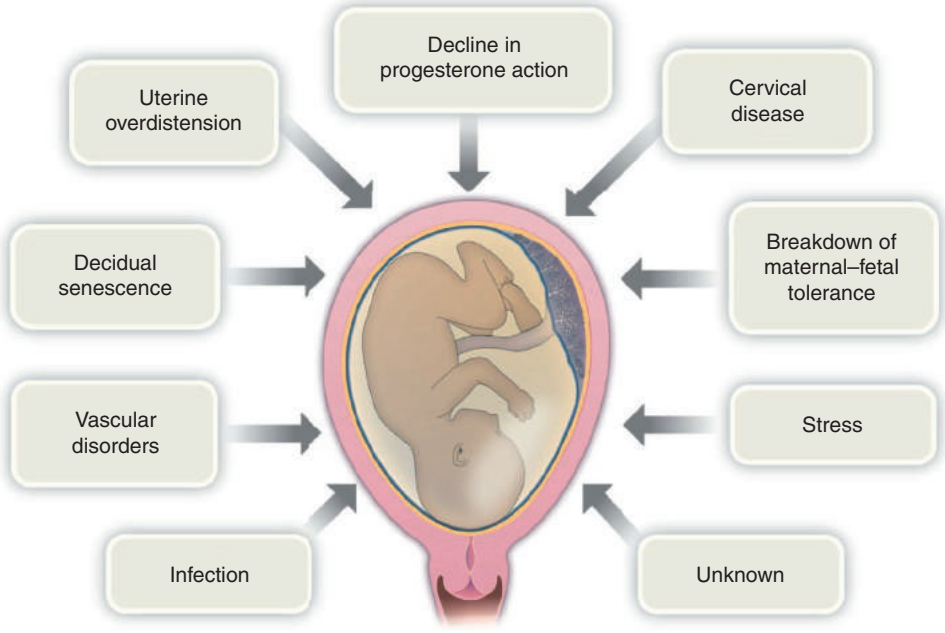
Multiple studies have demonstrated elevated risk of preterm birth among black women. The rate of preterm birth among non-Hispanic black women is as high as 14.4%, compared with 10.2% in the general United States population.[4] From 2015 to 2017, preterm births in the United States were most common among non-Hispanic black mothers and non-Hispanic black fathers and least common among non-Hispanic white mothers and non-Hispanic white fathers (10.8% vs. 6.5%).[26] Such disparities are augmented in early preterm birth (<28 weeks gestation), with preterm birth rates up to three to four times higher among black women.[26–28] Elevated risk remains after controlling for socioeconomic status and psychosocial risk factors, which themselves are associated with preterm birth.[11,26,29–31] Disparities are more modest to nonexistent for Hispanic women, despite social disadvantage within this group.[10] Other studies have found that the rate of preterm birth varies greatly based on geographic location and is driven by socioeconomic and demographic characteristics of those locales but possibly less so for blacks than whites.[32–34] Other factors associated with lower socioeconomic status, including educational background, extremes of maternal age, short interval between pregnancies (<6 months), and single marital status, have been independently associated with preterm birth.[35–41]



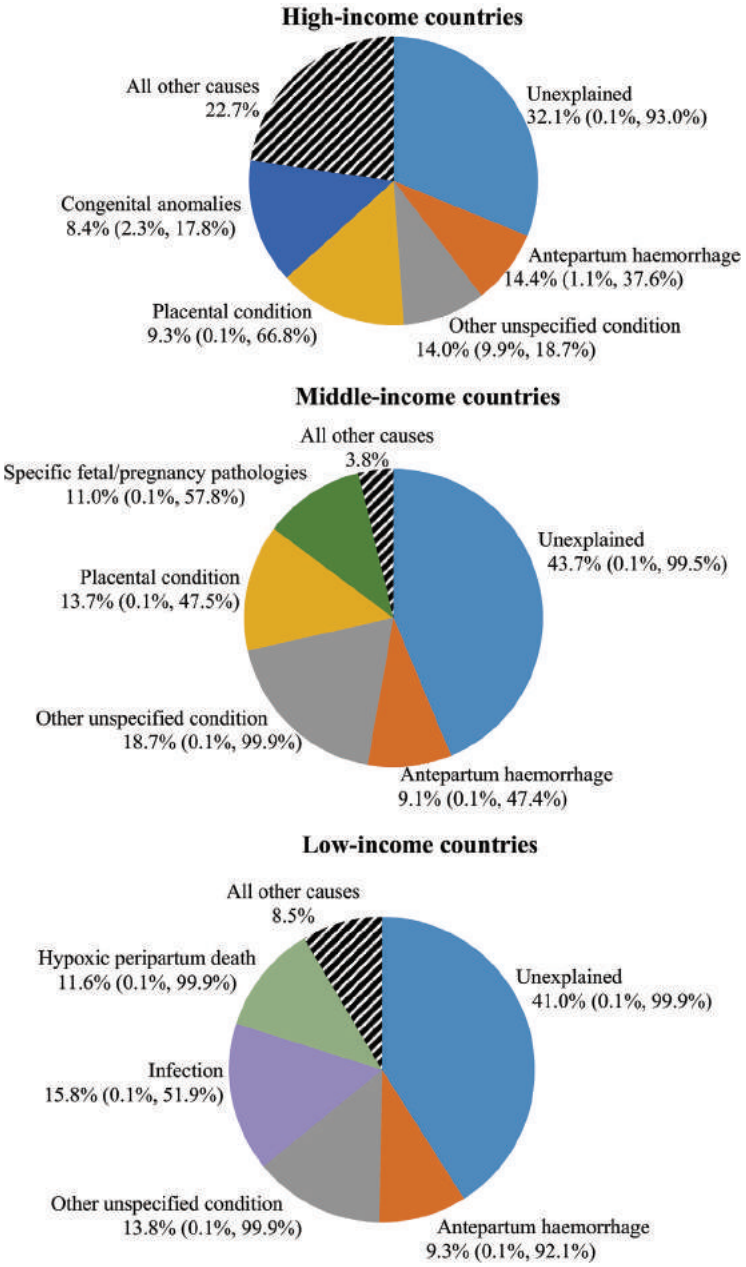
• **Fig. 6.3** Obstetric Precursors of Preterm Birth. (From Goldenberg RL, Culhane JF, Iams JD, Romero R. Epidemiology and causes of preterm birth. *Lancet*. 2008;371:75–84.)

Some portion of the racial/ethnic and socioeconomic disparities in preterm birth can be explained by increased psychosocial stress among these populations.[42,43] Several studies have found that maternal stress, as measured by a survey of life events and/or biomarkers of activation of the hypothalamic-pituitary-adrenal axis, is also strongly associated with preterm birth.[44–46] However, interventions to reduce stress have had limited success in preventing preterm birth.[47] Other factors proposed to contribute to disparities include nutrient deficiencies and infection, although further research is needed to clarify their roles.[48,49]

The longstanding disparities in preterm birth and perinatal outcomes which have been observed and studied over the last several decades have illuminated the role of structural racism on equity in health and health outcomes. While "race" has been considered in medical research as a demographic characteristic, it should be noted that "race" is not a biological construct per se but rather a sociopolitical construct that reflects the impacts of generational oppression, discrimination, and racism.[50] Maternal exposure to interpersonal racism has been associated with preterm



• **Fig. 6.4** Proposed Mechanisms of Disease Implicated in Spontaneous Preterm Labor. (From Romero R, Dey SK, Fisher SJ. Preterm labor: one syndrome, many causes. *Science*. 2014;345:760–765.)



• **Fig. 6.5** Causes of stillbirth, top five pooled estimates from representative country reports by income setting. (From Reinebrant HE, Leisher SH, Coory M, et al. Making stillbirths visible: a systematic review of globally reported causes of stillbirth. *BJOG*. 2018;125:212–224.)

birth rates and very low birth weight, and further, chronic "worry" about racial discrimination among African-American women was associated with elevated preterm birth rates.[51–53] The effect of structural racism on birth outcomes can also be gleaned from studies of preterm birth disparities by region. For example, in the United States, states with higher rates of police killings among unarmed black people were observed to have higher disparities in preterm birth rates.[54] Generational effects of racism, discrimination and oppression have affected economic mobility, housing security, access to education, exposure to stress and violence, environmental exposures, access to healthcare, quality of healthcare, and other factors which have been associated with adverse perinatal outcomes.[55–61]

Environmental Factors

Exposure to toxins—both self-inflicted and environmental—has been shown to increase the risk of preterm birth. Tobacco smoking during pregnancy is one of the most significant of these exposures.[62,63] The mechanism is unclear but may involve systemic inflammation (leading to spontaneous preterm birth) or placental vasoconstriction from nicotine causing fetal growth restriction, which, in turn, may lead to a medically indicated preterm birth.[10] Environmental tobacco smoke exposure has also been linked to adverse birth outcomes, including preterm birth.[64,65] Electronic nicotine delivery systems have been insufficiently studied in pregnancy, though increased risk of preterm

birth was described in electronic cigarette users participating in the United States Pregnancy Risk Assessment Monitoring System, a large nationally representative surveillance program for adverse pregnancy outcomes.[66] Marijuana use during pregnancy has been linked to preterm birth, including when used simultaneously with tobacco.[67–69]

Alcohol has not been consistently linked to risk of preterm birth, although cocaine and other illicit substances increase preterm birth risk, likely via alteration of placental blood flow and/or placental abruption.[70–72] Polysubstance use, including concurrent tobacco smoking and use of either stimulant or depressant drugs, is associated with preterm birth.[72,73]

Caffeine use during pregnancy has been associated with stillbirth in several observational studies; findings regarding its association with preterm birth have been inconsistent.[74] Higher levels of caffeine use are more frequently associated with these adverse pregnancy outcomes, suggesting possible causation.[74] American College of Obstetrics guidance, most recently reaffirmed in 2020, states that moderate caffeine consumption–less than 200 mg/day–is safe in pregnancy.[75]

Environmental air pollution has been associated with increased risk of preterm birth. There are myriad air pollutants that have been investigated, including ozone, carbon monoxide, sulfur dioxide, and lead, among others.[44,76–78] Both cumulative air pollution over the course of the pregnancy and during the week prior to delivery have been linked to increased risk of preterm birth.[78,79]

Exposure to other toxicants, including various pesticides and heavy metals, such as lead and cadmium, has also been associated with preterm birth.[80–82] Heavy metals can induce oxidative stress and inflammation in the placenta, thereby increasing risk of preterm birth.[82] Per- and polyfluoroalkyl substances (PFAS), which can be found in food packaging, commercial household products, and drinking water, have been linked to disruption of placental vasculogenesis and a proinflammatory state, though association with increased preterm birth has not been consistently demonstrated in studies to date.[83] Further studies are needed to examine the relationship between these exposures and risk of preterm birth and other adverse pregnancy outcomes.

Nutrition and Maternal Body Weight

Obesity, defined as body mass index (BMI) greater than or equal to 30 kg/m^2, is a major global health problem, affecting hundreds of millions of people worldwide. Prepregnancy obesity is a known risk factor for preterm birth.[84–88] Comorbidities associated with obesity, such as diabetes and hypertension, may contribute to this obesity-preterm birth association.[89–91] The biologic mechanisms behind the association may involve pathologic inflammation and altered vascular development.[92–95] Exercise during pregnancy for women who are overweight or with prepregnancy obesity is associated with a lowering of the risk of preterm birth and gestational diabetes.[96] Low maternal BMI is also a risk factor for spontaneous preterm birth.[97] Poor nutrient intake is associated with both low and high BMI and serves as one potential explanation for their association with preterm birth. Iron deficiency anemia, lower intakes of nutrients, such as folic acid and other B vitamins, zinc, calcium, magnesium, and polyunsaturated fatty acids, and higher dietary glycemic index have all also been associated with an increased risk.[33,48,98]

Infection and Microbiota

More than one-fourth of preterm births can be attributed to intra-amniotic infection, in which microorganisms perturb maternal-fetal homeostasis and trigger a cascade of biologic signals that initiate spontaneous preterm labor (PPROM) or fetal distress that leads to indicated preterm delivery.[99–103] Microorganisms and their immunogenic cellular components activate toll-like receptors, chemokines, and cytokines, which tips the balance of maternal-fetal tolerance and leads to uterine contractility, rupture of amniotic membranes, and preterm delivery.[13,104] Activation of the intra-amniotic, inflammasome, cytoplasmic, multiprotein complexes responsible for the inflammatory response has been implicated in both preterm labor with intact membranes and PPROM and is associated with intraamniotic microbial burden.[105–108]

The role of microorganisms in preterm birth is often subclinical, in which there are no signs or symptoms of overt maternal infection. One study showed that more than one fifth of preterm newborns have umbilical cords colonized by *Mycoplasma* spp.[109] *Ureaplasma* spp. have been isolated in the cervical fluid of women with preterm labor and confirmed intraamniotic infection as well as those without infection but with documented, sterile, intraamniotic inflammation.[110] Asymptomatic bacterial vaginosis was described as a risk factor for preterm birth in a 2007 metaanalysis, although treatment of bacterial vaginosis has not been shown to reduce risk of preterm delivery.[111,112] Asymptomatic vaginal *Candida* spp. colonization has not been associated with preterm birth.[113]

Sexually transmitted infections that have been associated with increased risk of preterm birth include chlamydia, gonorrhea, syphilis, and new human immunodeficiency virus (HIV) infection.[114–117] Urinary tract infections are a known risk factor for preterm birth.[118] Additionally, treatment of asymptomatic bacteriuria during pregnancy is associated with decreased risk of subsequent preterm birth; screening for asymptomatic bacteriuria in pregnancy thus is recommended in the United States.[119,120] Periodontal disease also increases the risk of preterm birth.[121,122] Unfortunately, treatment of periodontal infections does not reduce the rate of preterm birth.[123–125]

Infection has also been implicated in stillbirth. In a prospective population-based cohort study in the United States, infection was a probable or possible cause in 12.9% of stillbirths, with *Escherichia coli*, GBS, and *Enterococcus* spp. among the identified pathogens.[126] These bacterial pathogens were also associated with stillbirth in the context of fetal infection in a South African study.[127] Historically, listeriosis has been associated with stillbirth, although incidence of pregnancy-related listeriosis is declining in the United States and other high-income countries.[128,129] Syphilis rates during pregnancy, on the other hand, are on the rise in the United States, and maternal syphilis may increase the odds of stillbirth as much as five-fold.[115]

There is also evidence that the microbiome, and perturbations therein, plays an important role in maternal-fetal tolerance and preterm birth.[130–134] In particular, reduced prevalence of vaginal *Lactobacillus* spp. and resultant vaginal dysbiosis are associated with increased risk of preterm birth.[17,135] Recent advances have enabled sequencing and amplification of deoxyribonucleic acid (DNA) from myriad microbial communities, revealing complex potential interactions between these microbial communities and

clinical pregnancy outcomes.[17] Study of microbial communities during pregnancy, and microbiota in general, represents an important and exciting frontier in the effort to further our understanding of preterm birth.

SARS-CoV-2 Infection in Pregnancy

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection in pregnancy deserves special consideration. Other betacoronaviruses responsible for Middle East respiratory syndrome (MERS) and SARS were associated with adverse pregnancy outcomes, including fetal demise and preterm birth, although there are limited reports of these infections in pregnancy and no documented vertical transmission.[136,137] While vertical transmission of SARS-CoV-2 appears to be a rare outcome of maternal coronavirus disease 2019 (COVID-19), cases of both preterm birth and stillbirth with positive SARS-CoV-2 polymerase chain reaction (PCR) testing of the placenta, amniotic fluid, and cord blood have been described.[138] A multi-country cohort study revealed increased risk of preterm birth, including medically indicated preterm birth, among pregnant women with COVID-19 when compared to noninfected pregnant women controls.[139] Stillbirth, including in the context of maternal critical illness, has been reported with COVID-19, although it is unclear how stillbirth rates due to COVID-19 compare to the population incidence of stillbirth.[138] The broader impact of the COVID-19 pandemic on pregnant women, including on population-level preterm birth rates, continues to be explored, with some reports of decreased preterm birth rates and others suggesting no effect. [140–142]

Genetic Factors

Preterm birth tends to run in families and appears to be inherited across generations.[143,144] The heritability of preterm birth has been estimated to be 15% to 40%.[15,145] Women with a prior history of preterm birth have more than a twofold increased risk of preterm birth in the subsequent pregnancy, although environmental factors may also explain this finding.[146]

Advances in our ability to conduct genome-wide association studies—and to do so in a cost-effective manner—have led to increased interest in the study of single-nucleotide polymorphisms. Several large genome-wide association studies were recently conducted and found no definitive evidence of association between preterm birth and any one single nucleotide polymorphism.[147–151] However, additional analyses suggest that gene-gene interactions, particularly those related to cell motility and migration, glucocorticoid response, inflammation, and metabolic disorders, may affect the risk of preterm birth.[147,150,151] Several candidate genes have been identified by other investigators, but there are many inconsistencies in the data and findings.[152–155]

These inconsistencies may arise from the complex gene-gene and gene-environment interactions related to preterm labor and/or delivery.[149,155–157] One study found an association between a tumor necrosis factor-alpha allele, bacterial vaginosis, and preterm birth, although neither maternal carrier status nor bacterial vaginosis alone was associated with preterm birth.[158] Similar interactions with bacterial vaginosis were reported in a study on maternal carrier status of an interleukin-6 allele among black women.[153] Genomic studies exploring preterm birth lag behind progress for many other multifactorial diseases.[159] As we refine our ability to conduct genetic and epigenetic research and to do so more affordably, we will continue to take incremental steps toward solving the puzzle of preterm birth.

Placental and Pregnancy Factors

Several factors associated with disruption of the uterine environment during pregnancy have been associated with preterm birth.[14] Abnormal vascular development of the placenta is a frequent occurrence among women who deliver before term.[160,161] Abnormal placental villous maturation has been implicated in spontaneous preterm labor.[162] Perturbations of placentation may also account for many other adverse pregnancy outcomes that are seen in clinical practice and often cooccur with preterm birth, such as intrauterine growth restriction and preeclampsia.[163] Vaginal bleeding, isolated or associated with placental abruption or placenta previa, also carries increased risk of preterm delivery.[164]

Overdistension of the uterus, caused by multiple gestation or polyhydramnios, substantially increases the risk of preterm birth.[165–168] More than half of twin pregnancies result in delivery prior to 37 weeks gestation, likely because of mechanical distension from increased fetal mass and other comorbidities associated with multiple gestation. Among twin pregnancies, monochorionicity is a risk factor for preterm birth, as is twin-to-twin transfusion syndrome.[169,170] Anatomic abnormalities of the uterus and previous uterine or cervical surgical intervention also increase the risk of preterm birth.[171,172] Likewise, short cervix and cervical insufficiency carry increased risk, which varies greatly depending on degree of shortening.[173,174]

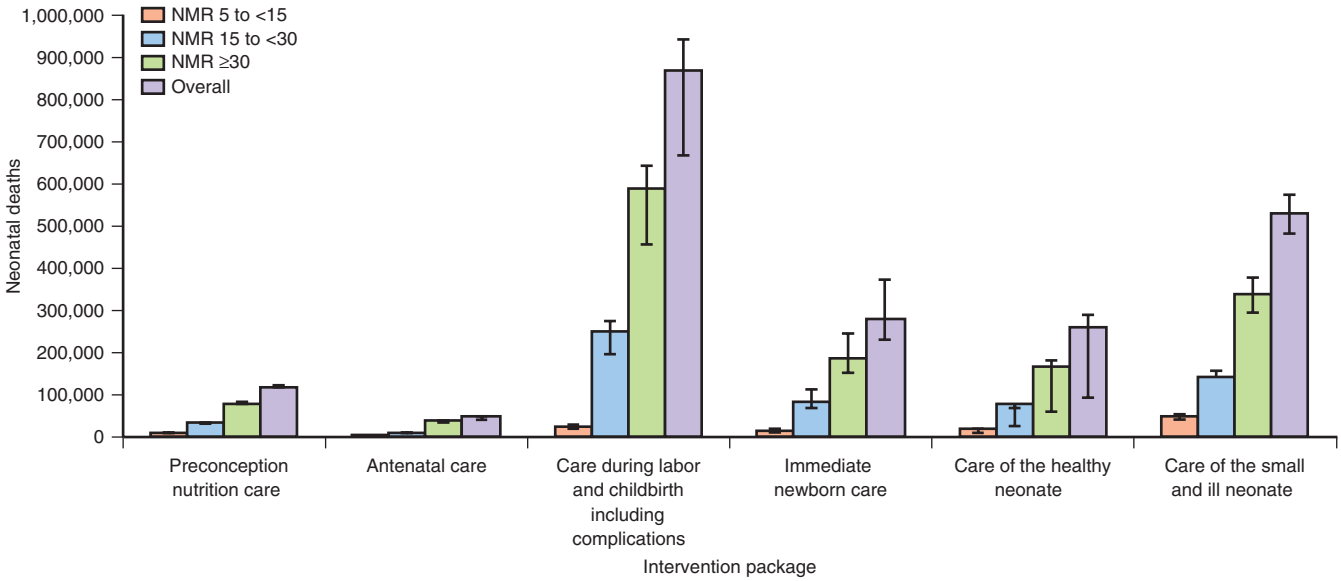
Prevention of Preterm Birth

Efforts to prevent—and predict—preterm birth have largely failed, although several targeted interventions, including low-dose aspirin and 17-hydroxyprogesterone, have been shown to reduce the risk of preterm birth among women at high risk for adverse pregnancy outcomes, such as women with a personal history of preterm birth or stillbirth.[175–179] Use of cervical cerclage among patients with short cervix and a history of preterm birth has effectively reduced the risk of preterm birth among this small subset of women.[179–181] Population-level interventions that address underlying disparities, such as improving access to prenatal care or other public policies related to smoking cessation or environmental pollution, may reduce the overall rate of preterm birth.[182] A recent systematic review of interventions during pregnancy to prevent preterm birth identified four interventions with clear evidence of benefit: midwife-led continuity models of care versus other models of care for all women; screening for lower genital tract infections for pregnant women less than 37 weeks gestation and without signs of labor, bleeding or infection; and zinc supplementation for pregnant women without systemic illness.[183]

Other obstetrical interventions have been successful at mitigating the neonatal morbidity and mortality associated with preterm birth, such as antibiotics, antenatal betamethasone, magnesium sulfate, and tocolytic agents, but these interventions rarely prevent delivery prior to 37 weeks gestation.

Over the last 25 years, considerable global attention has emerged on the plight of newborns in the world, starting with the inclusion of targets to reduce child mortality in the Millennium Development Goals (MDGs) in 2000 and more explicit targets for newborn mortality reduction in the Sustainable Development Goals in 2015. In addition to providing a "north star" for health

and development until 2015, the MDGs were successful at leveraging the attention, political will, and resources of the global community, nations, regions, researchers, aspiring innovators, and initiative leaders, all working toward a common goal. The Sustainable Development Goals (SDGs) were built on the successes and lessons learned from the MDGs and represented a new era of global health and development. While the SDGs are much more comprehensive, holistic, and ambitious and extend beyond the health and development sectors into a more integrated whole, some critics are challenged by the differences in their design, context, concept, scope, significance, targets, and approach. In 2016, a large array of countries and their newborn stakeholders, including Ministries of Health and Finance, academics, programmers, NGOs, funders, policymakers, clinicians, and, most importantly, civil society groups came together to develop and launch the Every Newborn Action Plan that galvanized efforts to reduce both neonatal mortality and stillbirths globally (Box 6.1).[184] Figure 6.6 outlines the estimated effect of intervention packages in 2014 on numbers of neonatal lives saved and the costs according to levels of care by the year 2025, with available interventions estimated to reduce deaths due to prematurity by 58% and prevent approximately 550,000 stillbirths.[185] While prevention of preterm birth



	Preconception nutrition care	Antenatal care	Care during labor and childbirth including complications	Immediate newborn care	Care of the healthy neonate	Care of the small and ill neonate
Estimated maternal lives saved by 2025	0	7500 (6700–9100)	150,000 (137,100–158,900)	NA	NA	NA
Estimated stillbirths prevented by 2025	23,000 (17,800–26,500)	240,000 (150,800–374,500)	550,000 (432,500–531,400)	NA	NA	NA
Estimated neonatal lives saved by 2025	110,000 (111,800–118,300)	43,000 (36,500–46,900)	790,000 (588,500–865,000)	190,000 (136,300–280,400)	230,000 (64,300–261,500)	580,000 (531,000–621,000)
Estimated additional child lives (postneonatal) saved by 2025	7500	1200	0	0	1900	0
Costs by 2025 (in billion US$)	1.88	0.4	2.29	0.035	0.11	0.96
Costs (billion US$) per 100,000 maternal and newborn lives and stillbirths saved	1.38	0.16	0.15	0.02	0.05	0.17
Costs (billion US$) per 100,000 newborn babies saved	1.65	0.92	0.29	0.02	0.05	0.17

• **Fig. 6.6** Estimated effect of the intervention packages on numbers of neonatal lives saved and costs according to levels of care by the year 2025. Error bars represent ranges. *NA*, Not applicable; *NMR*, neonatal mortality rate. (From Bhutta ZA, Das JK, Bahl R, et al. Can available interventions end preventable deaths in mothers, newborn babies, and stillbirths, and at what cost? *Lancet*. 2014;384:347–370.)

• BOX 6.1 The Every Newborn Action Plan

Vision

A world where there are no preventable deaths of newborns or stillbirths, where every pregnancy is wanted, every birth celebrated, and women, babies, and children survive, thrive, and reach their full potential.

Every Newborn Action Plan Goals and Targets

- End preventable newborn death: by 2035, all countries should have a neonatal mortality rate of 10 or less per 1000 live births
- End preventable stillbirth: by 2035, all countries should have stillbirth rates of 10 or less per 1000 total births
- A global neonatal mortality rate of 7 deaths per 1000 live births by 2035

Strategic Objectives

- Strengthen and invest in care during labor, childbirth, and the first day and week of life
- Improve the quality of maternal and newborn care
- Reach every woman and every newborn to reduce inequities
- Harness the power of parents, families, and communities
- Count every newborn: measurement, program tracking, and accountability

Guiding Principles

- Country leadership
- Integration
- Equity
- Accountability
- Innovation

and mortality attributable to preterm birth are not specific targets of global efforts, it is clear that targeted reductions in child and neonatal mortality cannot be achieved without specific focus on improving survival among preterm newborns. While *prevention* of premature delivery by a mother remains somewhat elusive and continues to attract ongoing research attention primarily in high-income countries, *management* of premature babies in all settings, particularly low- and middle-income countries, has received increasing attention over the past 15 years.

There are numerous inequities that must be combated in the global struggle to improve newborn health. In general, regions that have the highest neonatal mortality rates also have the lowest healthcare worker density.[186] Three-quarters of the world's physicians are working in high-income countries, serving approximately one-third of the world's population. Further analysis suggests that in Africa, 3% of the world's healthcare workforce has 1% of the world's expenditure on health to tackle approximately 24% of the global burden of disease.[187] Without a clear and concerted effort to address these long-standing inequities, the disparities in perinatal outcomes globally will persist.

Summary

Further study of the causal pathways of preterm birth and stillbirth, many of which are shared, may lead to discovery of more direct ways to control the biology of pregnancy. Preliminary evidence already exists for effective prevention of preterm birth through interference with some of these pathways.[177] Future interventions may take the form of targeted pharmaceuticals or community-based interventions that indirectly affect the biology, such as implementation of policies related to socioeconomic disparities or nutrition. Understanding when and where we are able to intervene to prevent preterm birth will require collaboration on large and transdisciplinary scales with concomitant integration of data from multiple levels of inquiry.[188] In particular, continued progress in prevention of preterm birth and stillbirth in low resource settings will require sustained investment in building capacity for maternal and child health on a global scale.

Suggested Readings

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7

Nonimmune Hydrops

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KEY POINTS

- Nonimmune hydrops fetalis results from many etiologies.
- Management involves correcting the underlying cause.
- The condition is associated with significant morbidity and mortality.
- Some etiologies are amenable to treatment in utero.

A newborn with hydrops has an abnormal accumulation of fluid. The condition varies from mild, generalized edema to massive anasarca with effusions in multiple body cavities and with peripheral edema so severe that the extremities are fixed in extension. Fetuses with severe hydrops may die in utero; if liveborn, they may die in the neonatal period from the severity of their underlying disease or from severe cardiorespiratory failure.

The first description of hydrops in a newborn twin may have appeared in 1609.[1] Ballantyne[2] suggested that the finding of hydrops was an outcome from many different causes, in contrast to the belief at that time that hydrops was a single entity. Potter[3] was the first to make the distinction between nonimmune hydrops and hydrops secondary to erythroblastosis fetalis by describing a group of infants with generalized body edema who did not have hepatosplenomegaly or abnormal erythropoiesis. Potter's description of more than 100 cases of hydrops included two sets of twins in which one had hydrops and the other did not, thus presenting the first description of twin–twin transfusion syndrome. With the nearly universal use of anti-D globulin and refinement of the schedule and doses for its administration, the occurrence of immune-mediated hydrops has steadily declined and may be as low as 6% to 10% of all cases of hydrops.[4]

Incidence

Determining the incidence of nonimmune hydrops in the general population is difficult because of biases in the pregnancy cohorts selected, as well as the accuracy of coding for nonimmune hydrops in those pregnancies. Most published studies come from single institutions with the at-risk populations ranging from that of a high-risk pregnancy clinic to infants in a neonatal intensive care unit and generally predating the more routine use of ultrasound investigation in the late first trimester of pregnancy.[5] Incidence rate of hydrops reported in those studies has been highly variable and generally higher than other studies, ranging from 6 per 1000 pregnancies in a high-risk referral clinic in the United Kingdom between 1993 and 1999[6] to 1 in 4000 pregnancies,[7] 6 per 1000 pregnancies,[8] 1.3 per 1000 pregnancies,[9] and 1 per 1700 pregnancies.[10] Two recent publications using population-based data found lower overall rates: a recent publication from Sweden using more than 1.9 million births extracted from the Swedish Birth Register for 1997–2015 found 309 cases of nonimmune hydrops at birth, which corresponded to an incidence of 1.6 per 10,000 births,[11] while data from California identified 1037 cases using ICD-9CM diagnosis codes from 4,090,950 live births, which corresponded to an incidence of 2.5 per 10,000 births between 2005 and 2012.[12] However, these lower rates may suffer from lack of information on fetuses who died in utero and from potential undercoding of the disorder in live birth infants. Geography also affects the incidence; for example, several causes of nonimmune hydrops, such as α-thalassemia, are more common in certain areas of the world. Hence, the actual incidence of nonimmune hydrops likely falls within a wide range of values reported from population and single center-based estimates.

Etiology

Nonimmune hydrops has been associated with a wide range of conditions (see Table 7.1). In many of these conditions, edema formation results from one of the following possible processes:

- Elevated central venous pressure (CVP) in which the cardiac output is less than the rate of venous return
- Anemia, resulting in high-output cardiac failure
- Congenital lymphatic flow disorder
- Capillary leak

The actual pathophysiology of hydrops for many of the conditions in Table 7.1, however, is still not understood.

The most common causes of nonimmune hydrops are chromosomal, lymphatic, cardiovascular, hematologic, thoracic, infectious, and conditions related to twinning.[11–15] As with reported incidence rates, the relative contribution of these causes varies by study. The studies that focus on early fetal presentation of hydrops (postconceptional age of less than 24 weeks' gestation) have found that chromosomal abnormalities, such as Turner syndrome and trisomies 13, 18, and 21, contribute 32% to 78% of all cases of hydrops.[5,6,10,16,17] For fetuses whose hydrops becomes evident after 24 weeks' gestation, cardiovascular and thoracic causes are most prevalent, with rates ranging between 30% and 50%.[17,18] Studies from Asia have noted a higher percentage of cases from hematologic causes, probably because of the higher rates of α-thalassemia in the population.[19,20]

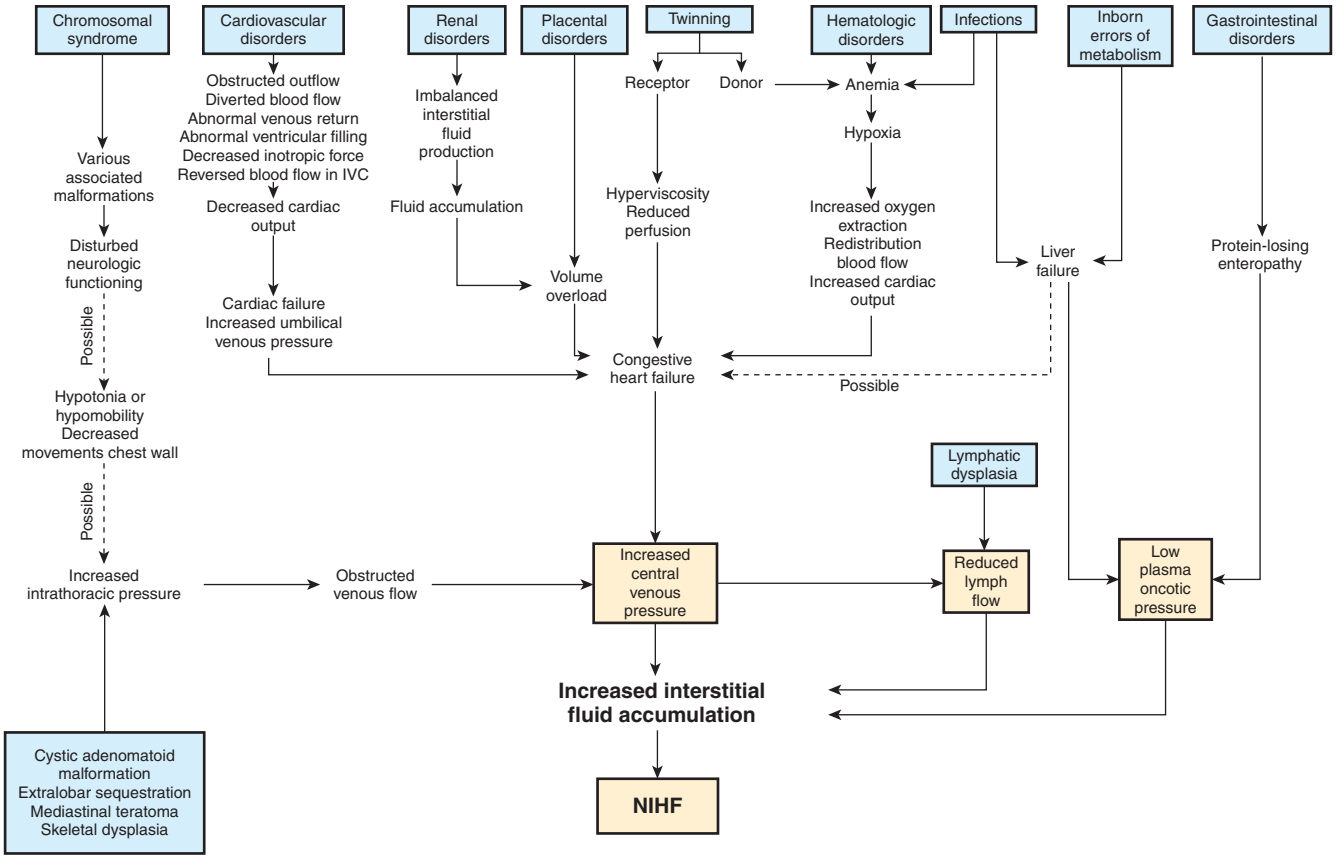
A review of studies published between 2008 and 2013 suggests a potential change in the etiologies of nonimmune hydrops when compared with studies published between 1979 and 2008.[4] These more recent studies reported a higher rate of lymphatic etiologies

TABLE 7.1 Conditions Associated With Hydrops Fetalis

Condition Type	Specific Conditions
Hemolytic anemias	Alloimmune, Rh, Kell, α-chain hemoglobinopathies (homozygous α-thalassemia) Red blood cell enzyme deficiencies (glucose phosphate isomerase deficiency, glucose-6-phosphate dehydrogenase)
Other anemias	Fetomaternal hemorrhage Twin–twin transfusion Diamond–Blackfan
Cardiac conditions	Premature closure of foramen ovale Ebstein anomaly Hypoplastic left or right heart Subaortic stenosis with fibroelastosis Cardiomyopathy, myocardial fibroelastosis Atrioventricular canal Myocarditis Right atrial hemangioma Intracardiac hamartoma or fibroma Tuberous sclerosis with cardiac rhabdomyoma
Cardiac arrhythmias	Supraventricular tachycardia Atrial flutter Congenital heart block
Vascular malformations	Hemangioma of the liver Any large arteriovenous malformation Klippel–Trénaunay syndrome Idiopathic infantile arterial calcification
Vascular accidents	Thrombosis of umbilical vein or inferior vena cava Recipient in twin–twin transfusion
Infections	Cytomegalovirus, congenital hepatitis, human parvovirus, enterovirus, other viruses Toxoplasmosis, Chagas disease Coxsackie virus Syphilis Leptospirosis
Lymphatic abnormalities	Congenital lymphatic dysplasia Lymphatic malformations Lymphangiectasia Cystic hygroma Noonan syndrome Multiple pterygium syndrome Congenital chylothorax Hereditary lymphedema type 1
Nervous system lesions	Absence of corpus callosum Encephalocele Cerebral arteriovenous malformation Intracranial hemorrhage (massive) Holoprosencephaly Fetal akinesia sequence
Pulmonary conditions	Cystic adenomatoid malformation of the lung Mediastinal teratoma Diaphragmatic hernia Lung sequestration syndrome Lymphangiectasia
Renal conditions	Urinary ascites Congenital nephrosis Renal vein thrombosis Invasive processes and storage disorders Tuberous sclerosis Gaucher disease Mucopolysaccharidosis Mucolipidosis
Chromosome abnormalities	Trisomy 13, trisomy 18, trisomy 21 Turner syndrome 46, XX/XY chimerism
Bone diseases	Osteogenesis imperfect Achondroplasia Asphyxiating thoracic dystrophy
Gastrointestinal conditions	Bowel obstruction with perforation and meconium peritonitis Small bowel volvulus Other intestinal obstructions Prune-belly syndrome
Tumors	Neuroblastoma Choriocarcinoma Sacrococcygeal teratoma Hemangioma or other hepatic tumors Congenital leukemia Cardiac tumors Renal tumors
Maternal or placental conditions	Maternal diabetes Maternal therapy with indomethacin Multiple gestation with parasitic fetus Chorioangioma of placenta, chorionic vessels, or umbilical vessels Toxemia Systemic lupus erythematosus
Miscellaneous	Neu–Laxova syndrome Myotonic dystrophy Hereditary lymphedema type 1
Idiopathic	

(15.0% compared with 5.7% in older studies) and a lower rate of thoracic or chromosomal etiologies. Idiopathic hydrops, or hydrops of unknown etiology, remained constant at between 18% and 20% of all cases. As with older studies, papers published between 2008 and 2013 varied widely in the prevalence of specific etiologies depending on the ability of the clinicians to complete their diagnostic evaluation, geography, and the inclusion of fetal deaths in the analysis.* Finally, population-based data from California births between 2005 and 2012 found similar etiological results for live-born infants. Aneuploidy was found in 116 of 1037 infants with nonimmune hydrops (11.2%), lymphatic causes in 9.2% of all cases, and 29.1% of cases with a diagnosis of hydrops of unknown etiology.[12] It is likely that with increased understanding and the availability of genetic testing for many of the conditions listed in Table 7.1, the number of infants diagnosed with idiopathic, nonimmune hydrops will continue to decline.

*References 5, 6, 8–10, 12, 17, 18, 20–23.



• **Fig. 7.1** Impact of Various Etiologies for Nonimmune Hydrops on Fluid Homeostasis. *IVC*, Inferior vena cava; *NIHF*, nonimmune hydrops fetalis. (Modified from Bellini C, Hennekam RCM. Non-immune hydrops fetalis: a short review of etiology and pathophysiology. *Am J Med Genet A*. 2012;158A:597–605.)

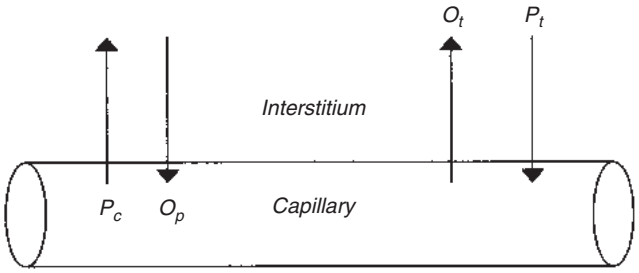
Pathophysiology

Normal Fluid Homeostasis

Abnormal body fluid homeostasis is the underlying cause of edema, whether localized or generalized. To understand the pathogenesis of hydrops, the clinician must consider the forces underlying normal fluid homeostasis that underpin a general pathophysiologic pathway for this disorder (Fig. 7.1) as proposed by Bellini et al.[24] The regulation of net fluid movement across a capillary membrane depends on the Starling forces, which were first described by E. H. Starling.[25] Flow between intravascular and interstitial fluid compartments is determined by the balance among (1) capillary hydrostatic pressure, (2) serum colloid oncotic pressure, (3) interstitial hydrostatic pressure or tissue turgor pressure, and (4) interstitial osmotic pressure, which depends on lymphatic flow. The Starling equation defines the relationship among these forces and their net effect on net fluid movement, or filtration, across a semipermeable membrane (such as the capillary membrane) as:

$$\text{Filtration} = K([P_c - P_t] - R[O_p - O_t])$$

where K = capillary filtration coefficient, representing the extent of permeability of a membrane to water and thus describing capillary integrity; P_c = capillary hydrostatic pressure; P_t = interstitial hydrostatic pressure or tissue turgor pressure; R = reflection coefficient for a solute, representing the extent of permeability of the capillary wall to that solute; O_p = plasma oncotic pressure as determined by plasma proteins and other solutes; and O_t = interstitial osmotic pressure (Fig. 7.2).



• **Fig. 7.2** Starling Forces and Net Effect on Fluid Homeostasis. *Arrows* represent net effect of movement of fluid across the capillary membrane for each factor under normal conditions. P_c, Capillary hydrostatic pressure; P_t, interstitial hydrostatic pressure or tissue turgor pressure; O_p, plasma oncotic pressure as determined by plasma proteins and other solutes; O_t, interstitial osmotic pressure.

Although an abnormality of any of the components of this equation may, in theory, result in the accumulation of edema fluid, the fetal–placental unit presents a unique physiologic condition that effectively eliminates two of the factors, assuming unimpeded fetal–placental flow and an appropriately functioning maternal–placental interface. Because approximately 40% of fetal

cardiac output is allocated to the placenta, there is rapid transport of water between the fetus and mother. Any condition resulting in elevated fetal capillary hydrostatic pressure or low plasma colloid oncotic pressure would likely cause the net flow of water from fetal villi in the placenta to the maternal blood stream where it can be effectively eliminated. This elimination of fluid would counteract the accumulation of interstitial fluid by the fetus. Although the placenta of a fetus with hydrops is also edematous, these changes are believed to occur with, and not before, fetal fluid accumulation.

Derangements in Fluid Homeostasis

Elevated Central Venous Pressure

The most commonly diagnosed causes of nonimmune hydrops that appear in fetuses older than 24 weeks' gestation are cardiac disorders. Any state in which cardiac output is lower than the rate of venous return results in an elevated CVP that raises capillary filtration pressures and, if high enough, restricts lymphatic return. Both of these mechanisms may then contribute to an interstitial accumulation of fluid. Structural cardiac causes of elevated CVP include right-sided obstructive lesions and valvular regurgitation. The most common and easily reversible cause of nonimmune hydrops is supraventricular tachycardia (SVT). In general, cardiac output rises with heart rate. At the increasingly high rates seen in SVT, however, cardiac output plateaus and then diminishes over time. The heart rates observed with SVT are often associated with decreased cardiac output. Impaired cardiac output results in elevated CVP, which can give rise to edema through mechanisms discussed previously.[26]

Infants with alloimmune hydrops (and several of the nonimmune hydropic conditions as well) have significant anemia that may also result in elevated CVP. It has been proposed that anemia leads to congestive heart failure with increased hydrostatic pressure in the capillaries, causing vascular damage that results in edema. However, the hematocrit values of infants with and without hydrops overlap significantly, suggesting that anemia alone is not the only explanation. A rapid decrease in hemoglobin concentration requires increased cardiac output in order to maintain adequate oxygen delivery. This increased cardiac output results in higher oxygen demands by the myocardium, which may be difficult to meet because of the anemia. The hypoxic myocardium can become less contractile and less compliant, with ventricular stiffness causing increased afterload to the atria. High-output congestive heart failure may then develop, resulting in elevated CVP. Raised CVP leads to increased capillary filtration pressures and impaired lymphatic return.[27]

An intrathoracic mass such as a pulmonary malformation may also increase CVP, resulting in impaired filling of the right ventricle and consequent increased capillary hydrostatic pressure. While the underlying reason for the elevated CVP differs between these two conditions, the resulting clinical presentations are similar.

Congenital Lymphatic Flow Disorders

If the rate of fluid filtration from plasma to tissues exceeds the rate of lymph return to the central venous system or if there is obstruction to lymphatic flow from the thoracic duct, then edema and effusions may form. To determine the effects of alterations in CVP on lymphatic return, Gest[28] inserted a catheter into the thoracic duct of fetal lambs and applied an opposing hydrostatic pressure by varying the height of the catheter. Thoracic duct flow was nearly constant over the physiologic range of CVP but sharply decreased at elevated pressures; therefore, lymphatic flow may be reduced or essentially blocked in pathologic states associated with elevated CVP.

Lymphatic causes of hydrops include congenital lymphovenous atresia—in which the thoracic duct outlet is not anatomically connected to the venous circulation—or complete absence of the thoracic duct. Pulmonary lymphangiectasia or pulmonary lymphatic perfusion syndrome may also result in hydrops fetalis if fetal pleural effusions are significant enough to cause mediastinal shift and compression of the heart and lungs.

Recent advances in the radiographic evaluation of the lymphatic system using magnetic resonance imaging (MRI) and lymphangiography have improved the assessment of this system and led to classification of neonatal lymphatic flow disorders.[29] In 1995 Constantin Cope showed that percutaneous transabdominal access of the thoracic duct through the cisterna chyli was not only feasible but led to better opacification of the thoracic duct in cases of chylothorax.[30] This method of imaging the lymphatic system has been used in neonates with congenital lymphatic flow disorders to diagnose abnormalities of the thoracic duct and/or the thoracic duct outlet.

Decreased Oncotic Pressure

A third potential etiology for nonimmune hydrops is hypoalbuminemia. The most convincing evidence for this etiology is in conditions where reduced compliance of a right ventricle may result in flow reversal in the inferior vena cava, which may in turn cause end-organ damage to the liver with consequent hypoalbuminemia and portal hypertension, enhancing formation of both edema and ascites. Hydrops has been produced in fetal lambs[31] in which the hemoglobin content was lowered in 12 fetuses through exchange transfusion using cell-free plasma; six became hydropic. Anemia developed more rapidly with a higher CVP in fetuses with hydrops than in the fetuses without hydrops. In the most severely anemic fetuses, it is probable that decreased oxygen transport causes tissue hypoxia, which in turn increases capillary permeability to both water and protein. These changes in capillary permeability may contribute to the development of hydrops.

However, human fetuses with hypoproteinemia as a result of nephrotic syndrome or analbuminemia rarely experience hydrops, supporting the hypothesis that hypoproteinemia alone is not sufficient to cause hydrops. To elucidate the role of isolated hypoproteinemia in the genesis of hydrops, Moise et al.[32] induced hypoproteinemia in sets of twin fetal lambs. One twin from each set underwent serum protein reduction through repeated removal of plasma and replacement with normal saline; the other twin served as the control. Over 3 days, plasma protein concentrations were reduced by an average of 41% with a 44% reduction in colloid osmotic pressure in experimental subjects. No fetal animals became edematous, and total body water content values were similar in experimental and control animals. Thus hypoproteinemia alone was insufficient to cause hydrops fetalis over the course of the study. Transcapillary filtration probably increased with hypoproteinemia but was compensated for by lymphatic return. Hypoproteinemia may lower the threshold for edema formation in the presence of impaired lymphatic return or increased intravascular hydrostatic pressures.

Increased Capillary Leak

One final potential etiology for hydrops is increased capillary leak. Increased capillary leak is typically identified not as a primary mechanism for hydrops but as part of the pathway between nonimmune hydrops and other insults, such as infection, hypoxic injury, or increased capillary hydrostatic pressure seen with elevated CVP or decreased or abnormal lymphatic flow.[24]

Prenatal Diagnosis

The prenatal diagnosis of hydrops fetalis is typically made via the ultrasonographic finding of excess fluid in the form of ascites, pleural or pericardial effusions, skin edema, placental edema, or polyhydramnios. Several definitions for ultrasonographic diagnosis based on quantity and distribution of excess fluid have been proposed. One widely accepted set of criteria consists of the presence of excess fluid in any two of the previously listed compartments. A 2015 study suggested that the involvement of more body compartments is associated with higher mortality.[33] Because this definition is based on the presence of excess fluid alone, the degree of severity is generally subjective.

Swain et al.[34] and Bellini and Hennekam[24] each outlined a multidisciplinary approach to the evaluation and management of a mother and fetus with hydrops. Table 7.2 provides recommendations for the antenatal investigation of fetal hydrops. Patient history should focus on ethnic background, familial history of consanguinity, genetic or congenital anomalies, and complications of pregnancy, including recent maternal illness and environmental exposures. Maternal disorders such as diabetes, systemic lupus erythematosus, myotonic dystrophy, and any type of liver disease should also be noted. Initial laboratory investigation includes blood typing and a Coombs test to rule out immune-mediated hydrops. Additional studies may include screening for hemoglobinopathies, a Kleihauer–Betke test to eliminate fetal–maternal hemorrhage, and testing for TORCH (i.e., toxoplasmosis, other agents, rubella, cytomegalovirus, and herpes simplex), including syphilis and parvovirus B19.

Rapid evaluation is necessary to determine whether fetal intervention is possible and to estimate the prognosis for the fetus. Many conditions, such as arrhythmias, twin–twin transfusion, large vascular masses, and congenital diaphragmatic hernias and other chest-occupying lesions, are discovered during the initial ultrasonographic evaluation.[35] Middle cerebral artery peak systolic velocity measurement can aid in detecting the presence of fetal anemia.[36] If the initial ultrasonic examination is not helpful in identifying a cause, it may be helpful to repeat it at a later date to reassess fetal anatomy, monitor progression of the hydrops, and evaluate the well-being of the fetus. Fetal MRI is being employed more commonly in cases of hydrops fetalis where the sonographic examination is unable to present a clear picture regarding etiology or to further delineate the anatomy in cases with a known anatomic etiology.

Fetal echocardiography should also be performed to evaluate for cardiac malformations and arrhythmia. Amniotic fluid can be obtained for fetal DNA analysis, cultures, and lecithin-to-sphingomyelin ratio to assess lung maturity. Fetal blood sampling allows for other tests, such as a complete blood cell count, routine chemical analyses, DNA analysis, bacterial and viral cultures, metabolic studies, and serum immunoglobulin measurements. Fetal thoracentesis may be done to establish a diagnosis. A pleural effusion cell count with a lymphocyte predominance would be suggestive of a lymphatic etiology of hydrops.

TABLE 7.2 Antenatal Investigation of Fetal Hydrops

Area	Testing
Maternal	History, including: Age, parity, gestation Medical and family histories Recent illnesses or exposures Medications Complete blood count and indices Blood typing and indirect Coombs antibody screening Hemoglobin electrophoresis Kleihauer–Betke stain of peripheral blood Syphilis, TORCH, and parvovirus B19 titers Anti-Ro and anti-La antibodies, systemic lupus erythematosus preparation Oral glucose tolerance test Glucose-6-phosphate dehydrogenase, pyruvate kinase deficiency screening
Fetal	Serial ultrasound evaluations Middle cerebral artery peak systolic velocity Limb length, fetal movement Echocardiography Fetal MRI Thoracentesis for pleural fluid cell count Thoracoamniotic shunt
Amniocentesis	Karyotype Alpha-fetoprotein Viral cultures; polymerase chain reaction analysis for toxoplasmosis, parvovirus 19 Establishment of culture for appropriate metabolic or DNA testing Lecithin-to-sphingomyelin ratio to assess lung maturity
Fetal blood sampling	Genetic testing Complete blood count Hemoglobin analysis Immunoglobulin M test; specific cultures Albumin and total protein measurements Measurement of umbilical venous pressure Metabolic testing

MRI, Magnetic resonance imaging; *TORCH*, toxoplasmosis, other agents, rubella, cytomegalovirus, and herpes simplex.

Adapted from Swain S, Cameron AD, McNay MB, Howatson AG. Prenatal diagnosis and management of nonimmune hydrops fetalis. *Aust N Z J Obstet Gynaecol*. 1999;39:285–290 and Bellini C, Hennekam RC, Bonioli E. A diagnostic flow chart for non-immune hydrops fetalis. *Am J Med Genet A*. 2009;149A:852–853.

Prenatal Management

The goals of antenatal evaluation and management of fetal hydrops depend on the underlying cause. In diagnoses for which therapy is futile, management goals include avoiding unnecessary invasive testing and procedures and informing parents of the prognosis and their options—such as pregnancy termination or a perinatal palliative care plan in the event of a live birth. If the underlying cause is amenable to fetal therapy, the risks and benefits of such therapy, as well as the warning that diagnostic error is possible, should be discussed with the family.

SVT is one of the most common known causes of nonimmune hydrops, and it is the most amenable to treatment.[37,38] Usually the mother is given antiarrhythmic agents, and the fetus is monitored closely for resolution of the SVT. Digoxin is most commonly administered, although other antiarrhythmics have been used, such as sotalol or flecainide, because transplacental transfer of digoxin may be impaired in the setting of hydrops. In extreme circumstances, such as fetal tachyarrhythmia refractory to maternal treatment, direct fetal administration of antiarrhythmic agents via percutaneous umbilical blood sampling or intramuscular injection, although untested and highly risky, has met with some success.

If anemia is the cause of hydrops, transfusions of packed red blood cells may be administered to the fetus. Often a single transfusion reverses the edema, although serial transfusions may be necessary. Parvovirus B19[39] and fetal–maternal hemorrhage are examples of diagnoses that are amenable to this therapy. Transfusions should be given with the use of ultrasonographic guidance into the intraperitoneal space or umbilical vein. Blood instilled into the abdominal cavity is taken up by lymphatics, but elevated CVP present in hydropic fetuses may impair this uptake. If uptake of intraperitoneal blood is incomplete, treatment for the hydrops is less successful. In addition, degeneration of the remaining hemoglobin may create a substantial bilirubin load, necessitating phototherapy or exchange transfusion after the fetus is delivered. Other diagnoses involving anemias that are refractory to transfusions, such as α-thalassemia, may require neonatal stem cell transplantation.

High morbidity and mortality rates in severe twin–twin transfusion with associated hydrops led to multiple international trials of laser photocoagulation of interfetal vascular connections. A systematic review of 34 studies of 3868 patients published between 1990 and 2014 found improved survival comparing the 1990–1995 with the 2011–2014 epoch. Of pregnancies requiring laser photocoagulation, both twins survived in 65% of the pregnancies in the more recent epoch, with an additional 23% of pregnancies reporting a survival of one of the fetuses.[40] However, there is limited data on the impact of treatment on the neurodevelopmental outcomes in survivors. As a result, a Cochrane review recommends considering treatment with laser coagulation at all stages of twin–twin transfusion using results from three studies,[41,42] even though further research is needed to assess the effect of treatment on milder versus more severe forms of the syndrome.

Fetal intervention has met with some success in surgical defects and other conditions associated with hydrops.[43–45] While fetal lung lesions such as congenital cystic pulmonary malformation (CPAM) and pulmonary sequestration frequently involute and may disappear before delivery, in the most extreme cases they can result in mediastinal shift, pulmonary hypoplasia, cardiovascular compromise, and hydrops. The highest risk lesions for the development of hydrops include those lesions with a CPAM volume ratio greater than 2, an everted hemidiaphragm, or a mass-to-thorax ratio greater than 0.56.[46,47] Early surgical resection for these most severe lesions has occurred in several centers. The latest therapy involves ex utero intrapartum therapy—or EXIT delivery—where the resection of the CPAM occurs immediately after delivery but before separation of the placenta. Reported outcomes vary widely between centers. Thoracoamniotic shunts for large unicystic lesions and pleuroamniotic shunts for hydrothorax have reportedly enhanced survival in extreme cases. Similarly, in cases of massive urinary ascites, urinary diversion via peritoneal shunts has been reported.

There are complications associated with fetal interventions. Preterm birth can be as high as 80% under 37 weeks' gestation and 15% under 30 weeks' gestation, particularly for open surgical procedures.[48,49] Maternal morbidities related to fetal intervention range from spontaneous and/or premature rupture of membranes, oligohydramnios, and uterine wound infection with dehiscence to mild postoperative interstitial pulmonary edema, especially in mothers with longer operative times.[48,50,51]

In cases in which the cause of hydrops can be corrected by appropriate care at the time of delivery, such as elimination of a chorioangioma, as well as those cases in which no cause can be ascertained, close observation for fetal demise is the focus of prenatal management. Many cases of nonimmune hydrops manifest in the third trimester as preterm labor. It is difficult to decide whether to attempt tocolysis and delay delivery so as to allow the potentially beneficial administration of steroids before birth or to deliver the fetus immediately. If tocolysis is possible, expectant management should include usual biophysical testing, although fetal decompensation may be difficult to assess. Abnormal fetal heart tracings, oligohydramnios, decreased fetal movement, and poor fetal tone are all ominous signs. Prolonging the pregnancy beyond attainment of a mature lung profile is not generally recommended unless there is evidence of clinical improvement or resolution of the hydrops.

Neonatal Evaluation

Table 7.3 summarizes the diagnostic evaluations recommended for newborns with nonimmune hydrops of unknown cause. There are a few key points:

- Prenatal test results may help guide initial evaluation.
- Early cardiac and hematologic evaluations will determine most known etiologies.
- A multidisciplinary approach is essential.
- Response to interventions may help guide the evaluation.

TABLE 7.3 Diagnostic Evaluation of Newborns With Nonimmune Hydrops

System	Type of Evaluation
Cardiovascular	Echocardiogram, electrocardiogram
Pulmonary	Chest radiograph, pleural fluid examination
Hematologic	Complete blood cell count, differential platelet count, blood type and Coombs test, blood smear for morphologic analysis
Gastrointestinal	Abdominal radiograph, abdominal ultrasonography, liver function tests, peritoneal fluid examination, total protein and albumin levels
Renal	Urinalysis, blood urea nitrogen, and creatinine measurements
Genetic	Chromosomal analysis, skeletal radiographs, genetic consultation
Congenital infections	Viral cultures or serologic testing, including TORCH agents and parvovirus
Pathologic	Complete autopsy, placental examination
Lymphatic	MRI, lymphoscintigraphy, ICG lymphography

ICG, Indocyanine green; *MRI*, magnetic resonance imaging; *TORCH*, toxoplasmosis, other agents, rubella, cytomegalovirus, and herpes simplex.

Adapted from Carlton DP, McGillivray BC, Schreiber MD. Nonimmune hydrops fetalis: a multidisciplinary approach. *Clin Perinatol*. 1989;16:839–851 and Bellini C, Hennekam RC, Bonioli E. A diagnostic flow chart for non-immune hydrops fetalis. *Am J Med Genet A*. 2009b;149A:852–853.

Intensive Care of the Infant With Hydrops Fetalis

After newborn resuscitation, stabilization, and placement of umbilical catheters, the clinical management should address both the cause and the complications of hydrops. Morbidity and mortality may result from the hydropic state, the underlying conditions giving rise to hydrops, or both. A fetus with hydrops that is delivered prematurely is subject to the additional complications of prematurity. If there is massive ascites or pleural effusions, initial resuscitation may require thoracentesis or peritoneal tap. Because of pulmonary edema, newborns with hydrops are susceptible to pulmonary hemorrhage and may require high levels of positive end-expiratory pressure.

Respiratory Management

Virtually all hydropic newborns will require mechanical ventilation because of pleural and peritoneal effusions, pulmonary hypoplasia, surfactant deficiency, pulmonary edema, poor chest wall compliance caused by edema, and likely persistent pulmonary hypertension of the newborn. The presence of persistent pleural effusions may necessitate the placement of chest tubes. Ascites may also compress the diaphragm and impair lung expansion. Breath sounds, chest wall movement, blood gas levels, and radiographs must all be monitored frequently so that ventilator support can be reduced in response to improvements in lung compliance and fluid clearance. Pneumothoraces and pulmonary interstitial emphysema remain potential complications as long as ventilator support is continued. Neonates who need a prolonged course of ventilation, particularly those born prematurely, may develop bronchopulmonary dysplasia. Chronic lung disease results in a longer and more complicated hospital course and contributes to the late mortality of neonatal hydrops.

Fluid, Electrolyte, and Medical Management

A primary goal of fluid management is resolution of the hydrops itself. Maintenance fluids should be restricted, with volume boluses given only in response to clear signs of inadequate intravascular volume. The hydropic newborn has an excess of free extracellular water and sodium. Fluids given during resuscitation further increase the amount of water and sodium that must be removed during the immediate neonatal period. Initial maintenance fluids should contain minimal sodium. Serum and urine sodium levels, urine volume, and daily weights should be monitored carefully to guide administration of fluids and electrolytes. Urinary sodium levels may help differentiate between hyponatremia caused by hemodilution and urinary losses.

If the hydrops is secondary to a lymphatic disorder, peritoneal or pleural fluid that is drained will contain white blood cells, immunoglobulins, albumin, and coagulation factors, and may also contain triglycerides if the neonate is fed. Fluid replacement may be necessary to replete ongoing losses. Fresh frozen plasma may be considered for volume resuscitation to restore coagulation factors. Fluid replacement using hypo-oncotic (4%) albumin or iso-oncotic (5%) albumin should be used cautiously, as they may worsen peripheral and pulmonary edema without correcting serum levels of albumin. Hyper-oncotic (20% or 25%) albumin can be used intermittently to correct hypoalbuminemia, although there is limited data on this practice in neonates with hydrops.[52]

Medical management of neonatal lymphatic disorders typically includes furosemide to decrease pulmonary lymphatic flow, particularly in cases of PLPS. A low long chain fatty acid diet should also be used to reduce lymphatic fluid burden. Octreotide, a somatostatin analog that causes splanchnic vasoconstriction, decreases hepatic lymphatic flow and has been used to treat patients with lymphatic flow disorders. However, there is insufficient data to support its use in hydropic neonates given the risk of pulmonary hypertension and necrotizing enterocolitis.

Cardiovascular Management

Hydropic infants may develop hypovolemia as a result of capillary leakage, poor vascular tone, impaired myocardial contractility from hypoxia or infection, impaired venous return caused by shifting or compression of mediastinal structures, and/or pericardial effusion. Thus, these infants may present in shock. Adequate intravascular volume must be maintained, and correctable causes of impaired venous return should be addressed. Peripheral perfusion, heart rate, blood pressure, and acid–base status should be monitored carefully.

Lymphatic Evaluation and Interventions

Advances in the use of T2-weighted MRI, dynamic contrast-enhanced magnetic resonance lymphangiograms (DCMRL), and lymphangiography have identified a group of infants with nonimmune hydrops who have abnormal lymphatic systems. These infants, most of whom have congenital anomalies of the thoracic duct or abnormally conducting pulmonary lymphatic channels,[29] may benefit from new procedures to relieve these obstructions.[29,53,54] There are few studies in neonates, although data from older infants—many of whom had single-ventricle physiology—suggest that the use of percutaneous lymphatic interventions after a bilateral intranodal lymphangiogram may be useful in these infants.[55,56]

Pinto et al.[29] categorized neonates with lymphatic disorders into two subgroups: neonatal chylothorax secondary to pulmonary lymphatic perfusion syndrome (PLPS) and central lymphatic flow disorder (CLFD). CLFD, which is a generalized lymphatic disorder characterized by abnormal lymphatic flow or lymphatic leak into more than one body compartment, is often characterized by dermal backflow through lymphatic collaterals seen on DCMRL. Two recent studies have shown that neonatal chylothorax secondary to isolated PLPS can be successfully treated with intranodal injection of ethiodized oil and is considered standard of care in some centers.[29,57] Ethiodized oil, a radiopaque contrast agent, has a high viscosity and results in a mechanical occlusion of abnormally conducting pulmonary lymphatic channels. A universal treatment strategy has not been established for CLFD given the various etiologies for this disorder. Finally, neonates born with hydrops may have congenital atresia of the thoracic duct outlet. In these cases, surgical thoracic duct-to-vein (lymphovenous) anastomosis has been used to relieve thoracic duct outlet obstruction.[29,58] Other treatment options once thought to be useful in hydropic infants with CLFD can worsen disease. There is some evidence that surgical thoracic duct ligation in patients with CLFD develop worsening anasarca from dermal backflow. As such, some experts recommend avoiding this procedure in infants.[57] Further work is needed in the neonatal population with nonimmune hydrops, specifically with central lymphatic flow disorder, to identify those infants who may benefit from established interventions as well the development of newer therapeutic options.

Clinical Course and Outcome

Despite improvements in diagnosis and management, mortality from nonimmune hydrops remains high. For example, one study of 92 infants born with nonimmune hydrops between 2000 and 2012 reported a 45% fetal death rate and a 36% survival rate at 1 year.[59] Other studies have reported survival rates between 20% and 30%.[60,61] Recently published population data, though, present a more optimistic set of outcomes. Swedish data from 1997–2015 found that 58.7% of live-born children with nonimmune hydrops were alive at 12 months of age,[11] while California population data from 2005 to 2012 found a 1-year survival rate of 56.8%.[12]

The best predictors of survival are the cause of the hydrops, the gestational age of the child at delivery, and the condition of the neonate at birth. Highest survival rates are seen in infants with parvovirus infection, chylothorax, and SVT. The lowest survival rates are for hydrops associated with a chromosomal diagnosis. While the figures may be biased because a significant number of pregnancies in such cases are terminated, several studies have reported elevated risk of mortality in live-born infants with nonimmune hydrops that resulted from aneuploidy, especially those with a cardiac diagnosis.[6,10,12] A review of 598 patients with nonimmune hydrops found other risk factors for increased mortality, including younger gestational age, lower 5-minute Apgar score, and the need for increased respiratory support,[13] and a smaller study from Taiwan also found that lower albumin levels were associated with a higher mortality rate.[62]

Interventions to improve outcomes in hydrops are limited by the rarity of the disease. Most hydropic neonates lose a minimum of 15% of their birthweight, and some lose as much as 30%. Ordinarily, diuresis begins on the second or third day after birth and continues for a period of 2 to 4 days. Once the edema has resolved, the neonates have normal levels of circulating protein and eventually recover from their apparent capillary leak syndrome. No specific management strategies during the neonatal period, such as the use of high-frequency oscillatory ventilation, have been shown to improve outcome.[23] However, extracorporeal membrane oxygenation may be a reasonable option for some infants with nonimmune hydrops, with one study showing a 54% survival rate in 28 cases.[63] More recent data have not been published.

For infants who survive the immediate neonatal period, long-term outcomes largely depend on the etiology of the condition. For example, an older study from Japan found that 13 of 19 surviving infants with nonimmune hydrops had normal development at 1 to 8 years.[20] More recent data, though, found only a 45% to 50% survival rate without developmental delay by 1 year.[59,64] In both these studies, hydropic infants with mild or severe delays had other morbidities, such as extreme prematurity, structural cardiac lesions, or chromosomal anomalies. Thus similar to mortality, long-term morbidities from nonimmune hydrops result primarily from the underlying cause of the hydrops, gestational age at delivery, and complications arising immediately after delivery.

Suggested Readings

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8 Maternal Diabetes

EMILY FAY, LAVONE SIMMONS, AND COLLEEN BROWN

KEY POINTS

- The incidence of diabetes in pregnancy is steadily rising, likely in parallel with the rising incidence of obesity among pregnant patients.
- Pregnant patients with diabetes are at increased risk for fetal complications (such as congenital malformations, fetal growth abnormalities, and stillbirth) and perinatal/neonatal complications (such as prematurity, respiratory distress, and metabolic abnormalities, including hypoglycemia and electrolyte derangements).
- Tight maternal glycemic control, achieved preconception and maintained throughout pregnancy, is key to optimizing fetal and neonatal outcomes.
- Breastfeeding should be encouraged and supported as it may reduce some of the possible adverse effects of intrauterine programming in the context of maternal diabetes.

Diabetes mellitus is an increasingly common disorder in the United States and around the world. According to 2018 data from the US Centers for Disease Control and Prevention (CDC), 26.8 million adults aged 18 years or older had diabetes and approximately half of these were women (Table 8.1).[2] An estimated 88 million adults—roughly 34.5% of the adult US population—have prediabetes, a condition associated with an elevated risk of developing type 2 diabetes. The rapidly rising rate of diabetes parallels the rising rates of obesity in counties across the United States (Figs. 8.1–8.3). Similarly, there has been a significant increase in the prevalence of gestational diabetes mellitus (GDM, elevated blood sugars due to insulin resistance diagnosed for the first time in pregnancy). Data from 2010 derived from birth certificates, as well as the Pregnancy Risk Assessment Monitoring System, estimated the prevalence of GDM in the United States to be as high as 9.2%.[1] Increasing rates of GDM are hypothesized to be due to increasing rates of obesity, which is a known risk factor (see Fig. 8.1), older maternal ages at delivery, as well as physical inactivity, smoking, and diets high in saturated fats.[3]

Given the statistics noted above, diabetes represents one of the most common medical diagnoses in pregnancy and is associated with profound implications on fetal and neonatal outcomes. Maternal pregestational diabetes is associated with an increased risk of congenital anomalies, abnormalities in fetal growth, and neonatal complications such as hypoglycemia, electrolyte abnormalities, respiratory distress, and cardiomyopathy. Additionally, the intrauterine environment, in the context of maternal diabetes, may impact pediatric neurodevelopmental outcomes as well as increase the risk of chronic disorders in exposed offspring such as obesity and metabolic syndrome.

Types of Diabetes

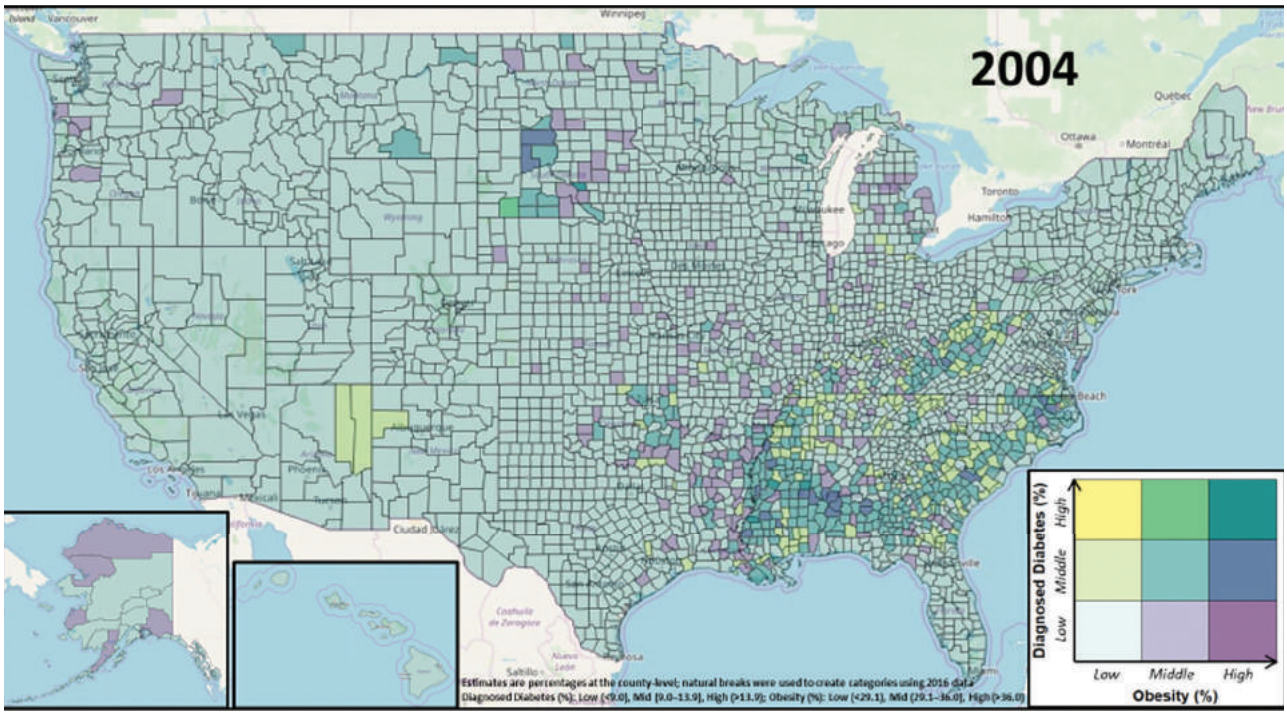
Type 1 Diabetes

Type 1 diabetes is characterized by insulin deficiency resulting from autoimmune destruction of pancreatic insulin-producing beta cells. Typically, people with type 1 diabetes will develop clinical signs and symptoms of diabetes in childhood. However, immune-mediated diabetes can arise at any time through adulthood. Immune-mediated diabetes in adults is characterized by a slower destruction of beta cells and subsequent progression to insulinopenia. People with type 1 DM are dependent on exogenously administered insulin. The underlying pathophysiology

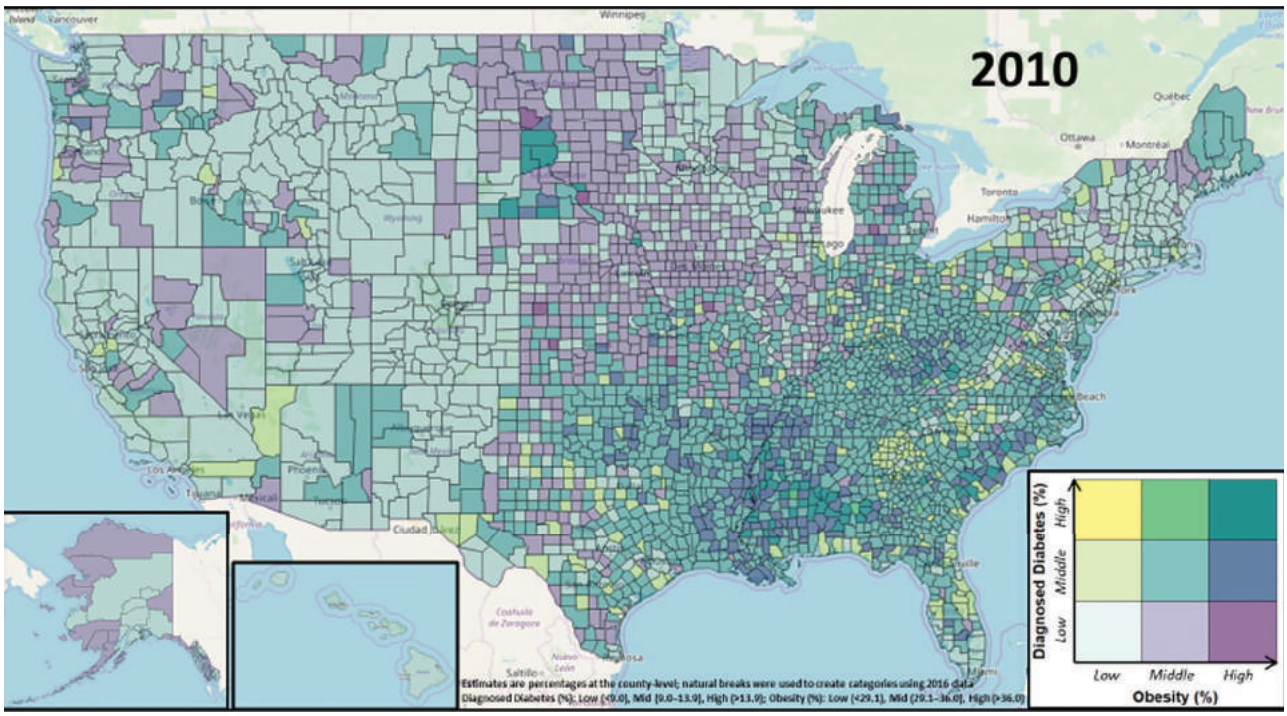
TABLE 8.1 Diagnosed and Undia gnosed Diabetes Among People Aged 18 Years or Older in the United States 2018

	Number with Diabetes (Million)	Percentage with Diabetes
Total		
≥18 years old	34.1	12.3
Age		
1–44 years old	4.9	4.1
45–64 years old	14.8	16.2
Gender		
Women	16.2	11.2

Source: 2013–2016 National Health and Nutrition Examination Survey estimates applied to 2018 US Census Data. CDC National Diabetes Statistics Report, 2020.



• **Fig. 8.1** Map of diabetes and obesity, by county, U.S. Year 2004. Diagnosed Diabetes (%): Low (<9.0), Mid (9.0–13.9). Obesity (%): Low (<29.1), Mid (29.1–36.0), High (> 36.0). (https://www.cdc.gov/diabetes/data/center/slides.html)

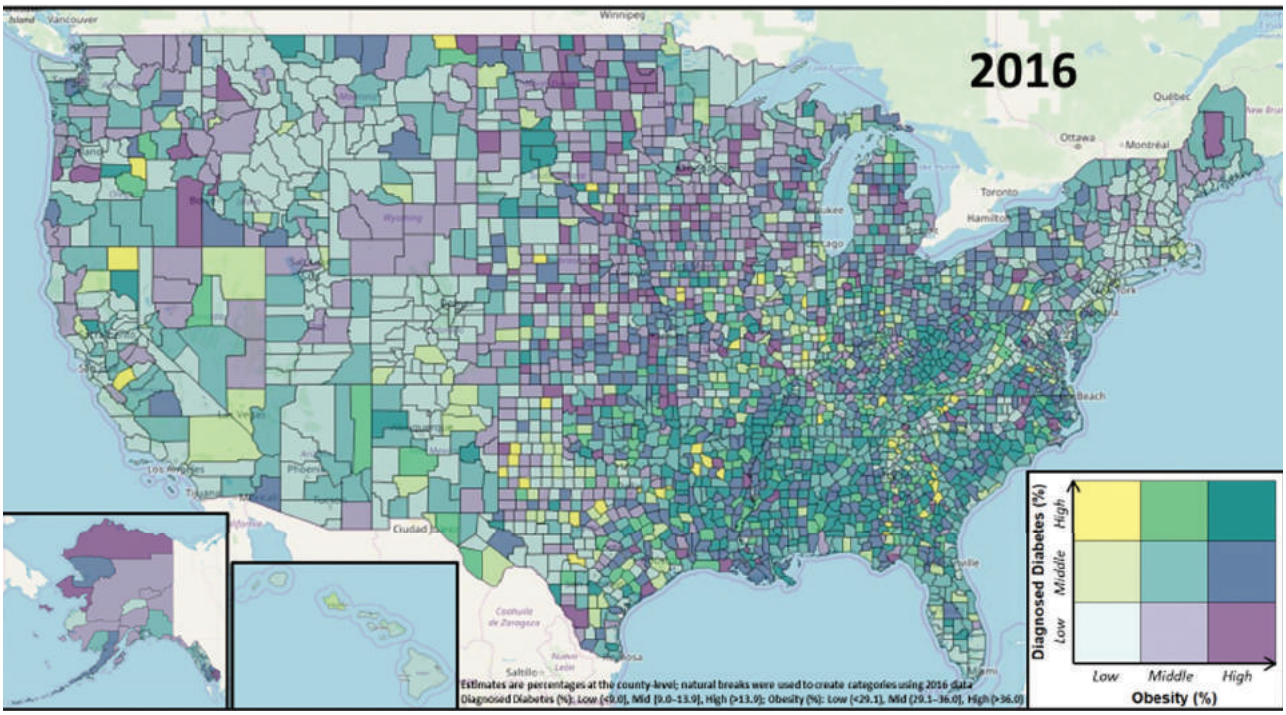


• **Fig. 8.2** Map of diabetes and obesity, by county, U.S. Year 2010. Diagnosed Diabetes (%): Low (<9.0), Mid (9.0–13.9). Obesity (%): Low (<29.1), Mid (29.1–36.0), High (>36.0). (https://www.cdc.gov/diabetes/data/center/slides.html)

of type 1 diabetes and the physiological changes of pregnancy place pregnant patients at elevated risk for diabetic ketoacidosis (DKA). DKA is associated with a significant impact on perinatal morbidity, contributing to higher rates of fetal demise, preterm birth, and NICU admission.[4]

Type 2 Diabetes

Type 2 diabetes is the most common form, accounting for 95% of cases in the United States. Type 2 diabetes is characterized by insulin resistance and is more significantly associated with obesity.



• **Fig. 8.3** Map of diabetes and obesity, by county, U.S. Year 2016. Diagnosed Diabetes (%): Low (<9.0), Mid (9.0–13.9). Obesity (%): Low (<29.1), Mid (29.1–36.0), High (> 36.0). (https://www.cdc.gov/diabetes/data/center/slides.html)

Other risk factors include advancing age, sedentary lifestyle, history of gestational diabetes, and comorbidities including hypertension and dyslipidemia. Historically, type 2 diabetes is associated with adult onset, however, there have been increasing rates of diagnoses of type 2 diabetes among children and adolescents that appear to parallel the rise in obesity rates in these age groups.[5]

Monogenic Diabetes

The etiology of ~1% to 2% of diabetes diagnoses are genetic, also referred to as monogenic diabetes (MODY: maturity-onset diabetes of the young). Multiple types of monogenic diabetes exist; all of the genes involved share the common feature of affecting beta cell development, function, or regulation. Clinical presentations of monogenic diabetes are heterogeneous, with phenotypes varying according to the specific mutation (Table 8.2). Most forms share the following clinical features: affected individuals are typically diagnosed before the age of 25 years and are not insulin dependent. Frequently, pregnant patients with monogenic diabetes may be first identified and diagnosed with diabetes during pregnancy. A notable difference from GDM, they are often found to have continued hyperglycemia postpartum. Genetic testing is prompted by the recognition of a strong family history of diabetes (often two or more consecutive affected generations), as well as an absence of obesity or other clinical features suggestive of insulin resistance.

Neonatal Consequences of MODY

The genetic mutations leading to monogenic diabetes are inherited in an autosomal dominant fashion, so there is a 50% risk of inheritance in the offspring. Fetal MODY mutations may impact intrauterine growth. Macrosomia and neonatal hypoglycemia are associated with *HNF4A* mutations. In an analysis of siblings discordant to the *HNF4A* mutation, affected infants had birth weights that were on average 790 g higher than unaffected infants; 56% of affected infants were macrosomic (birth weight >4000 g), as compared with 13% of those who were unaffected. Fifteen percent of affected infants demonstrated evidence of neonatal hypoglycemia.[6]

In contrast, fetuses who have inherited the *GCK* mutation do not appear to demonstrate the excessive fetal growth that typically results from maternal hyperglycemia; studies have reported that affected fetuses may have a birth weight that may be 500 to 600 g lower than unaffected fetuses of mothers with monogenic diabetes due to *GCK* mutations.[7] Fetuses inheriting the *GCK* mutation may demonstrate a decreased insulin response to hyperglycemia. Therefore, they may experience less macrosomia. Unaffected fetuses have a normally functioning glucokinase enzyme, are at risk for hyperinsulinemia in response to maternal hyperglycemia, and have a greater risk of macrosomia. Knowledge of the fetal genotype during pregnancy might theoretically allow providers and patients to tailor diabetes management. Definitive diagnosis of *fetal* genotype requires invasive testing such as first trimester chorionic villus sampling (CVS) or second trimester amniocentesis, with the associated risks of these procedures.

The management of diabetes during pregnancy may also have long-term effects on fetuses who inherit mutations associated with diabetes. Studies have demonstrated that individuals with maternally-inherited *HNF1A* mutations often have earlier age at diabetes diagnosis and at initiation of insulin therapy than those paternally-inherited mutations[8]; this observation may suggest that the intrauterine environment may impact the phenotype associated with these mutations, and further highlights the importance of tight maternal metabolic control during pregnancy in optimizing long-term outcomes.

TABLE 8.2 Maturity-Onset Diabetes of the Young

Gene	Associated Function	Associated Effect	Effect of Mutation	Patient Presentation and Considerations for Care
Glucokinase (GCK)	Glucokinase enzyme catalyzes the 1st (rate-limiting) step in glycogen storage and glycolysis	Links insulin secretion to elevations in serum glucose	Inactivation of GCK raises the glucose setpoint for insulin secretion	• Stable, mild hyperglycemia; prominent elevated fasting levels • Fetal inheritance associated with lower birthweight compared to unaffected • Unaffected fetus is at higher risk for macrosomia • Associated with suboptimal glucose control/ treatment failure[3]
Hepatocyte nuclear factor (HNF1A) ~50% of MODY cases	Transcription factor	Beta cell differentiation, development and function	Progressive beta cell dysfunction and decreased insulin secretion	• Relatively high penetrance: 63% of patients with HNF1A develop diabetes by age 25 and 79% by age 35.2 • More responsive to sulfonylureas (e.g., glyburide) than to biguanides (metformin)
Hepatocyte nuclear factor (HNF4A) ~10% of MODY cases	Transcription factor	Beta cell differentiation, development and function	Progressive beta cell dysfunction and decreased insulin secretion	• More responsive to sulfonylureas (e.g., glyburide) than to biguanides (metformin) • Associated with macrosomia and neonatal hypoglycemia
Hepatocyte nuclear factor (HNF1B) <1% of MODY cases (also known as *renal cysts and diabetes* [RCAD] *syndrome*)	Transcription factor	Beta cell differentiation, development and function	Progressive beta cell dysfunction and decreased insulin secretion	• Early-onset non-insulin dependent diabetes • Developmental renal disease (typically cystic) • Genitourinary abnormalities • Atrophy of the pancreas • Hyperuricemia • Gout

Data from Naylor and Philipson, 2011[130]; Murphy et al., 2008[131]; Bacon et al., 2015[7]; Diabetes Care 2022[5]

Gestational Diabetes

The reported incidence of diabetes in pregnancy is up to 9.2% with 80% to 90% of cases due to gestational diabetes (GDM).[1] GDM is defined by the presence of elevated blood sugars, resulting from insulin resistance diagnosed for the first time during pregnancy. The physiologic changes of pregnancy are associated with hyperglycemia in pregnant patients with GDM, prediabetes and diabetes. In pregnancy, insulin resistance increases with advancing gestation in response to increasing levels of human placental lactogen, progesterone, cortisol, and prolactin. Human placental lactogen and prolactin antagonize the effects of insulin at the cellular level. Progesterone decreases gastrointestinal motility, which may enhance carbohydrate absorption. It is believed that insulin resistance in pregnancy is likely a physiologic adaptation to maintain a steady supply of nutrients to the growing fetus.

Given the underlying pathophysiology of insulin resistance, there is likely some overlap between pregnant patients with type 2 diabetes and those diagnosed with GDM. Because of the likelihood that underlying type 2 diabetes may be first diagnosed in pregnancy (and therefore mistaken for GDM), early screening in pregnancy is recommended for pregnant patients with risk factors for type 2 diabetes (Table 8.3). Risk factors that merit early diabetes testing include the following: a previous history of GDM, history of impaired glucose tolerance, and obesity (body mass index [BMI] ≥30 kg/m^2). In conjunction with American Diabetes Association recommendations, to facilitate early diagnosis, diabetic screening is recommended for patients with BMI ≥25 kg/m^2 and have one of the following additional risk factors: physical inactivity, a first-degree relative with diabetes, history of delivery of a macrosomic infant (defined as birth weight ≥4 kg), or history of polycystic ovarian syndrome or chronic hypertension. A negative early screen for

TABLE 8.3 Criteria for the Diagnosis of Diabetes (American Diabetes Association)

Clinical signs and symptoms of hyperglycemia or hyperglycemic crisis and random plasma glucose ≥200 mg/dL

OR

Hemoglobin A_{1c} ≥6.5%

OR

Fasting plasma glucose ≥126 mg/dL

OR

75-g, 2-hr oral glucose tolerance test with 2-hr value ≥200 mg/dL

Adapted from American Diabetes Association. Standards of Medical Care in Diabetes 2011. *Diabetes Care* 2011;34:S11.

diabetes should be retested later in pregnancy at the recommended universal testing window: 24 to 28 weeks.

Currently in most of the United States, two-step testing is recommended with a 50-g glucose challenge test as the initial screen, followed by definitive diagnostic testing with a 100-g three-hour oral glucose tolerance test; this testing approach is endorsed by the American College of Obstetricians and Gynecologists (ACOG).[9] The International Association of Diabetes and Pregnancy Study Groups (IADPSG) recommends a one-step testing strategy utilizing a 75-g 2-hour oral glucose tolerance test; the diagnostic thresholds were selected based on odds ratio for various adverse outcomes evaluated in the Hyperglycemia and Adverse Pregnancy Outcome study.[10] Using a one-step approach may diagnose GDM in as many as 18% of pregnant patients. A recent randomized control trial evaluated the one- vs. two-step approach to diagnosis. The one-step approach did diagnose 16.5% of participants with GDM, as compared to 9.2% using the two-step. However, there were no significant between-group differences in primary outcomes related to perinatal and maternal complications.[10,11] In light of these findings, choice of screening test is left to each provider and/or practice based on the needs and adherence of their unique patient population.

Maternal Obesity

The obesity epidemic is contributing to the global rise in type 2 diabetes and GDM, thus resulting in an increased risk of the perinatal complications attributable to diabetes. Importantly, maternal obesity, even in the absence of diabetes, has been found to be an independent risk factor for adverse obstetric, fetal, and neonatal outcomes. Specifically, maternal prepregnancy obesity has been associated with an increased risk of congenital anomalies, stillbirth, macrosomia, hypertensive disorders of pregnancy (e.g., preeclampsia), stillbirth, cesarean delivery, as well as pediatric obesity.[12]

Pregnancies complicated by obesity are associated with increased odds of fetal open neural tube defects, hydrocephalus, cardiovascular anomalies, cleft lip and/or palate, and limb reduction anomalies.[13,14] The elevated risk of congenital malformation is likely compounded by the presence of suboptimal control of pregestational diabetes. There is an elevated risk of congenital malformations in offspring of obese pregnant patients with diabetes, and the detection of anomalies by ultrasound evaluation for fetal anomalies is more challenging in this population; studies estimate a 20% lower detection rate for anomalies.[15,16]

In several studies, the risk of perinatal mortality, including stillbirth, was shown to increase with increasing severity of maternal obesity.[17] Maternal prepregnancy obesity, as defined by a BMI of greater than or equal to 30 kg/m^2, has been identified as an independent risk factor for macrosomia and LGA birth weight.[18] In a cohort of singleton pregnancies, both maternal diabetes and maternal obesity were identified as independent risk factors for LGA birth weight; in the case of maternal obesity, the adjusted odds ratio for delivering an LGA infant is 1.6, while for pregestational diabetes, the adjusted odds ratio is 4.4.[19] Given the greater prevalence of obesity as compared with pregestational diabetes, it is likely that the proportion of LGA infants delivered by obese pregnant patients will exceed that of diabetic pregnant patients. Similar to what is observed in macrosomic infants of diabetic mothers (IDMs), LGA infants born to pregnant patients with obesity appear to have increased adiposity as compared with infants of patients without associated obesity; these children may go on to have an increased risk of childhood obesity and metabolic abnormalities.[20,21]

Association Between Perinatal Outcomes and Periconception Glycemic Control

In the setting of pregestational diabetes, suboptimal periconceptional glycemic control has been associated with a significantly increased risk of embryopathy. Embryopathy is associated with pregnancy loss and congenital anomalies. The background rate of congenital anomalies in the general population is around 2% to 3% compared to 6% to 9% among pregnant patients with pregestational diabetes.[22] Studies in the early 1980s demonstrated an association between congenital anomalies and increasing maternal periconception hemoglobin A_{1c} (HbA_{1c}); a first trimester HbA_{1c} greater than or equal to 7.5% is associated with a ninefold increased risk of congenital anomalies compared to optimal periconception glycemic control.[23] Extremely elevated maternal HbA_{1c} levels (i.e., ≥10%) in early pregnancy are associated with fetal anomaly rates of 20% to 25%, while periconception HbA_{1c} levels in the normal range are associated with a congenital anomalies rate similar to a nondiabetic population.[24,25] Contemporary studies have demonstrated similar associations. A nationwide population study of the United States demonstrated a 2.44 adjusted relative risk for congenital anomalies among patients with prepregnancy diabetes compared to patients without diabetes.[26] In a Danish prospective cohort study of 933 singleton pregnancies in patients with type 1 diabetes, 10.9% of the offspring with a periconceptional HbA_{1c} ≥10.4% had a major congenital anomaly as compared with 2.8% of the background population.[27]

There is no anomaly that is pathognomonic for pregestational diabetes. A wide range of congenital anomalies affecting multiple organ systems has been reported in pregnancies complicated by pregestational diabetes. Pregestational diabetes confers a 26-fold increase in odds of caudal regression syndrome, yet a large retrospective cohort study demonstrated that only 17% of all caudal regression cases occurred in pregnant patients with diabetes.[28] The most frequently encountered anomalies in the setting of maternal diabetes affect the cardiovascular system (3.5 times increase in odds in patients with diabetes as compared to the background population) as well as the CNS (Table 8.4). In one analysis, 13.6% of infants of diabetic patients have multiple congenital anomalies.[28]

The association of congenital anomalies in the setting of pregestational diabetes is thought to be due to teratogenic effects of glucose and ketone bodies. High levels of glucose and ketone bodies (e.g., β-hydroxybutyrate) are associated with teratogenicity in animal models. Hyperglycemia leads to the increased production of reactive oxygen species, which can alter cell membranes, cause mitochondrial dysfunction, or promote pathologic apoptosis; all of these alterations may contribute to embryopathy.[29] Animal studies have demonstrated that hyperglycemia-induced excessive apoptosis may affect neural progenitor cells, contributing to nervous system anomalies.[29] Hyperglycemia-related oxidative stress may demonstrate a direct effect on the proliferation and migration of neural crest cells, which play a critical role in the development of the fetal heart. In animal models, these changes resulted in cardiac outflow tract defects.[30]

Congenital malformation risk in patients with pregestational diabetes may be further compounded by exposure to medications that may be teratogenic. Patients with diabetes have a greater risk of comorbid medical conditions such as chronic hypertension or

TABLE 8.4 Congenital Anomalies Reported in Fetuses of Diabetic Mothers

Organ System	Anomalies
Central nervous system	Spina bifida Anencephaly Hydrocephalus
Cardiovascular system	Ventricular septal defect Tetralogy of Fallot Transposition of the great arteries Hypoplastic left heart syndrome Coarctation of the aorta Atrial septal defect Pulmonic stenosis Double-outlet right ventricle Truncus arteriosus
Genitourinary tract	Hydronephrosis Renal agenesis Ureteral duplication Hypospadias
Gastrointestinal tract	Intestinal atresias Anal atresia

complications of long-standing diabetes (e.g., nephropathy). In this setting, angiotensin-converting enzyme (ACE) inhibitors may be prescribed outside of pregnancy, however, these medications are known to be teratogenic and thus have limited application in pregnancy: in a nondiabetic population, first trimester exposure has been associated with a 2.7-fold increased risk of major congenital malformations, including a fourfold increase in the risk of CNS anomalies and a 3.7-fold increase in the risk of cardiovascular anomalies.[31] Exposure to ACE inhibitors beyond the first trimester of pregnancy also has adverse effects, including fetal renal failure, oligohydramnios with its associated consequences (e.g., pulmonary hypoplasia if early oligohydramnios is severe and persistent), fetal growth restriction, and increased risk of stillbirth.

Antenatal detection of congenital malformations usually requires a detailed second trimester ultrasound evaluation. Systematic reviews evaluating the accuracy of ultrasound diagnosis at GAs less than 24 weeks report detection rates of 16% to 44% for all anomalies[32,33]; the overall detection rate of major lethal anomalies is higher, at 84%.[34] Rates of detection may vary on GA at the time of ultrasound exam, expertise of the sonographer, and patient factors (e.g., maternal obesity).

Cardiac anomalies comprise up to 40% to 50% of congenital malformations encountered in IDMs; it is estimated that there is a fivefold increase in risk of congenital cardiac anomalies in IDMs as compared with infants of pregnant patients without diabetes.[35] In a case-control study of around 8000 live and stillborn infants weighing greater than or equal to 500 g and greater than or equal to 20 weeks' gestation, the absolute risk of a major cardiovascular system defect was 8.5 per 100 live births in offspring of insulin-dependent diabetic patients; in that population, the absolute risk of a cardiovascular malformation in infants born to nondiabetic patients was 0.8 per 100 live births.[36] A 2018 population-based historical Swedish cohort study using health registers demonstrated among liveborn infants of patients with type 1 diabetes, increasingly worse glycemic control in the periconception time was associated with a progressively increased risk of major cardiac defects with an adjusted risk ratio >2 for HgbA1c <6.5 and adjusted risk ratio >6 for HgbA1c >9.[37]

Given the increased risk of neonatal morbidity and mortality with some cardiac malformations, antenatal diagnosis appears vital for optimization of obstetric and neonatal care. Some studies have suggested that an antenatal diagnosis of complex cardiac malformations may be associated with improved neonatal outcomes as compared with infants in whom these malformations were first diagnosed postnatally. Given the increased risk of cardiac malformations in fetuses of patients with pregestational diabetes, many institutions recommend screening fetal echocardiography in addition to a detailed second trimester fetal anatomical survey. These established associations speak to the importance of preconception care to optimize periconception glucose control for optimal maternal, fetal, and neonatal outcomes.

Fetal Growth and Macrosomia

Maternal diabetes is associated with an increased risk for abnormal fetal growth. Pregestational diabetes with microvascular complications (especially diabetic nephropathy) or concomitant chronic hypertension may have an increased risk of intrauterine growth restriction, potentially related to abnormalities in placental structure or perfusion.[38]

Excessive fetal growth is an aberrant pattern more commonly encountered in diabetic pregnancies. Large for gestational age (LGA) is commonly defined as birth weight greater than the 90th percentile for GA. Macrosomia is more variably defined: birth weight greater than 4000 g or greater than 4500 g without adjustment for GA. Both pregestational and gestational diabetic pregnancies have a greater prevalence of LGA and macrosomic infants. Birth weights greater than or equal to the 90th percentile have been reported to occur in 42% to 62% of type 1 diabetic pregnancies, 30% to 56% of type 2 diabetic pregnancies, and in 10% to 20% of pregnancies complicated by gestational diabetes.[39] For infants born to patients without diabetes, the rate of macrosomia (when defined as birth weight ≥4 kg) has been reported to be 7% to 8%, and the rates of LGA have been reported to be 8% to 14%.[40]

Excessive fetal growth is probably stimulated by maternal hyperglycemia as explained by the classic Pedersen hypothesis. Maternal glucose readily crosses the placenta by facilitated diffusion; fetal glucose levels are maintained at a level that is approximately 70% to 80% of the maternal concentration of glucose. Maternal insulin does not cross the placenta. Fetal hyperglycemia stimulates fetal pancreatic beta cell hypertrophy and increased insulin production. Insulin acts as a potent growth factor in utero; thus, fetal hyperinsulinemia and concomitant increases in insulin-like growth factors (IGFs) probably result in increased protein, lipid, and glycogen synthesis contributing to increased fetal growth.[40] The excess fetal growth appears to be characterized by increased fat deposition and visceral enlargement. Fetal insulin activity in response to fetal glucose levels becomes evident at around 20 weeks' gestation. Insulin receptor levels in target organs such as the fetal liver appear to become maximal in the mid-trimester, with insulin receptor binding affinities increasing as pregnancy progresses.

Maternal glycemic control has been identified as a predictor of fetal growth. Studies have demonstrated a positive linear association between maternal glucose levels and risk of LGA birth weights.[41] Elevated maternal HbA_{1c} levels and nonfasting as well as mean glucose levels have been associated with risk of abnormal fetal growth.[39,42] However, reducing maternal hyperglycemia does

not always reduce LGA. More recent evidence from studies using continuous glucose monitoring suggest an association between glycemic variability, particularly in the second and third trimesters, and accelerated fetal growth.[43]

The clinical pattern of abnormal fetal growth in the context of maternal diabetes appears to be characterized by accelerated disproportionate growth in the second half of pregnancy. Studies evaluating fetal growth utilizing ultrasound assessment demonstrated pregnancies with evidence of accelerated growth as early as 17 to 24 weeks' gestation, with maximal growth occurring late in the third trimester. Observations of accelerated fetal abdominal circumference growth have been made in patients with diabetes, and associated with LGA birth weights.[44–46] In an evaluation of LGA neonates, IDMs had significantly increased fat mass and increased percentage body fat as compared with infants whose mothers had normal gestational glucose tolerance.[47] In general, fetal fat deposition seems to occur almost entirely within the third trimester and appears to be sensitive to the effects of fetal insulin.

Antenatal diagnosis of macrosomia is limited by the accuracy of fetal sonographic estimations of fetal weight. Measurements of the fetal head (head circumference and biparietal diameter), abdominal circumference, and femur length are incorporated into a formula (usually derived from nonlinear regression analysis) to estimate fetal weight; fetal weight percentiles are then determined from one of several fetal growth curves that, historically, have been derived from various geographic locations using representative subpopulations. Most experts consider a mean percent error in estimated fetal weight of ±10% to be clinically relevant for accuracy. Studies evaluating the accuracy of third-trimester ultrasound estimates of fetal weight show that sonographic estimates fall within 10% of birth weight in 50% to 75% of cases; the reported mean percentage differences between ultrasound-predicted and actual birth weight have ranged from 6% to 15%.[48]

Sensitivities for the antenatal diagnosis or prediction of birth weight greater than or equal to 4000 g has been estimated to be as low as 53% to 59%.[49] In a cohort of patients with gestational diabetes, third-trimester ultrasound estimation of fetal weight significantly overestimated the prevalence of LGA infants; of those patients identified as having an estimated fetal weight greater than or equal to the 90th percentile, only 23% actually delivered an LGA neonate.[50] Some data suggest that prediction of clinically relevant LGA (e.g., LGA infants who will have the greatest risk of shoulder dystocia) may be improved by the use of customized fetal growth curves, by taking into account clinical factors such as suspected fetal sex, maternal height, weight, and parity that are physiologic determinants of fetal growth.[51]

Pregnancies complicated by pregestational diabetes and suspected macrosomia have a greater risk of shoulder dystocia, birth trauma, cesarean delivery, and neonatal complications such as hypoglycemia requiring treatment and hyperbilirubinemia.[52] Shoulder dystocia is defined as difficulty and delay in delivery of the fetal shoulders/body following delivery of the fetal head; this often results from impaction of the anterior fetal shoulder behind the maternal pubic bone. Shoulder dystocia requires additional obstetric maneuvers to effect delivery, and delayed delivery is associated with an increased risk of perinatal asphyxia (potentially related to impaired oxygenation from occlusion of the umbilical cord during delayed delivery), and maternal birth trauma. Macrosomia and maternal diabetes have been identified as independent risk factors for shoulder dystocia. In a nondiabetic population, macrosomia, when defined as birth weight greater than 4500 g, was associated with a 13-fold increase in the odds of shoulder dystocia as compared with fetuses without suspected macrosomia; the rate of shoulder dystocia in a nondiabetic population with macrosomic infants was 23%.[53] When controlling for birth weight and other confounders, diabetes (pregestational and gestational) is an independent risk factor for shoulder dystocia, with a threefold increase in odds.[54] Shoulder dystocia rates as high as 35% have been reported in pregnant patients with diabetes who deliver an infant with a birth weight between 4750 and 5000 g.[55] In deliveries complicated by shoulder dystocia, pregnant patients with insulin requirement have a greater rate of birth trauma than infants of nondiabetic patients; in a review of shoulder dystocia cases in the state of California, 24% of neonates delivered to patients with diabetes experienced reported birth trauma and 7.3% of infants of nondiabetic patients had evidence of birth trauma.[54]

Birth trauma appears to occur at an increased rate in suspected macrosomic infants, even in the absence of shoulder dystocia. In a cohort of infants with a birth weight greater than or equal to 4000 g, 7% had evidence of a traumatic birth injury—most commonly Erb's palsy and clavicular or humeral fractures.[56] Birth trauma risks appear to increase on a continuum as the birthweight increases.[57,58] Macrosomic infants also have an increased risk of requiring cesarean delivery; cesarean delivery rates as high as 50% to 60% for infants with birth weights greater than or equal to 4 kg have been reported.[56]

Stillbirth and Perinatal Mortality

Pregnancies complicated by diabetes have been noted to have an increased risk of stillbirth (usually defined as fetal demise diagnosed at GAs at or beyond 20 weeks) as well as perinatal mortality. Perinatal mortality has varying definitions; the most inclusive definition is a fetal death at 20 weeks or more or an infant death before 28 days of life.[59] The most recent US National Vital Statistics Report from 2013 demonstrated a fetal death (stillbirth) rate of 5.96 per 1000 live births. The perinatal mortality rate was 9.98 per 1000 live births plus fetal deaths for the time period of evaluation.[60] This report did not specify fetal death rates or perinatal mortality rates for pregnancies complicated by specific medical or obstetric comorbidities. Studies evaluating the risk of perinatal mortality in pregnancies complicated by diabetes may have been limited by variable definitions of perinatal mortality as well as a lack of uniform agreement regarding how to determine if maternal diabetes was a direct contributor to the demise.

The stillbirth risk among patients with pregestational diabetes has been reported to be as high as five times the risk of stillbirth noted in the general population.[61,62] In the United States, a multicenter population-based case-control study identified maternal diabetes as an independent risk factor for stillbirth, accounting for 5.6% of all stillbirths in the cohort (for stillbirths occurring at GAs of ≥24 weeks, the adjusted odds ratio of stillbirth in patients with diabetes was a 3.47, 95% confidence interval [CI] 1.86 to 6.49).[63]

The reported risk of stillbirth may differ in patients with type 1 diabetes as compared with those with type 2 diabetes. In a large Danish cohort study, incidences of stillbirth among patients with type 1 diabetes was 28 per 1000 births as compared with a rate of 4.5 per 1000 births in the general population.[64] The risk of stillbirth is similarly elevated in patients with type 2 diabetes, although the magnitude of risk that has been reported has varied, with some studies suggesting worse outcomes in type 2 diabetes while others suggest comparable outcomes in both type 1 and

type 2 diabetes. Large retrospective cohort studies conducted in Denmark and in New Zealand demonstrated perinatal mortality rates two to three times higher in patients with type 2 diabetes as compared with those who have type 1 diabetes.[65,66] It is possible that the risk of perinatal mortality may be increased in pregnancies complicated by type 2 diabetes due to association with maternal obesity. Obesity has been shown to be an independent risk factor for adverse pregnancy outcomes, including stillbirth and gestational hypertensive disorders, which can also impact fetal growth and risks of prematurity.[17,67,68] A systematic review evaluating population-based studies reporting on risk factors for stillbirth in high-income countries (such as the United States, Canada, the United Kingdom, Australia, and the Netherlands) demonstrated that diabetes is a maternal medical comorbidity with one of the strongest associations with stillbirth. In this systematic review, patients with preexisting diabetes had an adjusted odds of stillbirth 2.9 times that of patients without diabetes; however, the calculated population-attributable risk of diabetes to stillbirths is 3% to 5%. In comparison to diabetes, maternal diagnoses of obesity and overweight (defined as BMI >25 kg/m^2) had a higher population-attributable risk for stillbirth (8% to 18%). Maternal overweight and obesity were identified as the highest ranking modifiable risk factor for stillbirth in high-income countries.[69]

The risk of perinatal mortality in pregnancies complicated by GDM is not clear. Some studies have reported an increased risk of perinatal mortality with GDM, while other studies have not. Patients may be misclassified as GDM when in reality they are type 2 diabetics first identified during pregnancy leading to discrepant results. A large retrospective cohort study evaluating outcomes of singletons delivered in California at 36 to 42 weeks' gestation demonstrated that there was an increased risk of stillbirth in women with GDM as compared with women without diabetes, though the absolute risks are low: women with GDM experienced 17.1 stillbirths per 10,000 deliveries, whereas nondiabetic women had 12.7 stillbirths per 10,000 deliveries (RR 1.34, 95% CI 1.2 to 1.5). This same study also evaluated the risk of expectant management in pregnancies complicated by GDM and concluded that at 39 weeks' gestation, the risk of expectant management (stillbirth and infant mortality risks) appears to exceed the risks of delivery.[70] Results from similar studies inform recommendations for consideration of delivery at 39 to 40 weeks' gestation in pregnancies complicated by GDM requiring medical therapy. Patients with diet-controlled GDM have not been shown to have greater risk of stillbirth.[71]

Several studies evaluating risks of adverse outcomes in the setting of maternal diabetes have identified an association between poor glycemic control and increased risk of perinatal mortality. The etiologies of perinatal morbidity that may be relevant include severe congenital malformations, growth abnormalities (e.g., fetal growth restriction in women with microvascular complications of diabetes), prematurity, and intrauterine asphyxia.[72] These etiologies are anticipated to occur with increased frequency in the setting of poor maternal glycemic control and diabetes severity. Poorly controlled diabetes early in pregnancy may contribute to abnormalities in placental development: high glucose levels have been shown to have an inhibitory effect on human trophoblast invasion.[73] Placental pathology of this nature might be hypothesized to impact placental function and therefore impact risk of intrauterine demise.

Intrauterine asphyxia may result from fetal hyperinsulinemia that is thought to increase fetal oxygen consumption and therefore decrease fetal oxygen tension; this proposed pathophysiology seems supported by the observation that some fetuses of patients with type 1 diabetes have higher plasma lactate levels and lower blood pH levels than normal when evaluated by third-trimester cordocentesis.[74] This lactic acidemia observed in some of the fetuses of diabetic patients may contribute to the risk of perinatal mortality.

One etiology of fetal death that is unique to diabetes is maternal diabetic ketoacidosis (DKA). Pregnancy represents a time of increased risk of DKA as several of the physiologic changes of pregnancy predispose to DKA.[75] Pregnancy results in increased insulin resistance, enhanced lipolysis, and ketogenesis. Pregnant patients are also in a state of compensated respiratory alkalosis; therefore, there is decreased buffering capacity (lower plasma bicarbonate levels) in the setting of acidosis. Complications unique to pregnancy such as nausea and vomiting, further increase risk of DKA.

The perinatal mortality rate in the setting of maternal DKA has been reported to be as high as 9% to 35%. The exact mechanism of fetal death in the setting of maternal DKA is not completely understood. It is known that maternal ketone bodies freely cross the placenta and contribute to fetal acidosis. Maternal DKA is also characterized by significant maternal hypovolemia (resulting from osmotic diuresis), which likely results in decreased uteroplacental perfusion. Maternal acidemia also shifts the maternal oxyhemoglobin dissociation curve leftward, thus further compromising oxygen delivery to the fetus. Decreased fetal oxygenation and metabolic acidemia are likely significant contributors to fetal mortality in this setting. Pregnant patients who develop DKA are often critically ill, and fetal status often reflects maternal illness. Fetuses often demonstrate evidence of intrauterine distress during monitoring. In this setting, obstetric management prioritizes maternal stabilization; emergent cesarean delivery for fetal distress resulting from DKA before adequate maternal treatment and stabilization is associated with high risk of maternal morbidity and mortality. Fortunately, early recognition of maternal DKA and aggressive, efficient initiation of treatment can lead to maternal improvement and effective intrauterine resuscitation of the fetus.

Prevention of perinatal mortality in pregnancies complicated by diabetes primarily relies on ensuring good maternal glycemic control throughout gestation. Increased antenatal surveillance to try to identify at-risk fetuses is also an accepted part of the obstetric management of pregestational diabetic patients. Given the conflicting data regarding risks of perinatal mortality in GDM patients, current expert guidelines recommend antenatal testing for GDM patients requiring medication therapy, and may be deferred with GDM patients controlled on dietary therapy alone who are otherwise at low risk for perinatal complications.

Maternal Preeclampsia

Preeclampsia is a gestational hypertensive disorder; the diagnosis of preeclampsia is made with the onset after 20 weeks' gestation of persistent elevations in systolic blood pressure (≥140 mmHg) or diastolic blood pressure (≥90 mmHg) in patients with previously normal blood pressure as well as the new onset of proteinuria, defined as greater than or equal to 300 mg of protein per 24-hour urine collection or a protein/creatinine ratio greater than or equal to 0.3. The diagnosis of preeclampsia may also be made in the absence of proteinuria if there are other complications such as thrombocytopenia, renal insufficiency, impaired liver function, pulmonary edema, or neurologic symptoms (such as cerebral or visual symptoms) or HELLP syndrome.[76] In addition to the aforementioned maternal complications, preeclampsia is also associated

with an increased risk of fetal complications such as fetal growth restriction and oligohydramnios that are likely to reflect altered placental perfusion in the setting of preeclampsia. Patients with severe forms of preeclampsia are also at increased risk of placental abruption. Delivery is the only definitive treatment for preeclampsia. In the setting of maternal or fetal complications resulting from preeclampsia, delivery is medically indicated as the risk of perinatal and maternal morbidity and mortality is elevated.[77]

Patients with pregestational diabetes are at increased risk for developing preeclampsia. The prevalence of preeclampsia in the diabetic population has been estimated at 17%, which is five to six times higher than the general population.[78] Among diabetic patients, the risk of preeclampsia appears to increase with increasing duration of diabetes as well as with the presence of microvascular complications. In a US prospective observational cohort study, 11% of patients with a duration of diabetes of less than 10 years developed preeclampsia as compared with 22% of patients with diabetes of duration >10 years. Thirty-six percent of patients with proliferative diabetic retinopathy and/or diabetic nephropathy developed preeclampsia in this cohort. Patients with diabetic nephropathy with a baseline level of proteinuria ≥300 mg in a 24-hour urine collection had an increased risk of small for GA infants and of preterm delivery.[79]

Obstetric Management of Diabetes in Pregnancy

Preconception Care

Care of the pregestational diabetic patients should ideally begin before pregnancy. As discussed previously, preconception care of diabetic patients can improve outcomes; if good glycemic control is achieved and maintained throughout conception and early pregnancy, rates of miscarriage and congenital malformations are often indistinguishable from the general population in patients with uncomplicated diabetes.[80] Preconception care also allows for identification of teratogenic medications and hopefully substitution with safer alternatives (e.g., discontinuation of ACE inhibitors and transition to other antihypertensives that are compatible with pregnancy). Given the elevated risk of neural tube defects in diabetic pregnancies, folic acid supplementation should be initiated before conception. Identification of microvascular complications of diabetes such as retinopathy and nephropathy before pregnancy allows opportunities for treatment of these conditions (e.g., laser therapy for treatment of cases of proliferative retinopathy); optimization of these conditions will hopefully prevent progression of disease during pregnancy. Diabetic patients with microvascular complications also appear to have an additional increased risk of adverse pregnancy outcomes; for example, patients with diabetic nephropathy have an increased risk of having a fetus who develops fetal growth restriction and also have a risk of gestational hypertensive disorders such as preeclampsia that exceeds the risk conferred by diabetes alone. Low-dose aspirin has been evaluated as a possible intervention to reduce the risk of preeclampsia in patients who have an a priori elevated risk of developing this obstetric complication, and its use has been found to confer a modest reduction in the risk of developing preeclampsia. Low-dose aspirin in pregnancy appears to have a good safety profile. The U.S. Preventive Services Task Force has recommended the use of low-dose aspirin in patients at elevated risk of developing preeclampsia, including pregestational diabetic patients (type 1 or type 2).[81,82]

Identification of patients who have additional risk factors for adverse pregnancy outcomes may allow for pregestational optimization of medical comorbidities as well as closer maternal and fetal monitoring during pregnancy.

Medical Therapy for Diabetes in Pregnancy

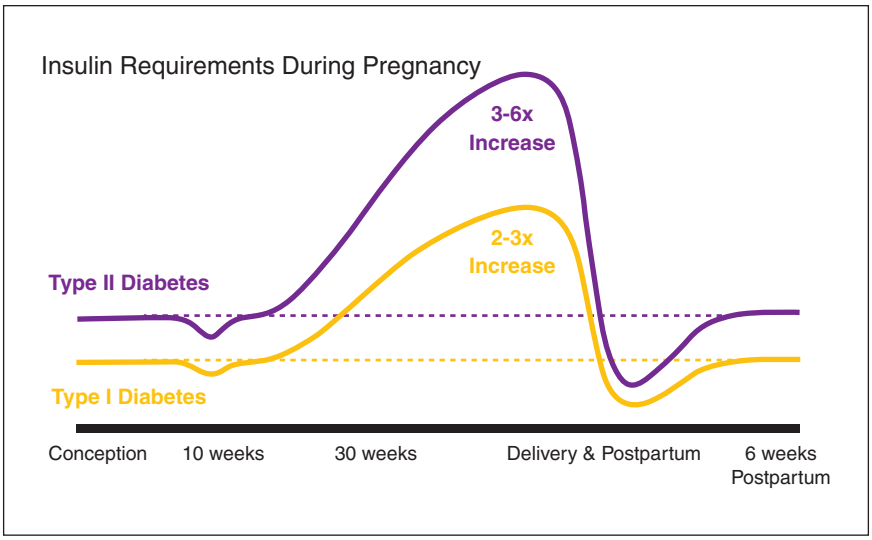
In general, maintaining tight glycemic control is a primary goal of the management of diabetes in pregnancy. The ideal target range for maternal blood glucose in pregnancy is not clear. Currently, expert consensus recommendations indicate the following targets for glycemic control in women with gestational and pregestational diabetes: fasting capillary blood glucose (CBG) of less than 95 mg/dL, 1-hour postprandial CBG levels below 140 mg/dL, or 2-hour postprandial levels less than 120 mg/dL; pharmacologic therapy is strongly recommended for patients with CBGs exceeding these thresholds.[5]

Results from the Hyperglycemia and Adverse Pregnancy Outcomes (HAPO) study raised the question of whether stricter glucose levels should be targeted. The HAPO study was a prospective multinational study that evaluated the relationship between maternal hyperglycemia and adverse pregnancy outcomes that has been attributed to maternal diabetes. The relationship between results from a 2-hour, 75-g oral glucose tolerance test and the following primary outcomes were evaluated: birth weight greater than the 90th percentile for GA; cord blood C-peptide levels greater than the 90th percentile; primary cesarean delivery; and clinical neonatal hypoglycemia. Maternal glucose values at each time point (fasting, 1 hour, and 2 hours after glucose challenge) were positively associated with the primary outcomes in a linear fashion. There was no apparent threshold glucose level above which risk for an adverse outcome was clearly elevated; frequencies of adverse outcomes rose steadily with worsening maternal hyperglycemia.[41]

As many adverse fetal/neonatal outcomes such as macrosomia and neonatal hypoglycemia are thought to result from fetal hyperinsulinemia in response to maternal hyperglycemia, it may be relevant to consider the maternal blood glucose levels in patients without diabetes. A review of 12 studies evaluating the glycemic profile of nondiabetic patients demonstrated fasting glucose levels of 71 ± 8 mg/dL (mean ±1 standard deviation); 1-hour postprandial levels of 109 ± 13 mg/dL; 2-hour postprandial levels of 99 ± 10 mg/dL; and 24-hr mean glucose of 88 ± 10 mg/dL, suggesting that further clinical study of maternal goals for glycemic control is warranted.[83]

Maintaining maternal glycemic control is made more difficult by physiologic changes of pregnancy that lead to worsening insulin resistance with advancing GA. Increasing levels of human placental lactogen and changes in levels of cortisol and prolactin are likely to exacerbate insulin resistance. Insulin sensitivity has been demonstrated to decrease by as much as 56% by 36 weeks' gestation. For patients with pregestational diabetes, third-trimester insulin requirements may be two to three times greater than prepregnancy doses (Fig. 8.4). Obese patients with type 2 diabetes may demonstrate extreme levels of insulin resistance.

The changing landscape of diabetes management brings new opportunities for improvement in maternal glycemic control, safety, and neonatal outcomes. Insulin pumps deliver insulin using a small, externally worn, computerized device to deliver micro-boluses of (typically) rapid-acting subcutaneous insulin over the course of 24 hours. Pump users can alter basal insulin rates throughout the day, rather than relying on the fixed



• **Fig. 8.4** Insulin Requirements During Pregnancy. (Courtesy LaVone Simmons, MD.)

pharmacokinetic curves of injectable insulin. Users inject a bolus for mealtime insulin using the same pump, allowing for fewer injections, flexibility in meal-timing and the ability to optimize and manage glucose control for individual exercise habits and diurnal variation. The continuous glucose monitor (CGM) is a separate device which assesses interstitial glucose values every 1 to 5 minutes, allowing the user far fewer needlesticks and the ability to evaluate temporal trends in glycemia throughout the day. The devices may be worn separately, or in some cases paired to allow for sensor-augmented and hybrid closed loop therapies.

Several studies note the safety and benefit of Continuous Glucose Monitoring during pregnancy. CGM use during pregnancy remains "off-label" in the United States; however, it is approved for use in Europe. A large, multicenter, randomized control trial, named the CONCEPTT study, demonstrated significant improvement in neonatal outcomes with use of real-time CGM as compared to self-monitored blood glucose (SMBG). Infants born to mothers in the CGM group were less likely to be large for gestational age, had lower rates of neonatal hypoglycemia, fewer NICU admissions, and shorter hospital stays.[84] While there was no significant impact on maternal outcomes or HgbA1C values, the study suggests that other glycemic factors, like the time spent in normal glycemic range (TIR), glucose variability, and temporal profiles may have a significant impact on neonatal outcome.[85] Spending 5% more time in normal range (63 to 140 mg/dL) each day, and 5% less time above range (>140 mg/dL) in the second and third trimesters, improves neonatal outcomes such has neonatal hypoglycemia, LGA, and NICU admission.[86] As advances in the use of diabetes technology in the perinatal period continue, researchers have the opportunity to gain clarity in the specific glucose variables that contribute to maternal and neonatal outcomes.

All diabetic patients require dietary therapy during pregnancy. Consultation with a registered dietician is an important part of care to ensure that the nutritional requirements of pregnancy are met and the distribution of calories from carbohydrates, proteins, and fats contributes to optimal glycemic control. In obese patients, in particular, attention to dietary therapy is also critical to the goal of preventing excessive weight gain. Excessive weight gain appears to be an independent contributor to some adverse outcomes such as fetal macrosomia.

Medical management of maternal hyperglycemia in patients with pregestational diabetes commonly relies on subcutaneous insulin, regardless of the method of insulin delivery. Insulin does not cross the placenta. Insulin has been well studied in pregnancy and has been shown to be effective in achieving good maternal glycemic control and in reducing the risk of adverse obstetric outcomes.

Patients with GDM or mild type 2 diabetes may be treated with oral medications such as metformin instead of insulin. The use of oral medications, especially for the treatment of GDM, has become increasingly popular following the publication of clinical trials that demonstrated the effectiveness of these medications. Metformin has long been used in type 2 diabetes; it acts primarily by decreasing hepatic glucose production, decreasing intestinal absorption of glucose, and by improving insulin sensitivity in peripheral tissues. Metformin has a low risk of producing hypoglycemia. In a randomized clinical trial comparing metformin with insulin for the treatment of GDM, there was no significant difference in neonatal outcomes, including hypoglycemia, birth trauma, respiratory distress, or hyperbilirubinemia.[87] It was notable that 46% of women in the metformin group ultimately required insulin in order to maintain glycemic control; these women were more likely to be overweight and appeared to have had higher fasting glucose values at study entry. Metformin therapy has the additional benefit of being associated with significantly less maternal weight gain than insulin therapy alone.[88]

Treatment conversations, particularly with regard to oral medications, require finesse. Both metformin and glyburide have been shown to cross the placenta. Patients should be counseled on the limited safety data of all oral medications in pregnancy. Evidence exists on the short-term consequences of fetal exposure to metformin, and is reassuring.[88] However, meta-analyses of infants exposed to glyburide vs. insulin for the treatment of GDM remain concerning. Infants exposed to glyburide had significantly higher rates of respiratory distress, macrosomia, hypoglycemia, and birth injury, despite no difference in maternal glycemic control.[89] Furthermore, smaller observational studies show higher rates of preeclampsia, hyperbilirubinemia, and stillbirth when glyburide is used, as compared with insulin. Therefore, numerous governing bodies do not recommend use of glyburide for treatment of gestational diabetes.

The choice between insulin and metformin is less clear. While insulin does not cross the placenta, its injectable nature makes adherence difficult for many when compared with oral medications. Unfortunately, long-term data on glucose homeostasis and the progression to type 2 DM in infants exposed to metformin in utero is lacking, despite reassuring short-term data in the neonatal period. Seven- to nine-year follow-up exists for infants exposed to metformin vs. insulin in the Metformin in Gestational Diabetes Trial (MiG). The results showed similar total body fat percentages and other metabolic measures.[87] The Society for Maternal Fetal Medicine continues to endorse metformin as a reasonable and safe first-line alternative to insulin in patients whom medical nutrition therapy fails.[90] Maternal and perinatal outcomes continue to be influenced by factors beyond drug choice. Those include timing of screening, screening test chosen, treatment protocols (initiation, adjustment, addition, or medication), as well as genetic predisposition. Continued investigation of not just long-term consequences of pharmacologic choice, but also of these other factors, is required.

Antenatal Monitoring

As discussed previously, pregnancies complicated by diabetes are at increased risk for abnormal fetal growth as well as perinatal asphyxia and stillbirth. These complications warrant increased antenatal surveillance in the third trimester. Results of testing or changes in the pattern of testing may help to inform decisions regarding timing and mode of delivery.

Fetal acidemia may produce characteristic changes on biophysical tests. Tests currently utilized include maternal assessment of fetal movement, fetal heart rate testing (nonstress test or contraction stress test), and ultrasound biophysical profile testing.

Maternal assessment of fetal movement is a frequently recommended component of fetal monitoring programs. Studies have noted that decreased fetal movement may be perceived in the period preceding fetal death, however, there does not appear to be a superior method. A suggested protocol considers 10 distinct movements within 2 hours reassuring.[91] Fetal heart rate testing relies on a mature and normally functioning fetal autonomic system, and assessment of heart rate baseline, variability, and the presence of accelerations and decelerations. Fetal gestational age is an important consideration, as the likelihood of a nonreactive fetal heart rate test is greater at preterm GAs. Given these considerations, most centers do not initiate these screening tests until 32 to 34 weeks unless there are indications for earlier testing (e.g., concern for fetal growth restriction, poorly controlled maternal diabetes, or hypertension, etc.). Biophysical profile testing relies on sonographic evaluation of amniotic fluid volume, fetal movement, demonstration of fetal tone, and fetal breathing movements. Each component of this examination is given a score, with a low score considered abnormal and requiring further assessment and, potentially, intervention.

It is not clear that antenatal fetal testing as described above is useful in preventing stillbirth or perinatal asphyxia, but these tests may be clinically useful in providing reassurance that fetal status is stable so as to allow for continued expectant management until a favorable term GA is reached. If stillbirth within 1 week of testing is used as the primary outcome of interest for antenatal fetal testing, both fetal heart rate testing and biophysical profile testing appear to have high negative predictive values (>99%) for reactive nonstress tests and biophysical profile scores of 8 to 10. In diabetic pregnancies (especially if poorly controlled), more frequent testing may be warranted as the fetal testing strategies above may miss fetal compromise precipitated by acute maternal metabolic changes. The appropriate frequency of fetal testing in pregnancies complicated by diabetes has been difficult to establish. Twice-weekly testing is commonly recommended in most centers. This testing strategy has been evaluated in a large retrospective cohort that demonstrated an overall low stillbirth rate, with the observation that none of the stillbirths occurred within 4 days of the last antepartum test.[92]

Evaluation of fetal growth also contributes to the assessment of fetal status. As discussed previously, third-trimester evaluation of fetal growth is significantly limited by increasing inaccuracy of predicted fetal weights as gestation progresses. Despite these limitations, third-trimester ultrasound assessment of growth is commonly recommended, given the risk of pathologic growth in diabetic pregnancies. For patients with microvascular complications of diabetes, concomitant chronic hypertension, and preeclampsia, fetal growth restriction may confer further elevated risk of perinatal morbidity and mortality, thus necessitating additional antenatal testing (e.g., fetal umbilical Doppler studies) to help inform obstetric management. While fetal macrosomia has been difficult to accurately diagnose antenatally, findings suggestive of predicted birth weight above 4500 g appear to be associated with an increased risk of shoulder dystocia. In this setting, it may be reasonable to consider cesarean delivery in an attempt to avoid shoulder dystocia and resultant birth trauma.

Delivery Planning

Patients with diet-controlled gestational diabetes appear to have a low risk of perinatal complications; therefore, early delivery is not indicated, and expectant management is usually appropriate. For pregestational diabetics and gestational diabetics requiring medication management, timed early delivery has been considered in an attempt to reduce perinatal morbidity and mortality. Strategies of scheduled delivery at 38 to 39 weeks for women with GDM have been evaluated in clinical trials; results have suggested that while there may be some potential reduction in macrosomia or LGA with earlier delivery, there does not seem to be a significant reduction in shoulder dystocia, neonatal hypoglycemia, or perinatal death.[92,93] Given recent evidence that suggests early term births (at 37 to 39 weeks) may be associated with differences in rates of neonatal complications (such as respiratory complications) as compared with deliveries at term (39 to 40 weeks)[94], it is probably reasonable for most well-controlled pregestational and gestational diabetics to plan for a delivery at around 39 weeks' gestation. Delivery at GAs greater than 40 weeks' gestation may be associated with a greater risk of macrosomia and shoulder dystocia and does not appear to be associated with other improvement in neonatal or maternal outcome.[95] Elective cesarean delivery in fetuses suspected of macrosomia has also been considered. As discussed previously, macrosomic infants have a greater risk of shoulder dystocia and IDMs have a greater risk of birth trauma resulting from shoulder dystocia. Based on evaluation of reported rates of shoulder dystocia and birth trauma at different birth weights, it has been proposed that it may be reasonable to consider cesarean delivery for fetuses with estimated fetal weights greater than 4500 g. The potential neonatal benefit of cesarean delivery in these cases must be balanced against potential maternal morbidity associated with cesarean delivery. Studies evaluating potential benefit of cesarean delivery for presumed macrosomia estimate that up to 588 elective cesarean deliveries for an estimated fetal weight of

4500 g may need to be performed to prevent one case of a permanent brachial plexus palsy.[96]

Intrapartum Diabetes Management

The goals of intrapartum diabetes management are to maintain strict glycemic control and to avoid ketosis. Titration of an intravenous infusion of short-acting insulin is a common strategy for maintaining blood sugars at less than 140 mg/dL (some expert opinions recommend levels <120 mg/dL). Maintenance of blood sugars at this level may be helpful in reducing neonatal hypoglycemia. Maternal hyperglycemia intrapartum has been shown to be associated with higher rates of neonatal hypoglycemia, probably by worsening the fetal hyperinsulinemic response. After delivery, the neonate will no longer have a steady supply of maternal glucose as a fuel, but higher insulin levels persist.

Neonatal Considerations

Hypoglycemia

Neonatal hypoglycemia is one of the more common neonatal morbidities encountered in diabetic pregnancies, with reports citing rates of neonatal hypoglycemia as high as 25% to 50%. Studies in which good maternal glycemic control were reportedly achieved suggest a lower incidence of neonatal hypoglycemia in the range of 5% to 15%.[56] The reported rates of neonatal hypoglycemia have varied in the medical literature; this is probably due to the continued debate about clinically meaningful definitions of hypoglycemia. Severe or persistent hypoglycemia is known to cause adverse neurologic and endocrine sequelae; however, it is not clear what levels of neonatal glucose are optimal to minimize risk of these complications. Defining these optimal newborn glucose levels is likely further confounded by the observation that preterm and low birth weight neonates are more likely to have lower blood sugars.[97,98]

Most studies have defined neonatal hypoglycemia as blood glucose (BG) levels below 35 to 45 mg/dL, but clearly not all infants who have hypoglycemia as defined by these levels demonstrate evidence of adverse effects.[98] In a large prospective cohort study evaluating neonates born at GAs of 35 weeks or older, hypoglycemia (defined as a BG < 47 mg/dL) when treated did not appear to be associated with an increased risk of neurosensory impairment when assessed at 2 years of age. It was also notable that the lowest BG concentration observed in these neonates as well as the number of hypoglycemic episodes did not predict the child's neurodevelopmental outcome at age 2 years.[99] Many infants with hypoglycemia (defined as BG <40 mg/dL) may initially be asymptomatic. Symptoms attributable to hypoglycemia may include tremor, jitteriness, irritability, lethargy, and hypotonia; severe cases of hypoglycemia may also lead to convulsions, apnea, or cyanosis.

Neonatal hypoglycemia resulting from diabetic pregnancies is most likely due to fetal hyperinsulinism, i.e., a response to fetal hyperglycemia resulting from maternal hyperglycemia. After birth, the infant no longer receives a steady supply of maternal glucose; if there is hyperinsulinemia originating in utero, then the neonatal period is characterized by a relative imbalance of insulin as compared with neonatal glucose supply. This imbalance leads to a risk of hypoglycemia evolving within the first few hours of life with a nadir in blood glucose levels reported at 1 to 3 hours of life in most infants.

Neonates with hypoglycemia have been observed to have elevated cord blood C-peptide and serum insulin levels at birth.[100] Based on the pathophysiology, it is not surprising that poor maternal glycemic control in the third trimester and in the intrapartum period is a predictor of neonatal hypoglycemia.[100,101] Maternal plasma glucose levels at delivery have been shown to correlate strongly and negatively with neonatal glucose concentrations at 2 hours of life; in observational studies, rates of neonatal hypoglycemia were significantly lower if maternal blood glucose levels were less than 130 mg/dL.[100,102] Intrapartum maternal hyperglycemia and resulting fetal hyperglycemia probably stimulate the fetal pancreas to release more insulin, with this effect persisting through delivery and for the first few hours thereafter. Macrosomia has also been shown to be a risk factor for neonatal hypoglycemia, with some studies suggesting a prevalence of 30% to 50% in LGA IDMs; this observation is not surprising, as excessive fetal growth has also been attributed to the effects of fetal hyperinsulinemia.[102,103]

In addition to optimizing maternal glycemic control throughout gestation, strict maternal glycemic control in labor or in the hours immediately preceding delivery (e.g., if scheduled cesarean section) will help to prevent or reduce rates of neonatal hypoglycemia. Current expert recommendations for maternal intrapartum glycemic control vary; ACOG recommends maintaining maternal blood glucose levels between 70 and 110 mg/dL for pregestational diabetics.[104] Attention to early neonatal feeding within the first hour with promotion of breastfeeding (and/or access to colostrum/supplementation) may also help to prevent neonatal hypoglycemia.[105–107]

Respiratory Distress

Neonates of diabetic patients may also be at greater risk of respiratory complications and respiratory distress syndrome (RDS) as compared with same GA infants born to nondiabetic patients. This increased risk of respiratory compromise appears to affect even late-preterm and early-term neonates, though studies evaluating risk of respiratory distress at GAs greater than 38 weeks vary in their results. In a large retrospective cohort evaluating infants born at GAs of 34 weeks or greater, gestational diabetes was identified as an independent risk factor for severe neonatal respiratory failure (requiring admission to the neonatal intensive care unit or the need for ventilator support at 24 hours of age).[108] The pathophysiology of neonatal respiratory distress related to perinatal diabetes is thought to result from a delay in pulmonary maturation and surfactant production, leading to a relative surfactant deficiency.[109] Insulin appears to have a role in regulating fetal lung maturation; in a cell culture model of type 1 alveolar cells, exposure to elevated levels of insulin reduced choline incorporation into surfactant phosphatidylcholine.[110] Fetal hyperinsulinemia may have an inhibitory effect on glycogenolysis, which contributes to a decrease in the synthesis of phosphatidylglycerol (PG).

Delivery prior to 39 weeks may be considered clinically when there is persistent suboptimal or worsening maternal glycemic control that is not responsive to usual medical therapy or when there is concern for significant risk of adverse fetal outcomes (e.g., in the setting of macrosomia and polyhydramnios), and this is usually performed without an amniocentesis to confirm fetal lung maturity.

Antenatal Corticosteroids for Reduction in Risk of Respiratory Distress Syndrome

In clinical settings in which early preterm birth (at GAs <34 weeks) is anticipated, antenatal corticosteroids may be considered to reduce the risk of RDS and other neonatal complications of

prematurity. In the setting of maternal diabetes, the decision to administer antenatal corticosteroids must also take into consideration the potential maternal impact of these medications. Corticosteroid administration can lead to maternal hyperglycemia by promoting hepatic gluconeogenesis and impairing insulin sensitivity.[111] Significant maternal hyperglycemia resulting from the administration of antenatal corticosteroids must be anticipated because if left untreated there may be an increased risk of maternal complications such as DKA in patients with type 1 diabetes.[112,113] An evaluation of the impact of antenatal corticosteroid use in pregnant diabetic patients demonstrated a significant increase in insulin requirements for the 5 days following medication administration. Peak insulin requirements were noted on days 2 to 3 after administration, with an approximate 40% increase in insulin dosage needed to achieve glycemic control.[114]

Because of the potentially adverse maternal effects resulting from corticosteroid administration, women with diabetes were excluded from the recent National Institute of Child Health and Human Development multicenter trial evaluating the role of corticosteroid therapy in pregnancies at risk of late preterm birth between 34 + 0 and 36 + 5 weeks' gestation. This trial demonstrated a significant decrease in the need for neonatal respiratory support and a decrease in the rate of severe respiratory complications in those infants exposed in utero to corticosteroids prior to delivery; however, neonatal hypoglycemia was significantly more common in the group of exposed infants (24% in the betamethasone group vs. 15% in the placebo group, RR 1.60, 95% CI 1.37 to 1.87).[115] These results have led ACOG to advise the consideration of betamethasone administration in singleton pregnancies at risk for late preterm birth. However, ACOG acknowledges that patients with pregestational diabetes were not included in the Antenatal Late Preterm Steroids trial, so the effect of late preterm corticosteroids in this population is unknown.[116] In this setting, the potential adverse effects of late preterm corticosteroids are likely difficult to justify in patients with pregestational diabetes.

Hypertrophic Cardiomyopathy

Neonates of diabetic patients have an increased risk of developing hypertrophic cardiomyopathy. The most common echocardiographic findings include asymmetric interventricular septal enlargement, with hypertrophy of the ventricular free walls noted to a lesser extent. On average, most series reporting on the prevalence of hypertrophic cardiomyopathy note that 30% to 40% of neonates of diabetic patients have evidence of these cardiac changes on imaging, but only approximately 5% of infants will manifest symptoms suggestive of congestive heart failure. Heart failure may be caused by interventricular septal hypertrophy, leading to obstruction of the left ventricular outflow tract. Most symptomatic infants require only supportive care. In almost all cases, the myocardial hypertrophy is found to resolve spontaneously within 6 to 12 months of life.

The underlying etiology of hypertrophic cardiomyopathy is still incompletely understood; it is hypothesized to be related to fetal hyperinsulinemia.[117] As noted in other organs, increased insulin and related factors such as IGFs have been found to stimulate hyperplasia and hypertrophy. In myocardial cells, insulin inhibits the enzyme glycogen synthase kinase-3, which is a negative regulator of myocardial growth; its inhibition might contribute to abnormal myocardial hypertrophy. Further evidence in support of fetal hyperinsulinemia in the pathogenesis of hypertrophic cardiomyopathy comes from the observation that infants with congenital hyperinsulinism (in the absence of maternal diabetes) also appear to have an increased risk of hypertrophic cardiomyopathy. In a series of 25 infants with congenital hyperinsulinism who required neonatal echocardiogram evaluation for symptoms (e.g., arrhythmias, auscultated murmurs, etc.), 40% were found to have evidence of hypertrophic cardiomyopathy with features similar to that found in neonates of diabetic parturients.[118] Findings from serial fetal echocardiographic evaluations have also raised the hypothesis that structural cardiac changes may represent an adaptive response to altered fetal cardiac function early in gestation. Fetuses of diabetic parturients demonstrated impaired diastolic function (prolonged isovolumetric relaxation time and lower early/atrial [E/A] ratio) and poorer global cardiac function (increased myocardial performance index or Tei index) as compared with fetuses of nondiabetic parturients. The fetuses of diabetic parturients went on to develop evidence of interventricular septal thickening in the third trimester but had no persistent evidence of cardiac dysfunction later in gestation.[119] Maternal metabolic control during organogenesis is known to have a significant impact on fetal cardiac development, as discussed earlier in this chapter.

Predictors of symptomatic cardiomyopathy in neonates of diabetic parturients are limited at this time. Despite evidence that supports a role for fetal hyperinsulinemia in the development of hypertrophic cardiomyopathy, the association between maternal glycemic control and this fetal/neonatal complication is not clear. Clinical studies evaluating the correlation between fetal/neonatal hypertrophic cardiomyopathy and maternal HbA_{1c} values have been mixed in their conclusions. Investigators have reported evidence of septal hypertrophy even in fetuses of parturients with well-controlled diabetes. Third-trimester fetal echocardiography has been used to measure the myocardial performance index (as an indicator of global cardiac function) and the E wave/A wave peak velocity (E/A) ratio at the mitral valve, as an indicator of ventricular diastolic function, in an attempt to identify fetuses of diabetic parturients who are at increased risk of adverse perinatal outcome (including symptomatic cardiomyopathy). In a small series, these fetal echocardiographic measurements appeared to have reasonable sensitivity and specificity for the prediction of a composite of adverse perinatal outcomes, but given the low prevalence of the most serious of these outcomes (symptomatic cardiomyopathy, perinatal death), the predictive value and the cost-effectiveness of these measures are probably limited at this time.[120]

Hypocalcemia and Hypomagnesemia

Calcium and magnesium are actively transported across the placenta to the fetus throughout gestation. At delivery, the transfer of these minerals is terminated and neonatal levels of these minerals are expected to decrease in most infants, but IDMs appear to have an increased risk of more significant declines in levels of calcium and magnesium.

Neonatal hypocalcemia is often defined as a serum calcium level less than 7 mg/dL or an ionized calcium level less than 4.4 mg/dL (1.1 mmol/L). Studies have historically reported that up to 50% of IDMs may have hypocalcemia in the neonatal period, though more recent reports suggest a lower prevalence of 4% to 5%.[56] Most affected infants will be asymptomatic. This condition is often found to resolve spontaneously. It is suspected that neonatal hypocalcemia may result from a delayed transition from fetal to

neonatal parathyroid action in calcium metabolism as fetal parathyroid glands are relatively inactive until after delivery. The risk of neonatal hypocalcemia is further increased in the setting of prematurity as well as perinatal asphyxia.

Neonatal hypomagnesemia is defined as a serum magnesium level below 1.5 mg/dL. Hypomagnesemia has been reported to affect as many as 40% of neonates of diabetic parturients, although most usually remain asymptomatic. Neonatal hypomagnesemia appears to be most clinically relevant in the context of significant hypocalcemia, as concurrent hypomagnesemia makes the treatment of hypocalcemia more difficult. Magnesium deficiency probably has adverse effects on parathyroid function, thus exacerbating hypocalcemia. The pathophysiology underlying neonatal hypomagnesemia is not clear. Some experts have hypothesized that fetal hypomagnesemia may result from lower maternal magnesium levels in the setting of diabetes from increased maternal urinary losses or renal dysfunction.

Clinical signs and symptoms of hypocalcemia and hypomagnesemia are similar to those of hypoglycemia and include jitteriness, irritability, tachypnea, and possibly seizures in severe cases. Unlike hypoglycemia, which often presents within the first few hours of life, symptoms of hypocalcemia and hypomagnesemia typically present later at 24 to 72 hours after birth.[121]

Polycythemia

Polycythemia, defined as a venous hematocrit above 65% or hemoglobin greater than 20 g/dL, is also observed more often in IDMs. Polycythemia has been observed in approximately 3% of all infants born at sea level, with a slightly higher prevalence of 5% in infants born at higher altitudes. Among neonates of diabetic parturients, the reported prevalence has ranged from 5% to 40%.[122] Fetal hyperglycemia leads to increased catabolism and increased oxygen consumption resulting in decreased oxygen tension. If chronic, this relative fetal hypoxemia is thought to stimulate erythropoiesis, resulting in polycythemia. Some small series have reported an association between maternal diabetic control (as represented by HbA_{1c} levels) and neonatal hematocrit. Higher erythropoietin levels have been noted in the amniotic fluid of patients with diabetes. Neonates of diabetic parturients have also been shown to have increased erythropoietin levels at birth; erythropoietin levels in neonates of diabetic parturients have also been shown to correlate with amniotic fluid glucose and insulin levels, as well as cord blood insulin levels.

Infants with polycythemia may be at greater risk of complications resulting from hyperviscosity; reported complications include ischemia and infarction such as within the kidneys or within the CNS. These complications attributable to hyperviscosity may explain the increased incidence of renal vein thrombosis and stroke that have previously been reported in IDMs. Polycythemia in utero may also lead to deficient iron stores in the liver, brain, and heart; iron deficiency in these organs may then predispose to myopathies and altered neurodevelopment.

Infants with clinically significant polycythemia are likely to appear plethoric and may demonstrate sluggishness or lethargy. Treatment is currently initiated on the basis of symptoms and clinical status; treatment is not recommended on the basis of hematocrit value alone as the hematocrit level does not appear to correlate well with clinical hyperviscosity. Reported therapies include intravenous fluid administration and possible consideration of partial volume exchange transfusion in some severe cases.

Hyperbilirubinemia

Neonates of diabetic parturients also appear to be at greater risk for hyperbilirubinemia than infants born to nondiabetic patients. In studies that defined hyperbilirubinemia as a serum bilirubin level greater than 12 mg/dL, or any bilirubin level requiring phototherapy, the prevalence of hyperbilirubinemia in neonates of diabetic parturients was 25%[56]; other series report a hyperbilirubinemia prevalence of 10% to 13%. This increased risk might be attributable to polycythemia (larger source of bilirubin to be conjugated by the liver prior to excretion), ineffective erythropoiesis with an increased red blood cell turnover, as well as to immaturity of hepatic bilirubin conjugation and excretion. Macrosomic neonates of diabetic parturients appear to have the greatest risk of hyperbilirubinemia[123]; this probably reflects the role of poor maternal glycemic control during pregnancy as neonates of diabetic parturients are more likely to be macrosomic and polycythemic.

Breastfeeding

Breastfeeding is important to the health and well-being of all children; the benefits of breastfeeding may be greater in neonates of diabetic parturients. Early feeding in the immediate neonatal period may help to prevent hypoglycemia or effectively treat mild cases of hypoglycemia.

The positive effects of breastfeeding for neonates of diabetic parturients are likely to extend significantly beyond the neonatal period. Intrauterine exposure to maternal diabetes and/or obesity appears to be associated with an increased risk of childhood obesity; successful breastfeeding of durations greater than or equal to 6 months may mitigate some of this risk. There has been increasing evidence of the long-term impact of the intrauterine environment on the development of adulthood diseases. Several cohort studies have suggested an increased risk of diabetes and obesity in childhood or adolescence in the offspring of diabetic patients.[124–126] Studies conducted in the Pima Indian population demonstrated that offspring of diabetic patients had a significantly increased risk of type 1 diabetes (12-fold increased odds) when compared to offspring of nondiabetic patients.[127] Offspring of diabetic patients also were noted to be heavier than offspring of nondiabetics when evaluated at ages 5 to 9 years and at 10 to 14 years; the greater risk of obesity in offspring of diabetic patients persisted even among those who had a normal birth weight.[127]

A longer duration of breastfeeding has been associated with a decreased risk of offspring childhood obesity. A meta-analysis of studies conducted in general populations demonstrated that each month of breastfeeding was associated with a 4% decrease in risk of being overweight in childhood.[128] In offspring of diabetic parturients, breastfeeding duration of greater than or equal to 6 months has also been associated with decreased measures of adiposity such as waist circumference and measures of visceral and subcutaneous abdominal fat.[129] The obstetric community has increasingly recognized the importance of recommending and advocating for breastfeeding among diabetic parturients, as they are anticipated to benefit as well. During lactation, postpartum patients with diabetes have a significant decrease in insulin or oral hypoglycemic medication requirements; in fact, postpartum diabetic patients (especially type 1) are at an increased risk of hypoglycemia while breastfeeding. Predelivery counseling by their obstetric and/or endocrinologic providers can help to facilitate an

appropriate medical management and safety plan during lactation; close follow-up during the postpartum period with attention to issues unique to lactation in the context of diabetes can help to optimize breastfeeding success.

The authors would like to acknowledge the 10th edition authors, Drs. Zane Brown and Justine Chang.

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9

Maternal Medical Disorders of Fetal Significance

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KEY POINTS

- Diagnostic imaging carries a risk of fetal radiation exposure. However, in most cases the actual fetal dose is low. While x-ray and computed tomography imaging should be avoided during pregnancy if possible, these studies are not contraindicated if needed.
- Adverse pregnancy outcomes in patients with lupus are more frequently seen in those with renal disease, antiphospholipid antibody syndrome, thrombocytopenia, and active disease.
- Cardiovascular disease in pregnancy is increasing in frequency and has become a major contributor to maternal mortality.
- The evaluation and management of most cancers should not be delayed for the sake of pregnancy. Surgery and most chemotherapeutic agents have been successfully used during the second and third trimesters.
- Epilepsy is associated with an increased rate of poor reproductive outcomes regardless of medication use. Medications used to control seizures do incur an increased rate of fetal anomalies, but the benefits to mother and fetus usually outweigh their risk.
- Selective serotonin reuptake inhibitors are considered first-line medication therapy for depression in pregnancy.

Pregnancies complicated by an underlying maternal medical condition increase the potential of a suboptimal perinatal outcome. Obstetricians are increasingly faced with patients affected by a medical problem that is unrelated to pregnancy. While diagnostic and therapeutic options for a nonpregnant adult in most cases can be effectively applied during pregnancy, there are concerns about whether these will adversely affect the fetus or newborn. Furthermore, the alterations of anatomy and physiology during pregnancy may require adjustments to therapeutic options.

Two questions should be addressed when a specialist is caring for a pregnant patient with a known medical problem. First, is the condition itself affected by the anatomic or physiologic alterations of pregnancy? Second, how does the medical problem potentially affect the patient, the fetus, or the ultimate perinatal outcome? A further concern is how to evaluate a newly suspected medical problem during pregnancy. Pregnancy itself can be a confounder as many symptoms experienced are similar to those of certain medical or surgical conditions, leading to either a lack of or delay in diagnosis. Providers may be reticent to perform needed imaging studies, proceed with indicated surgical procedures, or prescribe medications over concerns regarding risk to the fetus. Delays of diagnoses of de novo medical problems due to pregnancy have been reported. Unfortunately, in some cases, a lack of timely recognition and initiation of appropriate therapy may result in poor maternal or fetal outcomes.

General Principles in the Diagnosis and Management of Medical Complications During Pregnancy

Diagnostic Imaging

Ultrasonography and magnetic resonance imaging (MRI) are not associated with fetal risk and should be considered the imaging of choice for pregnant patients.[1] Diagnostic radiography, computed tomography (CT) scans, and nuclear medicine studies may be associated with fetal radiation exposure, even when studies target specific anatomy remote from the pregnant uterus (Table 9.1).[2] The fetal consequences of radiation exposure are both dose and time dependent (Table 9.2).[3] However, for most diagnostic procedures, actual fetal exposure is relatively low.

If possible, the practitioner should limit the amount of radiographic testing performed during pregnancy, but indicated studies should never be withheld because of pregnancy. Lead shielding of the abdomen and pelvis and careful selection of the type of study should be undertaken to minimize the fetal dose. To simplify the various measures of exposure, 1 radiation absorbed dose (rad), 1 Roentgen equivalents man (rem), 10 milliGray (mGy), and 10 milliSievert (mSv) can be considered equivalent.[4]

If the amount of fetal exposure is less than 5 rad, there appears to be no significant increased risk of malformations. While an increased risk of childhood cancer has been reported in case-control studies with prenatal radiation doses of greater than 10 mGy, prospective studies and larger meta-analyses have not consistently replicated this association between fetal radiation exposure and risk of cancer.[5,6] Iodinated contrast agents have not been shown to be teratogenic. Despite a theoretical concern about fetal hypothyroidism, there have been no reported cases resulting from the use of these agents.[3]

MRI has not been shown to have adverse effects on the fetus and there are no precautions or contraindications specific to pregnant patients. While historically it was recommended that MRI be avoided in the first trimester, the American College of

TABLE 9.1 Estimated Fetal Radiation Doses During Common Radiologic Examinations

Type of Examination	Fetal Dose (mGy)
Very Low Dose (<0.1 mGy)	
Cervical spine (anteroposterior, lateral)	<0.001
Extremities	<0.001
Mammography (two views)	0.001–0.01
Chest (posteroanterior, lateral)	0.0005–0.01
Low to Moderate Dose (0.1–10 mGy)	
Radiography	
Thoracic spine (anteroposterior, lateral)	0.003
Abdomen (anteroposterior)	0.1–3.0
Lumbar spine (anteroposterior, lateral)	1.0–10
Limited IV pyelogram	5–10
Double contrast barium enema	1.0–20
CT Scan	
Head CT	0
Chest CT or CT pulmonary angiography	0.01–0.66
Limited CT pelvimetry	<1.0
Nuclear Medicine	
Very Low Dose <0.1 mGy)	
Low-dose perfusion scintigraphy for PE	0.1–0.5
Pulmonary digital subtraction angiography	0.5
Technetium-99m bone scintigraphy	4–5
High Dose (10–50 mGy)	
Abdominal CT scan	1.3–35
CT scan of the abdomen/pelvis	10-50
^{18}F PET/CT whole-body scintigraphy	10-50

CT, Computed tomography; *IV*, intravenous; PET, positron emission tomography; *mGy*, milliGray.
Adapted from Tremblay E, Thérasse E, Thomassin-Naggara I, et al. Quality initiatives: guidelines for use of medical imaging during pregnancy and lactation. *Radiographics*. 2012;32:897–911.

Radiology has stated no special consideration is recommended for any trimester in pregnancy.[7–9] The use of gadolinium contrast in pregnancy remains controversial given its ability to cross the placenta and concentrate in amniotic fluid. A large, retrospective study found prenatal gadolinium exposure associated with rheumatologic, inflammatory, or infiltrative skin disorders (adjusted hazard ratio, 1.36; 95% confidence interval [CI] 1.09 to 1.69) and increased risk of stillbirth and neonatal demise (adjust RR, 3.70; 95% CI 1.55 to 8.85) when compared to unexposed pregnancies.[10] Ultimately, gadolinium contrast should only be used during pregnancy when close consultation with radiology specialists determines that the benefits for diagnosis far outweigh the aforementioned risks.[11] In the postpartum period, the use of all contrast agents, including gadolinium, is considered safe during lactation and the American College of Obstetricians and Gynecologists (ACOG) recommends that breastfeeding not be interrupted.[1]

Surgery During Pregnancy

Approximately 2% of pregnant patients require surgery for a nonobstetrical condition. The most common indications are appendicitis and cholecystitis followed by orthopedic procedures.[12–14] Indicated surgery during pregnancy is relatively safe. While the potential for adverse perinatal outcomes is slightly increased when compared with patients not requiring surgery, the delay of necessary procedures clearly increases the risk of poor maternal or fetal outcomes. A review of 54 studies regarding surgery during pregnancy concluded the following:[15]

1. The risk of maternal death is low.
2. Surgery and general anesthesia do not appear to be major risk factors for spontaneous abortion.
3. Elective termination rates are similar to the general population.
4. The rate of congenital anomalies does not appear to be increased.
5. Acute appendicitis, particularly with peritonitis, appears to be a risk of surgery-induced labor or fetal loss.
6. Urgent surgical procedures should be performed when needed.

Since appendectomy is the most common nonobstetric surgery performed during pregnancy (1 in 766 births), the data regarding outcomes are relatively robust. Anatomic changes in pregnancy can confound the diagnosis while a reluctance to operate on a pregnant patient can delay treatment and worsen outcomes. The negative appendectomy rate in pregnancy is higher when compared with nonpregnant cases (23% vs. 18%). The rates of fetal loss and early delivery are higher for complex appendicitis (6% and 11%, respectively) versus simple appendicitis (2% and 4%, respectively). Unfortunately, surgery for appendicitis and the finding of a normal appendix is still associated with an increased risk of loss (3% to 4%).[16,17] While laparoscopy is being utilized increasingly during pregnancy, there may be an associated increased risk of loss when compared with laparotomy for suspected appendicitis.[18] Ultimately, the type of surgical procedure, whether laparoscopic versus open, should be determined by the surgeon, in consultation with the obstetrics team.

Guidelines to optimize outcomes for pregnant patients undergoing surgery are relatively straightforward. Anesthetic options are unchanged when compared with nonpregnant patients, as both regional and general anesthesia can be safely administered. If general anesthesia is being used, extra care must be taken to protect the maternal airway from aspiration given the anatomic and physiologic oropharyngeal changes, reduced gastrointestinal motility, and displaced stomach that may contain significant residual contents hours after eating. In the second and third trimesters, a lateral decubitus position is preferred to reduce venacaval compression, thus optimizing both maternal venous return and uteroplacental perfusion. Preoperative counseling with an obstetrician and neonatologist is recommended, particularly if the gestational age is greater than or equal to 22 weeks to give the surgical team direction regarding interventions should fetal distress be encountered. Fetal heart rate monitoring, if feasible, can also facilitate the assessment of fetal status.

TABLE 9.2 American College of Radiology Summary of the International Commission on Radiological Protection Suspected in Utero-Induced Deterministic Radiation Effects

		RADIATION DOSE		
Menstrual or Gestational Age	Conception Age	<50 mGy (<5 rad)	50–100 mGy (5–10 rad)	>100 mGy (>10 rad)
0–2 weeks (0–14 days)	Before implantation	None	None	None
3rd and 4th week (15–28 days)	1st–2nd week (1–14 days)	None	Probably none	Possible spontaneous abortion
5th–10th week (29–70 days)	3rd–8th week (15–56 days)	None	Potential effects are scientifically uncertain and probably too subtle to be clinically detectable	Possible malformations increasing in likelihood as dose increases
11th–17th week (71–119 days)	9th–15th week (57–105 days)	None	Potential effects are scientifically uncertain and probably too subtle to be clinically detectable	Increased risk of deficits in intelligence quotient or mental retardation that increase in frequency and severity with increasing dose
18th–27th week (120–189 days)	16th–25th week (106–175 days)	None	None	Intelligence quotient deficits not detectable at diagnostic doses
>27 weeks (>189 days)	>25 weeks (>175 days)	None	None	None applicable to diagnostic medicine

mGy, MilliGray; *rad*, radiation absorbed dose.
Data from Tirada N, Dreizin D, Khati NF, et al. Imaging pregnant and lactating patients. *Radiographics*. 2015;35:1751–1765.

Medication Usage

Maternal medication use during pregnancy requires the provider to understand two basic principles:

- Alterations of maternal anatomy and physiology may alter the effective dose.
- Maternal medications may enter the fetal circulation with resulting fetal exposure.

There are significant physiologic alterations in pregnancy that may affect the bioavailability, distribution, clearance, and half-life of a medication. Absorption is altered due to nausea and vomiting, gastric volume and pH changes, increased gastrointestinal transit time, and differences in the activity of drug-metabolizing enzymes in the gut. Increased body weight and plasma volume as well as reduced albumin alter the volume of distribution. Hepatic and placental enzymatic activity and increased glomerular filtration rates will influence drug clearance.[19] Examples of medication classes that often require increased dosing through gestation include thyroid function modulators, antiepileptics, antihypertensives, and medication assisted therapies (MAT) for substance use disorder.

With the discovery that certain medications may induce congenital malformations, notably thalidomide in the early 1960s, the Food and Drug Administration (FDA) developed a maternal drug classification system with escalating categories (A through D) signifying increased fetal risk, and a standalone Category X denoting medications that were contraindicated in pregnancy. Most drugs (65% to 70%) were labeled category C, meaning that animal studies had shown potential adverse effects, however, no adequate human studies were available to corroborate.[20] With half of all pregnant women receiving prescription drugs in categories C and D, the actual utility of this classification system became questionable.[21] In 2015 the FDA published the "Pregnancy and Lactation Labeling Rule," eliminating the ABCDX classification for any new drug applications and retroactively labeling prescription drugs approved after 2001.[22] The new labels include a summary of known fetal and newborn risks, considerations for lactation and breastfeeding, latest available data, and information for pregnant patients to participate in ongoing registries for the medication. In addition to pregnant patients, the expanded labeling also includes information on the potential impact medications may have on both male and female reproductive potential. Ultimately, such information should be utilized by both patients and physicians as part of a shared decision-making process, with the understanding that maternal benefit will most often outweigh weak or theoretical fetal risks when it comes to recommending indicated medical treatments in pregnancy.

Autoimmune Disorders

Systemic rheumatoid illnesses are seen more commonly in women than men and with presentations commonly in young adulthood and middle age. Therefore pregnancy complicated by an autoimmune condition is common. There are an estimated 4500 pregnancies complicated by systemic lupus erythematosus (SLE) in the United States annually.[23] Other autoimmune conditions seen during pregnancy include rheumatoid arthritis and scleroderma. While perinatal implications may differ, depending on the diagnosis, there are enough similarities among most rheumatologic disorders that there are common themes in maternal management, including work-up, medication usage, and fetal screening. Furthermore, some patients may have findings consistent with an autoimmune condition but lack enough criteria to warrant a specific diagnosis. Finally, there are individuals lacking clinical rheumatologic manifestations that exhibit autoantibodies that have maternal, fetal, or neonatal implications. The nuances of all

autoimmune disorders are too broad to allow discussion in this chapter. Therefore the focus will be on SLE and common antibody disorders with specific perinatal implications.

Systemic Lupus Erythematosus

SLE or lupus is a chronic multiorgan disease with a wide clinical spectrum. Advancements in reproductive and autoimmune care have improved maternal and fetal outcomes, reflected by the reduction of fetal loss rates from 43% to 17% between 1960 and 2000.[24] In the absence of antiphospholipid antibody syndrome (APS) or significant renal insufficiency, fertility does not seem impacted.[25] As with most autoimmune conditions, there is no uniform agreement on whether pregnancy alters the disease course. However, risk factors associated with a lupus flare include active disease within 6 months before conception, history of multiple flares, and discontinuation of hydroxychloroquine.

Risk factors for poor pregnancy outcomes in patients with lupus include proteinuria, renal insufficiency, APS, thrombocytopenia, or active maternal disease at the time of conception (Table 9.3).[26] Those with nephritis, APS, or who have anti-Sjögren syndrome related-antigen A (anti-SSA or anti-Ro antibodies) may have increased risks of altered perinatal outcomes.[27] Adverse outcomes include spontaneous abortion and intrauterine growth restriction (IUGR), and the risk of superimposed preeclampsia and stillbirth are potentially increased. Therefore, assessment of the patient's baseline disease activity, as well as exploration for evidence of multiorgan system dysfunction, is vital to aid in counseling and management. Comanagement with rheumatologists and/or nephrologists is vital to improving maternal and fetal outcomes.

Initial prenatal evaluation of a patient with lupus includes taking a history, such as disease activity, organ system involvement, and current medical therapy. The latter is important in determining whether there is an increased fetal risk, as some medications used are generally contraindicated during pregnancy (methotrexate, mycophenolate mofetil). Fortunately, most patients planning pregnancy are not using these agents. Prednisone, azathioprine, and hydroxychloroquine are more commonly used medications with reasonable safety profiles during pregnancy.

Chemical screening for disease activity should be performed. This includes anti-double-stranded DNA and complement levels. Antiphospholipid antibodies, anti-Ro, and anti-La (otherwise known as anti-Sjögren syndrome related-antigen B or anti-SSB) antibodies should be measured. The patient should also have baseline assessments for proteinuria, renal function, hepatic enzymes, and platelet count not only to assess her status but also to formulate a baseline profile (Table 9.4). Since a lupus flare may mimic preeclampsia, a comparison of her repeated studies against those obtained earlier may be helpful, as the management of these two conditions differs.

Medication management, including type and amount, is dictated by disease activity and the presence of APS. Most patients are managed with steroids, hydroxychloroquine, or azathioprine, and alterations of these should probably not be dictated by pregnancy. While there may be theoretical concerns with their usage, these drugs have to be balanced against the risk of uncontrolled lupus (Table 9.5).[28–30]

Disease activity should include serial assessment of maternal symptoms as well as complement and anti-double-stranded DNA levels. Fetal evaluation includes serial ultrasounds and antepartum testing. An increase in the patient's blood pressure or level of proteinuria may represent either a lupus flare or preeclampsia. Differentiation between the two is challenging, but every effort should be made to do so, as therapies for both are different.

Antiphospholipid Antibody Syndrome

APS was originally described as an autoimmune disease characterized by circulating antiphospholipid antibodies (aPL) and venous thrombosis, recurrent pregnancy loss, and occasionally thrombocytopenia.[31] This disorder may be isolated or associated with other autoimmune diseases. The presence of aPL is found in about 1% to 5% of healthy women. However, these antibodies are found in 15% of women who have recurrent abortions and in 30% to 40% of those with SLE.[32,33] The presence of aPL in the latter is a risk factor for a poor pregnancy prognosis.

Few women with aPL will develop the disease, and the prevalence of APS is estimated to be only 50/100,000.[34] The diagnosis of APS must include clinical criteria (vascular thrombosis, unexplained death of a morphologically normal fetus >10 weeks' gestation, premature birth <34 weeks due to preeclampsia or placental insufficiency, or ≥3 weeks otherwise unexplained abortions <10 weeks), as well as the presence of aPL (lupus

TABLE 9.3 Systemic Lupus Erythematosus and Adverse Pregnancy Outcomes

	Adverse Outcome	Percentage	OR
Systemic Lupus Erythematosus ± Antiphospholipid Syndrome			
	Prematurity	25%–35%	
	Preeclampsia	10%–15%	
	Eclampsia / HELLP	1%–1.5%	
Antiphospholipid ± Systemic Lupus Erythematosus			
	Prematurity	25%–30%	
	Preeclampsia	10%–20%	
	Eclampsia / HELLP	3%—5%	
Systemic Lupus Manifestations That Adjust Perinatal Risk			
Active flare	Preeclampsia		12.7
	Emergent cesarean		19
	Early fetal loss		3
	Preterm birth		5.5
Active nephritis	Any		5.3
Hypertension	Preeclampsia		4.8–7.3
Prednisone >10 mg/day	Preterm birth		3.5

Adapted from Andreoli L, Bertsias GK, Agmon-Levin N, et al. EULAR recommendations for women's health and the management of family planning, assisted reproduction, pregnancy and menopause in patients with systemic lupus erythematosus and/or antiphospholipid syndrome. *Ann Rheum Dis.* 2017; 76:476–485.

TABLE 9.4 Laboratory Testing of Patients With Systemic Lupus Erythematosus During Pregnancy

Timing	Purpose	Test	Comment
Preconception or initial prenatal visit	Screen for organ system dysfunction and baseline for comparison should a lupus flare or preeclampsia be suspected	Urinalysis	Assessment of renal disease activity
		24-h urine for total protein or urine protein:creatinine ratio	24-h urine collection for proteinuria recommended if abnormal protein:creatinine ratio
		Complete blood count	Assess for thrombocytopenia
		Chemistry panel	
	Screen for risk of neonatal lupus syndrome	Anti-SSA (Ro) and Anti-SSB (La) antibodies	If present, screen for fetal congenital heart block
	Screen for disease activity and baseline for comparison should a lupus flare or preeclampsia be suspected	Anti-double-stranded DNA antibody Complement levels	Complement levels may increase in a normal pregnancy
Monthly		Urinalysis	Urine protein:creatinine ratio if urinalysis suspects proteinuria
		Creatinine	If prior history of lupus nephritis or prior renal dysfunction is noted
Every trimester		Anti-double-stranded DNA antibody Complement levels	
		Complete blood count	
		Chemistry panel	

anticoagulant, anticardiolipin antibody, or anti-β_2-glycoprotein antibody) on greater than two occasions, at least 12 weeks apart.[35] For those with a prior history of venous or arterial thrombosis, prophylactic anticoagulation with heparin throughout pregnancy and continuing through 6 weeks' postpartum is usually recommended. In patients with recurrent pregnancy loss, low-dose aspirin and heparin may reduce pregnancy loss by 50%.[36] Therefore even in the absence of a thrombotic history, in those with a history of stillbirth or recurrent pregnancy loss, prophylactic heparin and low-dose aspirin should be offered during pregnancy for up to 6 weeks' postpartum. [37]

Neonatal Lupus

Anti-Ro and anti-La antibodies can be present in asymptomatic women. The former are more often seen in women with Sjögren syndrome but can be also found in women with other autoimmune disorders.[38] These antibodies cross the placenta and have been associated with congenital heart block. In the absence of a prior infant with congenital heart block, the prospective fetal risk is low, at 2%. However, in patients with a prior history of an affected fetus or neonate, the recurrence risk is 15% to 20%.[39,40] The morbidity and mortality of these infants are significant, the latter ranging between 10% and 29%.[41] Other consequences include the potential need for a pacemaker or cardiac transplantation.

The injury to the atrioventricular node most often occurs between 16 and 24 weeks, and there is the potential for fibrosis that can extend to the endocardium and myocardium. No prenatal interventions have been shown to reverse complete heart block.[42] However, there may be some improvement in outcome with the use of fluorinated steroids if second-degree heart block is detected. There are potential fetal consequences of maternal steroids, including adrenal insufficiency, as well as neurodevelopmental and growth abnormalities.[41] It is suggested that women exhibiting these antibodies undergo fetal echocardiography beginning in the second trimester (16 to 18 weeks) and repeated every 1 to 2 weeks until 28 weeks.[43] In those with a prior child affected with congenital heart block, the use of hydroxychloroquine may be associated with a reduced risk of recurrence.[39]

Immune Thrombocytopenia

Approximately 7% to 10% of pregnancies are complicated by thrombocytopenia.[44] The most common etiology is gestational thrombocytopenia (75%), followed by preeclampsia (15% to 20%) and immune thrombocytopenia (ITP) (3%). By definition, ITP is immune mediated, and the responsible antibody may cross the placenta. The incidence is approximately 1/1000 pregnancies, but more significant disease (maternal platelet count $<50 \times 10^9$/L) is rare occurring in 0.85/100,000 pregnancies.[45,46] ITP is associated with an increased risk of neonatal thrombocytopenia, with 15% to 50% of newborns having platelets less than 100×10^9/L, 8% to 30% less than 50×10^9/L, and 1% to 5% less than 20×10^9/L.[44] Unfortunately, there is no variable that accurately predicts the potential for neonatal thrombocytopenia. There is no correlation between the maternal and neonatal platelet count, nor does the use of steroids or IVIg not seem protective. A history of a prior affected child or maternal splenectomy may increase the neonatal risk of thrombocytopenia.[46] Over the years, a number of interventions had been proposed in the past to reduce the risk of spontaneous fetal or neonatal bleeding. Strategies such as performing a routine cesarean section, fetal scalp platelet assessment in labor, or percutaneous fetal scalp platelet counts were not found to be helpful and were potentially associated with increased maternal or fetal

TABLE 9.5 Medications Used for Rheumatologic Disorders

Drug	Maternal and Fetal Outcomes	Recommendations
Commonly Used During Pregnancy		
Prednisone, prednisolone	Possible risk for orofacial clefts	Treatment of flares or maintenance therapy
Fluorinated corticosteroids (betamethasone, dexamethasone)	Cross the placenta. May be associated with poor fetal growth or adverse neurologic outcomes if used in multiple doses	Use only if fetal treatment is being considered (neonatal lupus syndrome)
Hydroxychloroquine	Considered safe in doses commonly used in SLE and RA	Safe to continue in pregnancy
Azathioprine	May be associated with IUGR, transient immune alterations	Long track record of use in pregnancies complicated by SLE. Safe to continue if necessary
Nonsteroidal anti-inflammatory drugs	May be associated with fetal ductus arteriosus constriction and impaired renal function with third trimester use	Avoid in third trimester. Limit use to very short duration (48–72 h)
Sulfasalazine	No increased risk in doses commonly used in RA	Use folic acid supplementation preconception and during pregnancy
Heparin	Does not cross placenta	If using low-molecular-weight heparin, may require changing to unfractionated heparin after 36 weeks for maternal anesthesia
IVIG	No identifiable fetal risk	May be used in refractory cases of immune thrombocytopenia
Contraindicated		
Methotrexate	Increased risk for congenital malformations	Conception should be delayed for at least 3 months after last dose
Leflunomide	Embryotoxicity	Discontinue 2 years prior to conception
Mycophenolate mofetil	Increased risk for congenital malformations	
May Be Used		
Cyclosporine	Often used in patients with organ transplants. No increased risk of congenital malformations	May be used in patients with SLE
Cyclophosphamide	Use in first trimester associated with fetal anomalies or loss. Second- and third-trimester use may be associated with fetal growth issues and impaired neurologic development	Use only if mother's life is at risk and other options have been exhausted
Rituximab	Associated with transient B-cell depletion in the second and third trimesters	Inadequate safety data to support routine use in pregnancy Neonatal live virus vaccination should be delayed by 6 months

IUGR, Intrauterine growth restriction; *IVIG*, intravenous immunoglobulin; *RA*, rheumatoid arthritis; *SLE*, systemic lupus erythematosus.
Adapted from Baer AN, Witter FR, Petri M. Lupus and pregnancy. *Obstet Gynecol Surv*. 2011;66: 639–653. Makol A, Wright K, Amin S. Rheumatoid arthritis and pregnancy. *Drugs*. 2011;71:1973–1987. Hyrich KL, Verstappen SMM. Biologic therapies in pregnancy: the story so far. *Rheumatology*. 2014;53:1377–1385.

morbidity. Therefore it is recommended that the delivery mode should not be altered for the diagnosis of ITP.[47]

Maternal management for this condition in the first and second trimesters of pregnancy is essentially unchanged when compared with a nonpregnant patient.[48] If the maternal platelet count is greater than 30,000/μL, no treatment is necessary. Below this threshold, or if there is evidence of spontaneous bleeding, corticosteroids and intravenous immunoglobulin are usually first-line therapies. Both are relatively safe during pregnancy and usually associated with a good maternal response. Refractory cases are difficult to manage. Large-dose anti-D antibody administration and splenectomy are options when initial therapy fails.[48]

Gestational thrombocytopenia (GTP) is a benign condition that sometimes can be difficult to delineate from ITP. However, the salient features include a lack of prior maternal history of severe thrombocytopenia and a maternal platelet count that is usually above 80,000/μL. Other potential causes for a low platelet count should be explored, including severe preeclampsia, autoimmune disease, medication usage, and viral illness. True GTP has no associated risk of neonatal thrombocytopenia. One potential consequence of GTP may be the reluctance of some anesthesiologists to place a regional anesthetic if the platelet count is too low.

Cardiovascular Disease

Cardiovascular disease in pregnancy has become a leading cause of morbidity and mortality, affecting 1% to 4% of pregnancies in the United States and contributing to 26% of maternal deaths.[49,50]

While this increase may be explained, in part, by improved reporting and evaluation of maternal deaths, the general consensus is that higher rates of advanced maternal age accompanied by comorbidities such as diabetes, hypertension, and obesity have contributed the most to increasing rates of cardiovascular mortality in pregnancy. In 1970, approximately 1% of first-time mothers were greater than 35 years old. Four decades later, this incidence has increased to 9.1%.[51]

Hemodynamic alterations of pregnancy begin early in gestation. Maternal blood volume increases by 50%, with an accompanying decrease in systemic vascular resistance. The cardiac output increases by 30% to 50% and heart rate by 15%. The labor process further increases cardiac output.[52,53] Furthermore, there are intra- and extravascular fluid shifts that occur after delivery. A previously compromised heart may not tolerate these changes. Attempts to stratify maternal risk have resulted in various scoring systems. The World Health Organization (WHO) risk stratification system has been validated in pregnancy and defines risk based on the presence of cardiac lesions, overall cardiac function, and current health status (Table 9.6).[54] The highest risk (WHO IV) includes individuals with pulmonary hypertension, severe left ventricular dysfunction, previous peripartum cardiomyopathy with residual impairment of left ventricular dysfunction, left heart obstruction, or Marfan syndrome with a dilated aorta.[55] In these patients, pregnancy is not advisable given the high risk of maternal mortality.

Peripartum Cardiomyopathy

Peripartum cardiomyopathy (PPCM) refers to the onset of idiopathic dilated heart failure within 1 month prior to delivery or within 5 months after. The incidence of PPCM in the United States is increasing and is estimated to occur in 1/968 live births.[56] Risk factors include advanced maternal age, hypertension, preeclampsia, parity, and multiple gestations.[53] While the exact etiology is unknown, potential mediators include inflammation, autoimmune processes, endothelial dysfunction, oxidative stress, and underlying genetic mutations.[57,58] African-American race has long been associated as a risk factor for PPCM but without much biologic explanation. More contemporary analysis suggests that negative social determinants of health contribute to proinflammatory states and hypertensive disorders. When combined with delayed diagnosis and inadequate treatment due to socioeconomic barriers,

TABLE 9.6 Modified WHO Risk Stratification for Pregnant Patients With Cardiovascular Disease

Modified WHO Risk Classification (Cardiac Event Risk)	Examples of Cardiac Lesions	Suggested Follow-up and Delivery Planning
Class I (2%–5% risk)	• Small or mild pulmonary stenosis, patent ductus arteriosus, or mitral valve prolapse • Successfully repaired lesions	Cardiology 1–2 times in pregnancy
Class II (6%–10% risk)	• Unrepaired atrial or ventricular septal defect • Repaired tetralogy of Fallot • Most supraventricular arrhythmias • Turner syndrome *without* congenital cardiac disease	Cardiology every trimester Multidisciplinary cardiac team for delivery planning
Class II–III (11%–19% risk)	• Mild left ventricular impairment • Hypertrophic cardiomyopathy • Native/tissue valvular heart disease • Marfan syndrome without aortic dilation • Heart transplantation	Cardiology every trimester Multidisciplinary cardiac team for delivery planning
Class III (20%–27% risk)	• Mechanical valve • Systemic right ventricle (i.e., congenitally corrected transposition or simple repair) • Cyanotic heart disease • Post Fontan operation • Complex congenital heart disease	Cardiology every 1–2 months Multidisciplinary cardiac team for delivery planning

Class IV (>27% risk)
Pregnancy contraindicated
	• Pulmonary arterial hypertension
	• Severe systemic ventricular dysfunction
	  • Ejection fraction <30%, NYHA III-IV
	• Previous peripartum cardiomyopathy with residual left ventricular dysfunction
	• Severe mitral stenosis
	• Severe, symptomatic aortic stenosis
	• Systemic right ventricle with decreased ventricular function
	• Aortic dilation
	  • >45 mm in Marfan syndrome
	  • >50 mm in bicuspid aortic valve or tetralogy of Fallot
	  • Aortic size index (ASI) >25 mm/m^2 in Turner syndrome
	  • Vascular Ehlers-Danlos
	  • Severe re-coarctation
	  • Fontan circulation with any complication
		Cardiology monthly
		Multidisciplinary cardiac team for delivery planning
		Consider termination of pregnancy

Adapted from: American College of Obstetricians and Gynecologists. ACOG Practice Bulletin No. 212: Pregnancy and Heart Disease. *Obstet Gynecol.* 2019;133(5):e320–e356; Thorne S, MacGregor A, Nelson-Piercy C. Risks of contraception and pregnancy in heart disease. *Heart.* 2006;92:1520–1525.

these factors likely contribute to the racial disparities observed in PPCM incidence and recovery.[59]

Presentation of PPCM may be indolent, as symptoms such as fatigue, shortness of breath, tachycardia, tachypnea, and lower extremity edema can mimic those commonly encountered in pregnancy and the immediate postpartum period. Furthermore, patients with preeclampsia can have significant left-ventricular dysfunction and pulmonary edema, albeit transiently, which may delay diagnosis. Initial workup includes an electrocardiogram. There is a high prevalence of abnormalities in peripartum cardiomyopathy, including evidence of left-sided changes, conduction delays, reduced voltage, and abnormal Q waves. Echocardiography provides the best sensitivity for diagnosis, often revealing depressed left-ventricular function, significant ventricular dilation, and an ejection fraction less than 45%.[53] B-type natriuretic peptide levels, though normally increased in pregnancy, may be significantly increased as a consequence of left-ventricular end-diastolic pressure.[57] Treatment is supportive and similar to that of preexisting dilated cardiomyopathy. Complications include cardiogenic shock, arrhythmias, thromboembolism, and death. While 25% to 50% of patients with peripartum cardiomyopathy may recover by 6 months, one-quarter do not improve. The mortality may approach 15%.[57] There is an increased risk in subsequent pregnancies, particularly in those who have not had full recovery of reduced ejection fraction and heart failure.[60]

Congenital Heart Disease

Congenital heart disease (CHD) occurs in 81 in every 10,000 live births, affecting approximately 1% of the population.[61] The prevalence of CHD complicating deliveries is approximately 9 per 10,000 hospitalizations.[62] This represents a significant increase over the last two decades due to the improved diagnosis and treatment of CHD allowing more patients to reach childbearing age. With the exception of hypertensive disorders and thromboembolic events, obstetric complications do not seem to be increased in these patients. However, cardiac complications are found in about 11% of patients and include arrhythmias, heart failure, and cardiovascular events. [63] Myocardial infarction, cerebrovascular accidents, and mortality were noted in about 2% of patients. In those patients with more complex CHD, including Ebstein anomaly, transposition of the great vessels, pulmonary atresia with ventricular septal defects, and Eisenmenger syndrome, the preterm delivery rate ranged between 22% and 65%. The offspring mortality is higher, probably caused by prematurity. The infants have a higher incidence of CHD, between 0.6% and 8%, depending on the maternal lesion.[63]

Women with CHD should be evaluated before conception to advise them of the maternal and fetal risks of pregnancy. Many patients have limited or inaccurate knowledge of their disease. The history of the actual defect and subsequent surgical and other interventions should be obtained. Electrocardiography and echocardiography should be performed. The latter should focus on ventricular and valve functions, pulmonary artery pressure, and the presence of any prosthetic material. Since about 18% of patients have a defined chromosomal or Mendelian abnormality, formal genetic screening should be performed.[64]

Pregnancy management requires a multidisciplinary team, including cardiology, high-risk obstetrics, and anesthesiology. If necessary, cardiac catheterization or percutaneous interventions can be considered. Cardiopulmonary bypass can be performed during pregnancy, but there is an increased risk of prematurity and fetal death, particularly with urgent, high-risk surgery and maternal comorbidity.[65] Medical control of arrhythmias, if necessary, is usually safe. However, the use of β-blocker or calcium channel blocker may be associated with suboptimal fetal growth. In most cases, vaginal delivery is preferred. Cesarean section may be considered for women with severe aortic stenosis or pulmonary hypertension.

Coronary Artery Disease

Coronary artery disease (CAD) is a significant cause of maternal mortality and may account for up to 20% of maternal cardiac deaths.[66] Acute myocardial infarction may complicate up to 6/100,000 pregnancies.[67] It appears that spontaneous coronary artery dissection is more common during pregnancy. In one review of maternal deaths in the UK, all of the women who died had at least one risk factor for CAD. Unfortunately, in half of these cases, their care was deemed substandard due to delay in diagnosis or reluctance to treat due to pregnancy.[66] Patients with ST-segment elevation myocardial infarction should be treated similarly to those who are not pregnant. This includes percutaneous coronary intervention.[68] If stenting is required, bare metal stents are preferred in order to minimize the need for long-term dual antiplatelet therapy.[69] Aspirin and clopidogrel appear to be safe in pregnancy. Longer-duration antiplatelet therapy is generally recommended if a drug-eluting stent is placed. Once clopidogrel is no longer deemed necessary, single-agent antiplatelet therapy with aspirin is appropriate. β-Blockers are relatively safe to use in pregnancy but carry a theoretical risk of suboptimal placental perfusion.

Renal Disease

Chronic kidney disease (CKD) is a heterogeneous group of disorders with alterations in renal function. The prevalence in women of childbearing women is estimated between 0.1% and 4%.[70] It is estimated that 1/750 pregnancies is complicated by stages 3 to 5 CKD.[71] Since there is no accurate ability to measure glomerular filtration rate in pregnancy, women with CKD have been arbitrarily classified into three categories based on the serum creatinine level: mild, less than 1.5 mg/dL; moderate, 1.5 to 2.5 mg/dL; and severe, greater than 2.5 mg/dL (Table 9.7).[72] Whether the newer staging of CKD is better than the latter classification in

TABLE 9.7 Stages of Chronic Kidney Disease

Stage	Description	Glomerular Filtration Rate (mL/mm²)
1	Kidney damage with normal or raised GFR	>90
2	Kidney damage with mildly lower GFR	60–89
3	Moderately lower GFR	30–59
4	Severely low GFR	15–29
5	Kidney failure	<15 or dialysis

GFR, Glomerular filtration rate.
From Vellanki K. Pregnancy in chronic kidney disease. *Adv Chronic Kidney Dis.* 2013;20:223–229.

predicting pregnancy outcomes is unknown, but Table 9.8 summarizes maternal and pregnancy outcomes.[73]

Pregnancy may influence the rate of decline of renal function. While it appears unlikely when the baseline creatinine is less than 1.5 mg/dL, patients with hypertension are more likely to develop progressive disease.[74] However, the same cannot be said for those with moderate impairment (creatinine >1.5 mg/dL). A significant risk of deterioration of maternal renal function can be seen in up to 40% of patients with progression to end-stage disease in 10%.[75] The risk of irreversible deterioration of renal function is significant in those with marked impairment of renal function irrespective of the type of disease when accompanied by uncontrolled hypertension.[76]

When compared with women without CKD, those with kidney disease have a 52% increased odds of preterm delivery, 33% increased odds of cesarean section, 71% increased odds of neonatal intensive care unit (NICU) admission, and twofold increased odds of low birth weight.[77] The effect of mild-to-moderate CKD on pregnancy is dependent on the degree of renal impairment and the presence of underlying hypertension. In patients with mild renal dysfunction, successful outcomes are usually above 80% but with a complication rate of 26%.[78] However, there are increased rates of IUGR, preterm birth, and preeclampsia. In those with severe renal impairment, there are increased rates of infertility, miscarriage, and poor perinatal outcome. These pregnancies are complicated by growth restriction (65%), preterm birth (90%), preeclampsia (60%), and perinatal death (10%).[71]

Ideally, patients are evaluated prior to pregnancy. Preconception counseling regarding maternal and perinatal outcomes is dependent on the patient's baseline renal function, degree of hypertension, and various comorbid conditions. For example, those with severe renal impairment should consult with a nephrologist to determine whether medication therapy or dialysis should be considered. Implementing therapies that may optimize her medical status in the non-gravid state is recommended to improve outcome. Antihypertensive medication adjustments may be necessary to reduce fetal consequences, particularly with the use of angiotensin-converting enzyme inhibitors or receptor blockers that are known teratogens. If the degree of renal impairment is unknown, a 24-hour urine for creatinine clearance and total proteinuria should be performed. Furthermore, screening for active renal processes such as glomerulonephritis, lupus nephritis, reflux, and infection is advisable, as treatment may improve the degree of renal dysfunction. Low-dose aspirin (100 to 150 mg/day) starting at 16 weeks is recommended to potentially reduce the rate of preeclampsia.

Management of pregnancy includes initial office visits every other week and then weekly after 32 weeks, as well as serial ultrasounds to evaluate fetal growth. The urine should be evaluated every 4 to 6 weeks for infection, proteinuria, and hematuria. The blood pressure targets should ideally be between 120/70 mmHg and 140/90 mmHg, making adjustments to antihypertensive medications as necessary. Maternal creatinine and hemoglobin levels are also regularly assessed, the frequency dependent on the patient's baseline renal dysfunction.[71]

Hemodialysis in pregnancy has become more common. It appears that more intensive dialysis (36 to 43 hours/week) is associated with improved perinatal outcomes, increased birth rate and decreased risks of preterm birth and IUGR.[79] Pregnancy outcomes after renal transplantation are thought to be superior when compared to women undergoing dialysis. However, these patients remain at high risk for hypertension, preeclampsia, gestational diabetes, low birth weight, cesarean delivery, and neonatal intensive care admissions when compared with the general population.[70]

Cancer

Principles

Cancer complicating pregnancy is rare, with an estimated frequency of 1 case per 1000 live births. The trend of delaying childbearing to later maternal age and the increased rates of cancer, in general, have increased the incidence over the past four decades.[80] The sites or types of cancer in pregnancy, in descending order of frequency, are cervical, breast, ovarian, lymphoma, melanoma, brain, and leukemia (Table 9.9).[81–83] Pregnancy potentially complicates the diagnosis, given the overlap with normal maternal symptoms, compromised physical examination, hesitation to

TABLE 9.8 Renal and Pregnancy Outcomes According to Chronic Kidney Disease Stage

Outcome	Control	Stage 1	Stage 2	Stage 3	Stage 4-5
Progressed to next stage of CKD	N/A	7.6	12.6	16.2	20
New onset HTN	5.5	7.9	17.6	47.1	50
New onset or doubling of proteinuria	NA	20.5	37.9	86.5	70
Gestational age at delivery (wk)	39 ± 1.7	37.6 ± 2.6	35.7 ± 3.2	34.4 ± 2.4	32.6 ± 4.2
Delivery <37 weeks	6.1	23.5	50.6	78.4	88.9
Delivery <34 weeks	1	7.3	20.7	37.8	44.4
Birth weight (g)	3242 ± 480	2966 ± 659	2484 ± 707	2226 ± 582	1639 ± 870
SGA (<10%ile)	10.3	13.3	17.9	18.9	50
NICU	1.8	10.3	27.6	44.4	70

CKD, Chronic kidney disease, *NA*, not applicable; *HTN*, hypertension; *SGA*, small for gestational age; *NICU*, neonatal intensive care unit.
Data are % or mean ± SD.
Data from Piccoli GB, Cabiddu G, Attini R, et al. Risk of adverse pregnancy outcomes in women with CKD. *J Am Soc Nephrol*. 2015; 26:2011–2022.

TABLE 9.9 Cancers That Can Complicate Pregnancy

Site/Type	Incidence (Per Number of Gestations)
Cervix	1:2,000–1:10,000
Breast	1:3,000–1:10,000
Melanoma	1:1,000–1:10,000
Ovary	1:10,000–1:100,000
Colorectal	1:13,000
Leukemia	1:75,000–1:100,000
Lymphoma	1:1,000–1:6,000

Data from Pentheroudakis G, Pavlidis N, Castiglione M, ESMO Guidelines Working Group. Cancer, fertility and pregnancy: ESMO clinical recommendations for diagnosis, treatment and follow-up. *Ann Oncol.* 2009;20(Suppl 4):S178–S181.

obtain testing, and restrictions of imaging and biochemical markers. All of these factors may contribute to a delay in diagnosis.

Malignancy complicating pregnancy presents a unique set of issues. Will the gestation accelerate the malignant process? Are the accepted therapies safe for the unborn fetus? Will delay of therapy adversely affect the mother? Should the pregnancy be terminated, or should the child be delivered prematurely to maximize treatment of the mother with no resultant risk to the child?

Few pregnancy conditions require a multidisciplinary approach as cancer. Providers unaccustomed to managing pregnant women may be reticent in recommending definitive cancer therapy, for fear of the surgical or teratogenic risks to the fetus. Therefore input regarding diagnosis and treatment must be acquired not only from an oncologist but also from the obstetrician, perinatologist, pediatrician, neonatologist, and dysmorphologist. The patient and her family must be involved in decision making. Information should include the risks of the disease and its potential therapies, as well as the limitations of current knowledge about cancer in pregnancy and any uncertainties of outcomes. Furthermore, depending on the gestational age at diagnosis, discussions regarding termination and potential iatrogenic prematurity are important.

In most cases, there should not be any delays in diagnostic procedures. Most imaging modalities are relatively safe in pregnancy but can be modified to lessen the risk of fetal exposure, providing there is no reduction in an ability to make a diagnosis. Biopsies and indicated surgery should not be delayed for the sake of the pregnancy.

Chemotherapy

The fetal impact of chemotherapy is dependent on the gestational age of exposure and the agent administered. The rates of congenital malformations are 16%, 8%, and 6% in the first, second, and third trimester respectively.[84] The impact of exposure is dependent on the agent. Alkylating drugs and antimetabolites appear to have the highest rates of adverse outcomes, while platinums, taxanes, and antibiotics have the lowest.[80] The literature regarding most chemotherapeutic agents is limited, consisting of a few collected series. For a list of the more common chemotherapeutic agents that have been used in pregnancy, refer to Table 9.10.[85,86]

Little is known regarding the long-term outcomes of fetuses exposed to chemotherapeutic agents. The National Cancer

TABLE 9.10 Common Chemotherapeutic Agents and Uses

Class	Drug	Common Uses
Alkylating Agents		
Bind covalently to DNA, causing cross-links and strand breaks	Busulfan	Leukemias
	Chlorambucil	Lymphomas, leukemias
	Cyclophosphamide	Breast, ovary, lymphomas, leukemias
	Melphalan	Ovary, leukemia, myeloma
	Procarbazine	Lymphomas
Antimetabolites		
Act as a false substrate during DNA and RNA synthesis, resulting in truncated proteins	5-Fluorouracil	Breast, gastrointestinal
	6-Mercaptopurine	Leukemias
	Methotrexate	Trophoblastic disease, lymphomas, leukemias, breast
	6-Thioguanine	Leukemias
Antibiotics		
Bind with DNA, prevent RNA synthesis, generate highly reactive free radicals	Bleomycin	Cervix, lymphomas
	Daunorubicin	Leukemias
	Doxorubicin	Leukemias, lymphomas, breast
Antimitotic Agents		
Stop mitosis in M phase of cell cycle	Vincristine	Leukemias, lymphomas
	Vinblastine	Breast, lymphomas, choriocarcinomas
Taxanes		
Inhibit disassembly of microtubules	Tamoxifen	Breast, lymphomas, choriocarcinoma
	Paclitaxel	Breast, ovarian
Platinum Compounds		
Bind to DNA, causing cross-links and apoptosis	Cisplatin	Ovary, cervix, sarcoma
Other		
	L-Asparaginase	Leukemias
	Hydroxyurea	Leukemias
	All-*trans*-retinoic acid	Leukemias

Data from Neoplastic diseases. In Cunningham FG, MacDonald PC, Gant NF, et al., eds. *Williams Obstetrics.* 20th ed. Stamford, CT: Appleton and Lange; 1997; Ngu S, Ngan HY. Chemotherapy in pregnancy, *Best Pract Res Clin Obstet Gynaecol.* 2016;33:86–101.

TABLE 9.11 Principles in Management of Cancer in Pregnancy

Diagnostic	Therapeutic	Prenatal Care	Delivery and Postpartum
Standard diagnostic procedures should not be delayed	If possible, delay surgery until the second trimester	Ultrasound prior to interventions to confirm dating and screen for fetal malformations	Delivery timing should preferably based on obstetrical indications
Multidisciplinary discussion regarding further diagnostic, staging and therapeutic options	Surgery ≥23 weeks requires discussion regarding need for intraoperative fetal monitoring	Assess fetal well-being after each cycle of chemotherapy	Chemotherapy should not be administered within 3 weeks of delivery
	Chemotherapy should be avoided if possible in the first trimester	Serial ultrasounds for fetal growth	Placenta should be evaluated for evidence of metastatic disease
	Radiation therapy should be avoided if possible during pregnancy	Neonatology consultation, particularly if preterm birth is being considered	Breast feeding is contraindicated with chemotherapy

Institute in Bethesda, Maryland, USA, maintains a registry in the hopes of determining the delayed effects. A small series of fetuses exposed to chemotherapeutic agents for acute leukemia revealed normal mental development with follow-up between 4 and 22 years.[87] A recent case-control study involving 129 children, 96 of whom were exposed to chemotherapy in utero, suggested that maternal cancer with or without treatment did not impair cognitive, cardiac, or general development.[88]

The risk of teratogenicity does not appear to be higher with combination chemotherapy than with single-agent therapy.[84] Studies performed thus far involve small numbers of patients, with power insufficient to show a statistical difference, but there seems not to be a trend. There is an increased rate of small for gestational age babies and premature labor in mothers receiving cytotoxic chemotherapy.[89] Theoretical consequences include bone marrow or immune suppression. As a result, the timing of chemotherapy should account for the anticipated date of delivery. Data regarding safety for breastfeeding the neonate of a mother receiving cancer chemotherapy are limited. For this reason, the majority of agents are contraindicated in nursing mothers (Table 9.11).

Radiation Therapy

The deleterious effects of fetal irradiation have been discussed previously. Adjuvant radiation during pregnancy should be avoided if possible. However, there are small case series of radiotherapy in pregnancy, primarily for lymphoma.[90] There are considerations regarding radiation therapy during pregnancy. The dose used in estimating risk should be the amount that the fetus actually receives. For example, axillary or neck irradiation for lymphoma involves a lower direct fetal exposure than direct pelvic irradiation for cervical cancer. Secondly, the magnitude of radiation scatter to the pelvis should be estimated. External shielding does not prevent the internal reflection of the ion beam. Thirdly, the advancing size of the uterus actually increases the amount of radiation exposure to the fetus, because of the closer proximity of the nonpelvic irradiation. Therefore an 8-week-old fetus may actually receive a smaller radiation dose from supraclavicular irradiation than a 30-week-old fetus. Fourthly, will the fetus concentrate the radiation and therefore increase its delivered dose? This is exemplified by the use of radioactive iodine (^{131}I) for maternal thyroid conditions. The actual rad dose is markedly higher in the fetus because the fetal thyroid concentrates the iodine.

Cervical Cancer

Cervical cancer is one of the more common gynecologic malignancies in pregnancy. The incidence of cervical intraepithelial neoplasia is approximately 130 per 100,000 gestations and of invasive disease, 3.3 per 100,000 gestations.[91] Colposcopy with cervical biopsy remains the mainstay of diagnosis. The latter may be associated with increased bleeding given the increased blood flow to the uterus and cervix. However, if cancerous invasion is suspected, or uncertain, a biopsy is necessary. If microinvasive disease is confirmed, cone biopsy is required to rule out frankly invasive disease. This procedure is undertaken with caution during pregnancy, because of the associated high rate of bleeding complications and miscarriage. Cervical conization may raise the risk of incompetence or preterm labor.

The therapy is dictated by the disease stage and gestational age as well as the patient's decision regarding pregnancy continuation and future fertility. Treatment options include external beam radiation, internal radiotherapy (brachytherapy), or surgery. In most cases, delay of definitive therapy by 4 to 14 weeks may be acceptable. Pregnancy does not seem to accelerate the growth of the tumor. Shared decision making is important. In the extremely previable gestation, the likelihood of achieving a safe gestational age for the fetus without worsening the stage or spread of the cancer in the mother must be balanced against parental desires based on ethical or religious beliefs. Conversely, it might be reasonable to delay definitive therapy until a time when delivery would not likely result in a long-term disability because of extreme prematurity.

Breast Cancer

Breast cancer is the most common malignancy of women, with approximately 1 in 8 women affected in their lifetimes: 0.2% to 3.8% of cases are diagnosed during pregnancy.[92,93] The incidence of breast cancer during pregnancy or within a year of delivery is 1.3 in 10,000 live births or 10 to 30 per 100,000 pregnancies.[94,95] Pregnancy does not seem to influence the actual course of the disease; however, there appears to be a higher risk of diagnostic delay and a trend toward more advanced stages in pregnant, compared with nonpregnant, women.

The diagnostic procedures for breast cancer should not be altered during pregnancy. Any suspicious mass should undergo a biopsy. Mammography, although discouraged for routine screening

in pregnancy, can be used safely if indicated. The fetal radiation exposure is negligible – approximately 0.001 to 0.01 mGy.[96] Mammography may be more difficult to interpret due to the physiologic changes of pregnancy, and ultrasound examination may be a useful alternative. Metastatic evaluation may also be limited because of a reluctance to utilize bone and liver scans during pregnancy. MRI can be used safely in the second and third trimesters.

Surgical therapy for breast cancer should not be delayed because of pregnancy. The risks of mastectomy and axillary node dissection appear to be low.[94] Radiation therapy is usually not recommended during pregnancy. If the patient has evidence of tumor invasion in the lymph nodes, adjuvant chemotherapy is often given. The timing of delivery should account for the following factors:

- When would the fetus have a reasonable chance for survival with a low risk of severe permanent morbidity?
- Can the number of cycles of chemotherapy be minimized with an earlier delivery? In addition, avoiding delivery just before or just after administration of chemotherapy is important to reduce the risk of immunosuppression and infection.
- How long can radiotherapy be safely delayed without increasing the risk of metastatic spread of the tumor?

Approximately 10% of women treated for breast cancer become pregnant, the majority within 5 years of diagnosis. Data suggests that pregnancy does not influence recurrence rate.[97] It seems reasonable to delay childbearing for at least 1 to 2 years, which is the time of the highest rate of recurrence.[98]

Ovarian Cancer

Most ovarian cancer occurs in women older than 35 years. Delayed childbearing has been more widely accepted, exemplified by British birth rates doubling in women older than 30 years and tripling in women older than 40 years since 1975, in addition to a twofold increased birth rate among US women older than 40 years since 1981.[99,100] It would not be surprising for the rate of ovarian and other cancers during pregnancy to increase. However, the current estimate of actual ovarian malignancies in pregnancy is low and estimated to range from 1 in 10,000 to 1 in 50,000 deliveries.[99] Whereas most ovarian cancers are epithelial in origin, borderline epithelial and germ cell tumors (dysgerminomas and malignant teratomas) are more common in pregnancy.

The widespread use of ultrasonography, particularly in the first two trimesters, has been helpful in identifying adnexal masses. Fortunately, most are functional cysts (13% to 17%) or benign teratomas, serous or mucinous cystadenomas, endometriomas, or paraovarian cysts.[101] Actual malignancy is rare and is estimated at 5% of the ovarian masses found. Surgery for an adnexal mass occurs in approximately 1 per 1000 pregnancies. Most procedures are performed not for suspected malignancy but because of concern about torsion or rupture. The incidence of adnexal torsion ranges between 7% and 22%, with increasing rates in masses greater than 6 cm.[102,103] Often these events occur at the end of the first trimester when the uterus elevates beyond the true pelvis, and at the time of delivery.

The characterization of an ovarian process can be aided by ultrasonography or MRI, but these modalities are not definitive. Ultrasound scoring systems that use size and character poorly predict malignancy but have a better negative predictive value.[104] Although an ovarian cyst, particularly if it is simple in nature, is probably not cancer, however, malignancy cannot be excluded. Indications for surgical exploration include a complex mass, a persistent simple cyst greater than 8 cm, or one that is symptomatic.[105] The optimal time for laparotomy is during the second trimester. If patients opt for more conservative management, they should be counseled that they have a potential risk of requiring emergent surgery for an acute event such as torsion or rupture.

If a malignancy is confirmed, staging and therapy are no different than for a nonpregnant woman. Frozen-section diagnosis, peritoneal washings, omentectomy, and sub-diaphragmatic biopsy are performed. Depending on the cell type and the stage, treatment can range from removal of the affected adnexa to complete hysterectomy and bilateral oophorectomy. Fortunately, most epithelial ovarian cancers found in pregnant women are usually of a lower stage, with 59% of reported cases being stage I.[99]

Survivors of Childhood Cancer

Given the improvements of therapy for childhood cancer, a large number of these individuals have survived into adulthood. Some are unable to conceive because of high-dose radiation or cytotoxic chemotherapy. The risk of decreased fertility for patients exposed to pelvic radiation therapy may be as high as 32%.[106] Those who remain fertile may have concerns regarding whether their treatment increases the risk of adverse pregnancy outcomes. Although data are limited, female cancer survivors treated with radiation therapy appear to have increased risks of premature delivery, low birth weight, and miscarriage. There is no evidence that female partners of male cancer survivors treated with radiation have these excess risks.[107]

Maternal Seizure Disorders

Epilepsy is the most common major neurologic disorder in pregnancy. Approximately 18 million women are affected worldwide, and 40% of those are of childbearing age. The estimated prevalence in pregnancy is 0.2% to 0.7%.[108] The pattern of maternal seizures ranges from complex partial to generalized tonic-clonic (grand mal) and generalized absence (petit mal) seizures. Physiologically, seizures arise from paroxysmal episodes of abnormal brain electrical discharges; when associated with motor activity, they are termed convulsive.

The effect of pregnancy on the frequency and severity of the seizure disorder has been difficult to ascertain because of limited prospective data. EURAP, an international registry of Antiepileptic Drugs and Pregnancy, reported on more than 3,700 patients whose seizure frequency and treatment were recorded. Sixty-six percent of patients had no seizures during their pregnancy. When using first-trimester seizure activity as a reference, 71% had no change in frequency in the second and third trimester, 13% improved, and 16% deteriorated.[109] In patients with worsening seizure activity, decreased plasma concentrations of antiepileptic drugs (AEDs) have been hypothesized as causative. The fall in plasma drug levels during pregnancy may be due in part to increased protein binding, reduced absorption, and increased drug clearance. The adequacy of prepregnancy seizure control can influence a patient's course during gestation. Patients whose seizures were poorly controlled tended to have more frequent seizures during pregnancy, whereas patients who had no seizures for 2 years before pregnancy had only a 10% chance of experiencing seizures during gestation. These latter patients may be candidates for stopping therapy or considering monotherapy if they have previously required multiple antiepileptic drugs.[110,111]

Perinatal Risk

For reasons that are not clear, women with seizures have more obstetric complications and a higher rate of poor perinatal outcomes. A recent systematic review comparing women with epilepsy to those without revealed increased odds of spontaneous miscarriage, antepartum and postpartum hemorrhage, hypertensive disorders, labor induction, cesarean section, preterm birth, and fetal growth restriction.[112] Preexisting conditions may play a role in these outcomes, as one large population-based study found significant associations between pregestational diabetes, hypertension, psychiatric diagnoses, and substance use disorders among pregnant patients with epilepsy compared to those without epilepsy.[113]

Earlier publications suggested an increased risk of congenital malformations in children of mothers with epilepsy even without prenatal use of AED. More recent data from larger, registry-based sources refute this. A study comparing patients with epilepsy to matched controls revealed that women receiving no medication had no increased rate of congenital malformations; however, monotherapy was associated with an increased risk of embryopathy (odds ratio [OR] 2.8). Furthermore, the frequency was even higher with the use of two or more drugs (OR 4.2).[114] A meta-analysis revealed the OR of malformations in those with untreated epilepsy was similar to nonepileptic controls (OR 1.92, 95% CI 0.92 to 4) but a higher prevalence of a major congenital malformation in those exposed to antiepileptic medications (OR 3.26, 95% CI 2.15 to 4.93).[115]

Malformation risk correlates with the number of medications used and is dose-dependent in those receiving monotherapy Recent updates from five international registries have reported malformation rates ranging from 1.8% to 13.8% with monotherapy and 6% to 9.8% with polytherapy.[116,117] Historically, malformations associated with AED include a fivefold rise in the rate of orofacial clefts, an increase in the rate of congenital heart disease, particularly with trimethadione, and a 3.8% incidence of neural tube defects in fetuses exposed to valproic acid.[118–120] Facial abnormalities (e.g., midface hypoplasia) are not specific to any particular AED; they have been seen with phenytoin, carbamazepine, and trimethadione. Some antiepileptic medications can adversely affect postnatal cognitive development. Although conclusive data are lacking, there may be an increased adverse effect, particularly with valproate.[121,122]

Overall rates of congenital malformations have declined as prescribing patterns of AED have changed. In a large observational study from the EURAP study group examining AED use from 2000 to 2013, as the rate of valproic acid and carbamazepine monotherapy decreased (25.9% to 6.7% and 41.1% to 13.5%, respectively), there was a 27% decrease in congenital malformations over the same time period.[123]

Fetal Hydantoin Syndrome

The classic features of the fetal hydantoin syndrome are facial clefting, a broad nasal ridge, hypertelorism, epicanthal folds, distal phalangeal hypoplasia, and growth and mental deficiencies; however, these effects also result from the use of other antiseizure medications (Table 9.12).[124] The postulated cause of this syndrome is the teratogenic action of a common epoxide intermediate of these medications. The hydantoin syndrome was found to develop in fetuses with inadequate epoxide hydrolase activity.[125] This enzymatic deficiency appears to be recessively inherited. It appears that preconception folic acid supplementation can reduce the risk of major congenital malformations in women prescribed AED.[126]

Management

Management of the pregnant patient with epilepsy centers on minimizing seizure activity to reduce physical risk and lower the incidence of fetal complications. Preconception counseling is vital and should entail (1) adjusting medication doses into the therapeutic range, (2) attempting to utilize monotherapy, if possible, and (3) choosing an agent with the least risk of teratogenesis. If the patient's disease is adequately controlled with a specific agent, changing medications based solely on teratogenicity risk should be weighed against the risk of increasing seizure activity. Typically, the benefit of preventing seizure activity outweighs the potential for reducing congenital malformations, though ultimately this must be a shared decision between patient and provider. Levetiracetam has increasingly been utilized as a first-line AED in pregnancy given its clinical effectiveness, convenient range of dosing, and favorable malformation risk profile.

Folic acid supplementation reduces the risk of open neural tube defects in the general population. Given the known inhibition of folate synthesis by many AED, patients with epilepsy on antiepileptic therapy should be encouraged to take folic acid supplementation (400 μg daily) at least 3 months prior to conception. Data remain insufficient about the efficacy of high dose folic acid (e.g., 4 mg daily) in patients taking AEDs, therefore both the American Academy of Neurology (AAN) and American College of Obstetricians and Gynecologists (ACOG) continue to recommend 400 μg daily supplementation for patients prescribed AED.[127] Additionally, there is some evidence of potential drug–drug interactions and animal evidence that high levels may have adverse effects on fetal brain development.[128] Maternal serum alpha-fetal protein (MsAFP) screening for neural tube defects, a fetal anatomic ultrasound, and a targeted fetal echocardiogram in the mid-trimester should be considered for all patients prescribed AED.

TABLE 9.12 Clinical Features of the Fetal Hydantoin Syndrome

Craniofacial abnormalities	Broad nasal ridge
	Wide fontanel
	Low-set hairline
	Broad alveolar ridge
	Metopic ridging
	Short neck
	Ocular hypertelorism
	Microcephaly
	Cleft lip with or without palate
	Abnormal or low-set ears
	Epicanthal folds
	Ptosis of eyelids
	Coloboma
	Coarse scalp hair
Limb abnormalities	Smallness or absence of nails
	Hypoplasia of distal phalanges
	Altered palmar crease
	Digital thumb
	Dislocated hip

Data from Briggs GC, Freeman RK, Yaffe SJ. *Drugs in Pregnancy and Lactation*. 7th ed. Baltimore: Lippincott Williams & Wilkins; 2005.

Serum AED levels should be checked monthly, and the dose adjusted accordingly to maintain therapeutic levels, particularly with the use of lamotrigine, carbamazepine, and phenytoin.[126] Although the evidence is less clear with other agents such as phenobarbital, valproate, primidone, and ethosuximide, serial level assessment should not be discouraged. Medications should not be changed unless they prove ineffective at the optimal serum level. If a patient reports greater seizure activity, the serum drug level should be checked immediately. A common reason for increased seizures is discontinuation or inconsistent use due to patient hesitancy towards AED use in pregnancy. As with any chronic medical condition, continuous reinforcement of the benefits of treatment combined with ongoing dialogue about patient concerns can often mitigate potential interruptions in care.

Mothers taking older generation AED, such as phenytoin, phenobarbital, or primidone, may have a higher incidence of neonatal coagulopathy as a result of vitamin K-dependent clotting factor deficiency. Although maternal vitamin K supplementation in the third trimester may be reasonable, there is insufficient evidence to determine whether it will reduce neonatal hemorrhagic complications.[126] Pediatricians should be made aware of maternal epilepsy and AED use in order to adequately assess the newborn for AED side effects, reassure patients about the safety of breastfeeding with AED, and counsel patients about the importance of maintaining their therapy to ensure safe care of the newborn. Additionally, when counseling about postpartum contraception, in particular oral combination pills (OCP), care must be taken to assess whether an AED reduces their efficacy, or conversely if the OCP reduces AED concentrations. For this reason, long-acting reversible contraception such as an intrauterine device or subcutaneous implant should be considered first-line options.[129]

Perinatal Mood and Anxiety Disorders

Perinatal mood and anxiety disorders (PMADs), including depression, anxiety, obsessive-compulsive disorders, posttraumatic stress disorder, bipolar disorders, and psychosis, affect approximately one in five pregnancies, including up to 1 year postpartum. It is estimated that maternal mental health conditions contribute to nearly 13% of all maternal deaths in the United States, with suicide accounting for 5% to 7% of these deaths.[130] Coexisting factors such as substance use disorders, socioeconomic barriers, and racial disparities often delay the diagnosis and treatment of PMADs, leading to worse perinatal outcomes and suboptimal parent-child bonding. (Table 9.13).[131] Routine screening of all pregnant patients for PMADs in both the antepartum and postpartum periods is recommended by ACOG, as well as the American Academy of Pediatrics (AAP), and U.S. Preventive Services Task Force.[132–134] Additionally, screening for thyroid dysfunction or substance use disorder may aid in addressing coexisting medical conditions. Ultimately, a multidisciplinary approach is advantageous, including social work, licensed therapists, addiction specialists, peer support groups, and in cases of incarceration or question of competency, legal professionals, and ethics boards.

Depression

Depression remains one of the leading causes of disability worldwide, with prevalence continuing to rise.[135] Rates of depression during pregnancy are 7% to 11% in the first trimester, 9% to 13% in the second trimester, and 9% to 12% in the third trimester. The prevalence over the entire pregnancy course is 18.4%.[136,137] Risk factors for perinatal depression include a history of depression or anxiety, unintended pregnancy, lower socioeconomic and education status, domestic violence, single status, and limited social support network.[138] The presence of a chronic medical condition in conjunction with side effects from various medications has also been associated with perinatal depression. All pregnant patients should be screened routinely with a validated screening tool at their first prenatal visit and during the postpartum period, at a minimum. Additional screening should be considered at 1, 2, 3, and 6 months postdelivery, or with any significant negative pregnancy event or change in mood or health status (Table 9.14).[134]

TABLE 9.13 Impact of Psychiatric Illness on Pregnancy Outcome

Illness	Teratogenic Effects	IMPACT ON OUTCOME		Treatment Options
		Obstetric	Neonatal	
Anxiety disorders	N/A	Increased incidence of forceps deliveries, prolonged labor, precipitate labor, fetal distress, preterm delivery, and spontaneous abortion	Decreased developmental scores and inadaptability; slowed mental development at 2 years of age	Benzodiazepines Antidepressants Psychotherapy
Major depression	N/A	Increased incidence of low birth weight, decreased fetal growth, and postnatal complication	Increased newborn cortisol and catecholamine levels, infant crying, rates of admission to NICUs	Antidepressants Psychotherapy ECT
Bipolar disorder	N/A	See *Major depression* entry above	See *Major depression* entry above	Lithium Anticonvulsants Antipsychotics ECT
Schizophrenia	Congenital malformations, especially cardiovascular	Increased incidence of preterm delivery, low birth weight, small for gestational age, placental abnormalities, and placental hemorrhage	Increased rates of postnatal death	Antipsychotics

ECT, Electroconvulsive therapy; *N/A*, not applicable; *NICUs*, neonatal intensive care units.
From Use of psychiatric medications during pregnancy and lactation. ACOG Practice Bulletin No. 92. American College of Obstetricians and Gynecologists. *Obstet Gynecol.* 2008;111:1001–1020.

TABLE 9.14 Validated Screening Tools for Perinatal Depression

Screening Tool	Items	Time to Complete (min)	Sensitivity and Specificity	Spanish Available
Edinburgh Postnatal Depression Scale	10	<5	Sensitivity 59%–100% Specificity 49%–100%	Yes
Postpartum Depression Screening Scale	35	5–10	Sensitivity 91%–94% Specificity 72%–98%	Yes
Patient Health Questionnaire 9	9	<5	Sensitivity 75% Specificity 90%	Yes
Beck Depression Inventory	21	5–10	Sensitivity 47.6%–82% Specificity 85.9%–89%	Yes
Beck Depression Inventory–II	21	5–10	Sensitivity 56%–57% Specificity 97%–100%	Yes
Center for Epidemiologic Studies Depression Scale	20	5–10	Sensitivity 60% Specificity 92%	Yes
Zung Self-Rating Depression Scale	20	5–10	Sensitivity 45%–89% Specificity 77%–88%	No

From Gestational hypertension and preeclampsia. ACOG Practice Bulletin No. 222. American College of Obstetricians and Gynecologists. *Obstet Gynecol* 2020;135:e237–60.

Cognitive behavioral therapy (CBT) should be considered a first-line treatment to address perinatal depression. Oftentimes, CBT alone is not effective for major depression. Adding medication to regular therapy is the most common and effective adjunct treatment for major depression. However, due to concerns about fetal exposure to antidepressants, patients or providers commonly limit or discontinue medication use when pregnancy is diagnosed. Unfortunately, these patients have a 68% risk of relapse of major depression as opposed to a 26% risk in those who continue their medication regimen. Of those who discontinued medication, half will relapse in the first trimester and 90% by the second trimester.[139] Another study found that among those who discontinued antidepressants, over half had to restart them in pregnancy.[140]

Medical treatment of depression is effective in approximately 70% of cases. The most commonly prescribed antidepressants during pregnancy are selective serotonin reuptake inhibitors (SSRIs). As a group, SSRIs have not been consistently associated with specific birth defects.[141] Paroxetine has been associated with an increased risk of congenital heart defects, particularly with doses greater than 25 mg daily in the first trimester, and should be avoided as a first-line antidepressant, if possible.[142]

Persistent pulmonary hypertension of the newborn (PPHN) has been associated with SSRI use in pregnancy. Initial studies placed the incidence of PPHN at 10 in 1000 in fetuses exposed to SSRIs after 20 weeks.[143] A subsequent metaanalysis from pooled studies accounting for varying study sizes and quality estimated a far lower risk estimate of 1 case of PPHN for every 1000 pregnant patients exposed to SSRIs.[144] In reviewing the potential relationship between SSRI and PPHN, there are also a number of risk factors for PPHN that are also associated with depression, including obesity, smoking, cesarean section, and early delivery.[145] When assessing the potential link between SSRIs, prematurity, and low birth weight, systematic evaluation suggests that the medication is not the most likely cause, but rather one of the many confounders that accompany both a diagnosis of depression and risk for prematurity.

Approximately 30% of newborns exposed to SSRIs prior to birth develop poor neonatal adaptation syndrome (PNAS).[146] This is usually transient and most often does not require specific medical care. PNAS affects the central nervous system, motor function, and the gastrointestinal and respiratory systems. NICU admissions are more common.[147] Reduction of medication dose in the latter third trimester in the hope of reducing adverse neonatal outcomes does not appear to be beneficial.[148] Long-term outcome data suggest that maternal antidepressants do not cause autism or affect the neurodevelopment of offspring.[149] Therefore given the lack of consistent data suggesting significant fetal harm, SSRIs are currently considered the first-line antidepressant for use in pregnancy.[146] Furthermore, reducing the dose or discontinuing SSRIs in the latter part of pregnancy does not appear to improve neonatal outcome.

Electroconvulsive therapy (ECT) has been used in pregnancy, usually for refractory severe cases of psychotic depression, schizophrenia, and bipolar disease. There are some potential complications with ECT, including fetal bradycardia (2.7%), preterm contractions, or labor (3.5%), and potential complications of anesthesia including aspiration.[150]

There have been concerns regarding the use of SSRIs during breastfeeding. Fluoxetine has an active metabolite with a long half-life and is found in higher concentrations in infants.[151] Short-term neonatal effects have been reported, including increased crying, decreased sleep, and irritability, particularly with fluoxetine and citalopram. However, the relative infant dose is less than 10% of the maternal dose, and this level is lower than the fetal exposure.[146] In cases of prematurity, where there is immaturity of drug elimination pathways in the newborn, there may be potential for relative toxicity leading to irritability, agitation, and sleep disturbances. However, this is usually mitigated with medication adjustment or change, if possible, or supplementing breastfeeding with donor milk. The benefits of breastfeeding must be accounted for in decisions regarding the use of SSRIs, especially considering the

significant risk for postpartum depression. The currently available data do not support a contraindication for lactation.[146]

Postpartum Psychosis

Postpartum psychosis is rare, with a recent metaanalysis placing the incidence at between 0.89 to 2.6 per 1000 live births.[152] This condition is distinguishable from postpartum depression by a patient's inability to discern reality from periods of delirium. Patients at risk for postpartum psychosis typically have an underlying major depressive disorder, mania, or schizophrenia. Other risk factors include younger age and family history. The recurrence rate is approximately 25%. The peak onset of symptoms is between 10 and 14 days after delivery. Common clinical features of postpartum psychosis include confusion, disorganization, depersonalization, insomnia, irritability, delusions or hallucinations, and mood abnormalities such as mania, agitation, or depression.[153] Recognition of this disorder is extremely important to the protection of the patient and her family. Prompt hospitalization and intensive pharmacologic therapy are the mainstays of early intervention.

Schizophrenia

The prevalence of schizophrenia in a general population ranges between 4 and 7 per 1,000 persons, depending on the estimate used.[154] It is most commonly associated with delusions, hallucinations, and incoherence. Morbidity is higher than that of any other PMAD. There appears to be a genetic component to the etiology, with schizophrenia developing in approximately 10% of offspring of an affected person. Concordance of schizophrenia in identical twins reaches 65%. In a large, contemporary case-control study, schizophrenia was associated with higher rates of adverse pregnancy outcomes, including preeclampsia, venous thromboembolism, preterm birth, and both small and large for gestational age newborns.[155]

With a peak age of incidence of approximately 20 years, a higher incidence in women than men, and lower rates of routine primary or gynecologic care, obstetricians often face complicated situations of unintended pregnancies, discontinued or suboptimal psychiatric treatment, and situations where a patient may not fully grasp that they are pregnant. There appear to be higher rates of cesarean section and operative vaginal delivery in affected patients.[156] Offspring of women with schizophrenia may have a higher rate of sudden infant death syndrome and congenital malformations.[157] However, it is difficult to ascertain whether these risks are independent of other factors such as smoking, low socioeconomic status, poor social support, and the use of certain medications.

Treatment is achieved primarily using psychotropic medication. While antipsychotic medications cross the placenta, the potential for teratogenesis appears low and is often outweighed by the benefits of maintaining treatment. Lithium, used primarily in mania, is associated with a higher rate of Ebstein anomaly. Although the incidence of this consequence is low, either safely deferring the medication in the first trimester or continuing its use with careful counseling is a viable alternative.[158] Fetal echocardiography should be performed in women who have used lithium in early pregnancy.

As with antidepressants, the benefits of continuing most antipsychotic medications during lactation and breastfeeding appear to outweigh the minimal newborn risks reported in the literature (Table 9.15).[151,159]

TABLE 9.15 Summary of Current Knowledge of Drug Excretion Into Breast Milk, Drug Concentrations in Infant Serum, Adverse Effects in the Child, and Breastfeeding Recommendations for Different Psychotropic Drugs

Class or Drug	Drug Transfer Into Breast Milk	Infant Plasma Concentrations	Adverse Effects in the Child	Breastfeeding Recommendations
SSRIs	Low	Low plasma concentrations	Case reports of adverse effects in infants exposed to fluoxetine and citalopram	Compatible with breastfeeding; however, fluoxetine and citalopram may not be drugs of first choice
TCAs	Low	Low plasma concentrations (except doxepin)	No suspected immediate adverse effects observed (except doxepin)	Compatible with breastfeeding; however, doxepin should be avoided
Other antidepressants	Limited data	Limited data	Limited data	Insufficient data
Benzodiazepines	Low	High plasma concentrations with longer-acting drugs with active metabolites	Case reports of CNS depression reported for diazepam	Sporadic use of short-acting benzodiazepines unlikely to cause adverse effects
Lithium	Low	Dose received by the infant is high	Limited data; some reports of toxicity in the infant	Limited data; however, breastfeeding should be avoided
Carbamazepine, sodium valproate	Low	Low plasma concentrations	Some case reports of various adverse effects in the infant	Generally more compatible with breastfeeding than lithium
Lamotrigine	High	High plasma concentrations	Limited data	None able to be made
Novel antipsychotics	Moderate	Variable plasma concentrations	Limited data	None able to be made

CNS, Central nervous system; *SSRIs*, selective serotonin reuptake inhibitors; *TCAs*, tricyclic antidepressants.
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10

Hypertensive Complications of Pregnancy

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KEY POINTS

- Hypertensive disorders of pregnancy are classified as chronic hypertension, gestational hypertension, preeclampsia with and without severe features, and chronic hypertension with superimposed preeclampsia.
- Pharmacologic management of chronic hypertension should be reserved for women with sustained elevations in blood pressure at or greater than 160 mm Hg systolic or 110 mm Hg diastolic.
- Magnesium sulfate administration to prevent eclampsia is used for those preeclampsia cases with severe features.
- Daily low-dose aspirin is recommended in women at high risk of preeclampsia, initiated between 12 and 16 weeks of gestation and continued until delivery.
- When preeclampsia without severe features is diagnosed, delivery should be accomplished at or beyond 37 completed gestational weeks. When severe features are present, delivery should be accomplished expeditiously (regardless of gestational age) once maternal stabilization has been achieved.

Hypertension is the most common medical problem in pregnancy, affecting 10% to 15% of all pregnant women. As the third most common cause of maternal mortality after thromboembolic disease (15%) and hemorrhage (11%), hypertensive disorders account for almost 10% of maternal deaths in the United States.[1] Complications arising from hypertensive disorders have profound effects on the fetus and neonate and thus are a major source of perinatal mortality and morbidity. Preeclampsia (PE) is a leading cause of perinatal morbidity and mortality, with an estimated 50,000 to 60,000 PE-related deaths per year worldwide. With the greatest morbidity and mortality, preeclampsia affects 5% to 7% of all pregnant women but is responsible for more than 70,000 maternal deaths and 500,000 fetal deaths worldwide every year. In the United States, it is a leading cause of maternal death, severe maternal morbidity, maternal intensive care admissions, cesarean section, and prematurity.

Unfortunately, the incidence of preeclampsia has increased by 25% in the United States during the past two decades.[2] Furthermore, because preeclampsia has been shown to be a risk factor for future cardiovascular and metabolic disease in women, recognizing the importance of proper perinatal management and prevention is essential.[3]

Classification of Hypertensive Disorders of Pregnancy

The diagnostic criteria for the hypertensive complications of pregnancy have undergone significant revision in the past several years.[4–7] The American College of Obstetricians and Gynecologists (ACOG) has recently summarized the current consensus regarding the definitions of hypertensive disorders encountered in pregnancy.[8,9] Hypertension during pregnancy is currently defined into four categories (Table 10.1):

1. Chronic hypertension which predates pregnancy or is present prior to 20 weeks of gestation
2. Gestational hypertension which occurs after 20 weeks of pregnancy and resolves postpartum
3. Preeclampsia
4. Chronic hypertension (predating 20 weeks of gestation) with superimposed preeclampsia

Importantly, proteinuria is no longer required for the diagnosis of preeclampsia if other abnormal findings are present. Preeclampsia is now defined as hypertension plus proteinuria *or* hypertension plus other systemic abnormalities noted as follows.

Hypertension in pregnancy is defined when blood pressure is:

- ≥140 mm Hg systolic or ≥90 mm Hg diastolic on two occasions at least 4 hours apart after 20 weeks' gestation in a woman with a previously normal blood pressure.
- The requirement for documenting hypertension twice over an interval of at least 4 hours is shortened to minutes if systolic pressure is ≥160 mm Hg or diastolic pressure is ≥110 mm Hg.

Proteinuria is defined when urinary protein is:

- ≥300 mg per 24-hour urine collection

or

- Protein/creatinine ratio ≥0.3

or

- Dipstick reading of 1+ in a voided urine specimen if more precise laboratory-based results are not readily available

When the hypertension criteria above are present but proteinuria is absent or not tested, preeclampsia is diagnosed when any of the following are present:

- Platelet count <100,000/μL.
- Liver transaminase levels are twice normal values or higher

TABLE 10.1 Classification of Hypertensive Disorders of Pregnancy

Category	Definition
Hypertension in pregnancy	Systolic blood pressure ≥140 mm Hg or diastolic BP ≥90 mm Hg, or both, measured on two occasions at least 4 hours apart
Chronic hypertension	Hypertension diagnosed or present before pregnancy or before 20 weeks of gestation, or hypertension diagnosed for the first time during pregnancy and that does not resolve in the postpartum period
Gestational hypertension	New-onset hypertension after 20 weeks of gestation in the absence of proteinuria that normalizes postpartum. Blood pressure elevation first noted after 20 weeks that does not resolve after delivery is defined as chronic hypertension.
Preeclampsia	New-onset hypertension with new-onset proteinuria (300 mg/24 hours or a protein/creatinine ratio of ≥0.3) or one or more of the following: thrombocytopenia ($<100 \times 10^9$/L) elevated liver transaminases (twice normal) renal insufficiency (serum creatinine >1.1 mg/dL or doubling creatinine level without preexisting renal disease) pulmonary edema new-onset headache or visual disturbances
Chronic hypertension with superimposed preeclampsia	Preeclampsia occurring in a woman with hypertension predating 20 weeks of pregnancy

Data from American College of Obstetricians and Gynecologists' Committee on Practice Bulletins—Obstetrics. ACOG practice bulletin No. 203: chronic hypertension in pregnancy. *Obstet Gynecol.* 2019 and Gestational hypertension and preeclampsia. ACOG Practice Bulletin No. 222. American College of Obstetricians and Gynecologists. *Obstet Gynecol.* 2020;135:e237–e260.

- Newly elevated serum creatinine is >1.1 mg/dL or a doubling in the absence of other renal disease
- Pulmonary edema
- New-onset cerebral or visual disturbances

Because fetal growth restriction is managed similarly in pregnant women with and without preeclampsia, it has been deleted as a criterion for severe preeclampsia.

Chronic Hypertension

Up to 5% of pregnant women have chronic hypertension, which is diagnosed when hypertension is present before pregnancy or recorded before 20 weeks of gestation. However, when hypertension is first noted in a patient after 20 weeks' gestation, it may be difficult to distinguish chronic hypertension from preeclampsia. In such cases, the precise diagnosis might not be made until after delivery and well into the postpartum period. Hypertension that is first diagnosed during the second half of pregnancy and persists postpartum is diagnosed as chronic hypertension.

Chronic hypertension has adverse effects on pregnancy outcome. A report of 55 studies of chronic hypertension comprising 795,221 pregnancies found that 26% of women entering pregnancy with chronic hypertension developed superimposed preeclampsia, 41% required cesarean section, and 28% had preterm delivery. Of the neonates, 17% had birth weight less than 2500 g, NICU admission occurred in 21%, and perinatal death occurred in 4.0%.[10] The adverse fetal and maternal perinatal outcomes are related to the severity of the preexisting hypertension. When chronic hypertension is secondary to maternal chronic renal disease, a large (506,000 pregnancies) systematic review found significantly elevated odds for preeclampsia (odds ratio [OR] 10.36), premature delivery (OR 5.72), small for gestational age (OR 4.85), and pregnancy failure (OR 1.80).[11] Women with untreated severe chronic hypertension are also at increased risk for cardiovascular complications during pregnancy, including stroke.[12]

The majority of cases of chronic hypertension seen in pregnancy are idiopathic (essential hypertension), but other causes should always be sought because pregnancy outcome is worse in women with secondary hypertension. Renal disease (e.g., chronic renal failure, glomerulonephritis, renal artery stenosis), cardiovascular causes (coarctation of the aorta, Takayasu arteritis), and, rarely, Cushing disease, Conn syndrome, and pheochromocytoma should be excluded through physical examination, history, and more detailed testing if needed.

Patients with chronic hypertension should be evaluated thoroughly early in pregnancy with serum urea, creatinine, and electrolyte measurements, urinalysis, and 24-hour urine collection for protein and creatinine clearance determinations. Reassessment of renal function should be performed in each trimester and more frequently if the patient's condition deteriorates.

Antihypertensive Treatment of Chronic Hypertension in Pregnancy

A 2014 Cochrane review of 49 trials found that treatment of mild-to-moderate hypertension reduced the risk of developing severe hypertension but had no effect on the incidence of preeclampsia, preterm birth, fetal death, fetal growth restriction, or any other measured outcome.[13] However, a recent multicenter trial[13a] (chronic hypertension in pregnancy study [CHAP]; 2408 women before 23 weeks' gestation with mild chronic hypertension randomized to receive antihypertensive medications if blood pressure was >140/90 mm Hg [active treatment] versus no treatment unless severe hypertension developed [SBP >160 or DBP >=105]) documented a reduction in composite outcome (severe preeclampsia, medically indicated delivery <35 weeks' gestation, placental abruption, or fetal/neonatal death) with maternal antihypertensive medication treatment–30.2% versus 37.0%; adjusted risk ratio (RR), 0.82; 95% CI, 0.74–0.92; P<.001. Importantly, antihypertensive medication treatment had no effect on birth weight <10th percentile and other serious maternal and neonatal complications.

The CHAP study demonstrated for pregnant women with chronic hypertension that a treatment threshold of 140/90 mm Hg yields improved outcomes compared to initiating medication

only when blood pressure rises above 160/105 mm Hg, as previously recommended. One strength of the CHAP study includes the fact that the majority of enrolled patients had chronic hypertension on medication at the time of trial entry (56%) and 41% were enrolled prior to 14 weeks' gestation.

Thus, while antihypertensive medications were reserved in the past for patients whose blood pressures placed them at significant risk of maternal stroke (systolic blood pressure of ≥160 mm Hg or diastolic pressure of ≥110 mm Hg), the American College of Obstetricians and Gynecologists recently published a Practice Advisory[13b] recommending 140/90 as the threshold for initiation or adjustment of medical therapy for chronic hypertension in pregnancy, rather than the previous threshold of 160/110 mm Hg. Patients taking blood pressure medications at the start of pregnancy should be maintained on their medications, rather than discontinuing them and waiting to initiate treatment for blood pressures in the severe range.

Antihypertensive Medications in Pregnancy

The choice of antihypertensive agent for use in pregnancy is governed by reducing risk of severe maternal sequelae (e.g., cerebral hemorrhage, myocardial infarction) while avoiding adverse effects on the fetus. Because lowering of maternal systolic blood pressure to <140 mm Hg or diastolic to <90 mm Hg can compromise uterine perfusion and fetal growth and oxygenation, maternal pressures should be maintained in the 120 to 155 mm Hg systolic and 90 to 105 mm Hg diastolic range.[8]

The antihypertensive medications most commonly used in pregnancy are listed in Table 10.2.

α- and β-Adrenergic Blockers

Labetalol is a mixed α_1-adrenergic, β_1-adrenergic, and β_2-adrenergic blocker and is the most frequently used medication for chronic hypertension in pregnancy. Pure β-blockers have been associated with increased risk of IUGR (e.g., atenolol), and the mixed adrenergic blockade produced by labetalol is thought to mitigate this unwanted effect.[14] Labetalol is also used intravenously to manage severe hypertension accompanying preeclampsia.

Calcium Channel Blockers

Nifedipine in extended-release forms is effective in managing chronic hypertension in pregnancy.[15] In immediate-release form, nifedipine 10 to 20 mg orally or as a constant infusion of 0.5 to 10 mg/hr is recommended for urgent management of severe-range maternal blood pressures exceeding 160/110 mm Hg.

Methyldopa

α-Methyldopa is a centrally acting antihypertensive agent, it has a slow onset of action with prolonged time to therapeutic effect (days), and compliance with methyldopa therapy may be impeded by side effects such as sedation in some patients.

Hydralazine

Hydralazine, a potent peripheral vasodilator, is used intravenously to treat acute hypertensive emergencies in pregnancy (blood pressure >160/110 mm Hg). Its role as an oral agent in the management of chronic hypertension is limited to a second- or third-line choice. Long-term use of hydralazine may be associated with a lupus-like syndrome in some patients.

Diuretics

Diuretics are often used in hypertension in nonpregnant adults, but there is little role for their use in pregnant women with hypertension. Because diuretics can reduce the plasma volume expansion of normal pregnancy and thus impede fetal growth, use of diuretics in pregnant patients should be limited to those with cardiac dysfunction or frank pulmonary edema.[16]

Angiotensin-Converting Enzyme (ACE) Inhibitors and Angiotensin Receptor Blockers (ARBs)

ACE and ARBs should not be used during pregnancy because of their association with fetal renal failure, oligohydramnios, pulmonary hypoplasia, and intrauterine or neonatal death.[17] Transitioning women taking these agents in the prepregnancy period is recommended. Patients who conceive while taking an ARB or ACE inhibitor should be switched to a safer alternative as soon as possible.

Low-Dose Aspirin

Low-dose aspirin prophylaxis (81 mg/day) after 12 weeks of gestation has been shown to modestly reduce the risk of preeclampsia in women at increased risk, without adverse fetal or maternal effects. The US Preventive Services Task Force and ACOG recommend daily low-dose aspirin for women with a history of early-onset preeclampsia requiring delivery <34 weeks of gestation, or with more than one prior pregnancy complicated by preeclampsia.[18] The guidelines recommend initiating aspirin prophylaxis (81 to 162 mg daily) between 12 and 28 weeks of gestation (optimally before 16 weeks) and to continue until delivery.

Antenatal Fetal Surveillance in Chronic or Gestational Hypertension

Because women with chronic hypertension are at increased risk of slowing of fetal growth and of superimposed preeclampsia, antenatal surveillance in women with chronic hypertension is recommended. However, limited data exist regarding optimal timing and intervals of testing.

The procedures of providing antenatal fetal biophysical testing in chronic hypertension should also include maternal screening for signs of superimposed preeclampsia.

Detection of tapering fetal growth requires periodic sonographic fetal growth assessments, typically every 3 to 4 weeks from 24 weeks

TABLE 10.2 Drugs Commonly Used to Treat Chronic Hypertension in Pregnancy and Their Modes of Action

Drug	Mode of Action	Typical Dose
Methyldopa	Centrally acting antihypertensive	0.5–3 g/day in 2–3 divided doses
Labetalol	Mixed α- and β-adrenergic blockers	200–2400 mg/day in 2–3 divided doses
Nifedipine	Calcium channel blocker	30–120 mg/day in slow-release form

Adapted from American College of Obstetricians and Gynecologists; Task Force on Hypertension in Pregnancy. Hypertension in pregnancy. Report of the American College of Obstetricians and Gynecologists' Task Force on Hypertension in Pregnancy. *Obstet Gynecol.* 2013;122:1122–1131.

onward. Because arrest of fetal growth is an indication for delivery, tapering of growth on sonogram should prompt more frequent and intensive evaluations. If the sonographic estimated fetal weight tapers below the 20th percentile or the abdominal circumference is at a significantly smaller percentile than the head circumference, more intensive growth sonography and frequent fetal biophysical surveillance is indicated. In such cases, sonography is typically performed at 10- to 21-day intervals with attention to amniotic fluid volume, trending of each biometric parameter (especially abdominal circumference), and umbilical artery Doppler waveforms.

Weekly fetal biophysical testing in IUGR has been shown to reduce perinatal mortality while not increasing labor induction or cesarean delivery.[19] Indications for delivery in the hypertensive patient with fetal IUGR include absence of growth of the head and abdomen over a 10-day interval, oligohydramnios, biophysical score of less than 6, or reversal of end-diastolic velocity on the umbilical Doppler waveform.

Women with renal impairment and chronic hypertension have a markedly higher risk of poor perinatal outcome than women without renal impairment. The incidence of impaired fetal growth is closely related to the degree of renal impairment, and women undergoing dialysis are at particular risk for fetal growth failure, preterm delivery, and fetal death, even with optimal management. Those who start dialysis during pregnancy are at the greatest risk, with only a 50% chance of a surviving infant.[20]

Gestational Hypertension

Management of women with gestational hypertension and preeclampsia is summarized in Box 10.1.

Gestational hypertension is defined as new-onset hypertension occurring after 20 weeks of pregnancy in the absence of signs of preeclampsia.[4,9] During pregnancy, gestational hypertension is indistinguishable from preeclampsia-in-evolution. Because a woman with apparent gestational hypertension at 36 weeks of gestation can rapidly devolve into preeclampsia at 39 weeks of gestation, the diagnosis of gestational hypertension should always evoke caution and vigilance. Only if the patient's blood pressure returns to normal postpartum, without development of signs of preeclampsia during the pregnancy, should the final diagnosis of gestational hypertension be applied.

The earlier that gestational hypertension is evident, the greater the risk of preeclampsia. When the diagnosis is made before 32 weeks' gestation, more than one-third of patients will develop preeclampsia, whereas the risk is less than 10% when the diagnosis is made after 38 weeks of gestation. Use of antihypertensive agents to treat patients with gestational hypertension should be avoided, given the risk of concurrent preeclampsia and the lack of evidence supporting improved fetal outcome. Gestational hypertension tends to recur in subsequent pregnancies and predisposes women to hypertension in the future.[21]

Preeclampsia-Eclampsia

Apparently unique to humans, the underlying causes of preeclampsia remain poorly elucidated. It is evident that the clinical manifestations of preeclampsia arise from vascular endothelial dysfunction that ultimately involves the maternal central nervous, renal, hepatic, and cardiovascular systems. In its full-blown form, preeclampsia can produce a profound coagulopathy and liver, respiratory, or cardiac failure and maternal death.

Preeclampsia is categorized as having severe features when systemic complications (e.g., renal, hepatic, pulmonary, or hematologic) are present (Box 10.2). This distinction is important because, if the presence of severe features is documented at any gestational age, the only appropriate treatment option is delivery, whereas expectant management may be acceptable in a woman who has mild disease and is remote from term.

• BOX 10.1 Management of Pregnant Women With Chronic Hypertension

Monitoring
- Daily home BP monitoring with a validated cuff after 5 minutes in sitting position
- Fetal growth sonography every 4 weeks
- Fetal biophysical testing at least weekly from 32–34 weeks

Avoid
- Low sodium diets
- Weight loss prescriptions
- Limitations of moderate exercise

Prophylaxis
- If prior pregnancy had preeclampsia with severe features and delivery was <34 0/7 weeks, begin daily low-dose aspirin (81–162 mg) at 8–16 weeks of gestation

Antihypertensive Medications
- Maintain systolic BP 120–160 mm Hg; diastolic BP 80–105 mm Hg
- Use labetalol, nifedipine, or methyldopa if antihypertensives needed
- Avoid ACE inhibitors, ARBs, renin inhibitors, and mineralocorticoid receptor antagonists

Delivery
- At 37 0/7 weeks unless preeclampsia with severe features
- If <37 0/7 weeks, administer antenatal corticosteroids
- If preeclampsia with severe features
  - Promptly after maternal stabilization if uncontrollable BP, eclampsia, pulmonary edema, abruptio placentae, nonreassuring fetal status
  - Administer magnesium sulfate antepartum and postpartum

ACE, Angiotensin-converting enzyme; *ARBs*, angiotensin receptor blockers; *BP*, blood pressure.

Etiology

Although the precise etiology of preeclampsia remains uncertain, numerous factors are associated with elevated risk (Table 10.3). Up to 10% of primigravid patients have mild disease, and approximately 1% have severe disease. The most widely accepted theory for the pathophysiology of preeclampsia is based on pathologic observations in the placenta indicating hypoperfusion in the maternal spiral arteries with resultant hypoxia. The placenta then releases substances into the maternal circulation that adversely affect maternal endothelial function, leading to the clinical syndrome of widespread vascular dysfunction, which is recognized as the syndrome of preeclampsia.[22] Individual responses to the process of progressive maternal vascular dysfunction vary in severity and timing in a manner that seems to have genetic, familial, and immunologic components. For example, preeclampsia occurring in a first-degree relative confers a fourfold increase in risk of the disease in siblings and children.[23] Population studies have suggested that women exposed to the antigenic effects of sperm before conception have a lower rate of preeclampsia than do women who conceive with lower degrees of exposure, although the evidence is inconclusive.[24]

The endothelial dysfunction that characterizes preeclampsia[25] manifests as greater reactivity to circulating vasoconstrictors such

• BOX 10.2 Management of Pregnant Women With Gestational Hypertension or Preeclampsia

Women With Gestational Hypertension or Preeclampsia Without Severe Features

Monitoring and Management

- BP checks twice weekly
- Renal and liver function tests and platelet counts weekly
- Antepartum testing weekly from 32 weeks of gestation
- Fetal growth sonography every 2–4 weeks after 24 weeks of gestation
- If fetal growth restriction is diagnosed, monitor umbilical artery Doppler
- Antihypertensive medications if blood pressures >140/90 mm Hg
- Avoid strict bed rest

Delivery

- At or beyond 37 0/7 weeks unless preeclampsia with severe features
- Magnesium sulfate for the prevention of eclampsia
- Regional neuraxial anesthesia (spinal or epidural) may be performed
- Monitoring of BP should be performed for the first 72 h and again at 7–10 days

Women With Preeclampsia and Severe Features

- Administer parenteral magnesium sulfate and continue without interruption until at least 24–48 h postpartum
- If 34 0/7 weeks or more, deliver after maternal stabilization
- If less than 34 0/7 weeks
  - Delivery may be delayed in facilities with adequate maternal and neonatal intensive care while antenatal corticosteroids for fetal lung maturity are administered
  - Delivery should not be delayed if there is uncontrolled hypertension, eclampsia, pulmonary edema, abruptio placentae, or nonreassuring fetal status
- If previable gestational age, deliver promptly after maternal stabilization
- If sustained systolic BP >160 mm Hg and/or diastolic BP >110 mm Hg, administer antihypertensive medication

Prevention of Recurrent Preeclampsia

- If preeclampsia in prior pregnancy, begin daily low-dose aspirin (81–162 mg) prior to 16 weeks

BP, Blood pressure.

TABLE 10.3 Risk Factors for Development of Preeclampsia

Factor	Relative Risk
Primigravida	3
Age >40 years	3
African-American race	1.5
Family history	5
Chronic hypertension	10
Chronic renal disease	20
Antiphospholipid syndrome	10
Insulin-dependent diabetes mellitus	2
Multiple gestation	4

as angiotensin, reduced production of endogenous vasodilators such as prostacyclin and nitric oxide, increased vascular permeability, and an increased tendency toward platelet consumption and coagulopathy.[26] The end result is hypertension, proteinuria secondary to glomerular injury, edema, and a tendency toward extravascular fluid overload with intravascular hemoconcentration.

Prediction

Perhaps one of the most important contributions that prenatal care makes to maternal and fetal outcomes is the timely detection of preeclampsia and the prevention of eclampsia.[27] A wide variety of biochemical and physical tests have been proposed as screening tools for early detection of preeclampsia, but even the most widely used biochemical tests have poor predictive values.[28] Uric acid levels are elevated in many cases of preeclampsia, but the sensitivity of the measurement is low.[29] Clinicians should be aware of the limitations of routine urine testing for detection of proteinuria, with standard dipstick testing being notoriously inaccurate.[30]

Doppler ultrasonographic assessment of the vascular dynamics in the uterine arteries during the second trimester has been proposed as a screening tool in populations in which obstetric ultrasonography is routine.[31] Up to 40% of women who develop preeclampsia have abnormal waveforms, and this finding was reported to be associated with a sixfold rise in the risk of preeclampsia.[32] As no randomized trials to date have demonstrated convincingly the ability of uterine artery Doppler studies to predict preeclampsia, its use is currently not recommended.[9]

The role of angiogenic factors in the pathophysiology of preeclampsia has become increasingly clear. Vascular endothelial growth factor (VEGF) and placental growth factor (PlGF), which bind to fms-like tyrosine kinase-1 (Flt-1) and soluble fms-like tyrosine kinase-1 (sFlt-1) receptors, have a critical role in angiogenesis and placental development. Flt-1, VEGF, and PlGF factor promote angiogenesis and placental vasculogenesis, whereas sFlt-1, VEGF, and PlGF inactivate those proteins, resulting in disordered angiogenesis and endothelial dysfunction. Levels of sFlt-1 are elevated in women with preeclampsia, and these elevated levels of sFlt-1 precede the features of clinical preeclampsia.

Zeisler et al.[33] recently reported a multicenter, prospective study of the ratio of sFlt-1 to PlGF in women between 24 and 37 weeks' gestation who presented with a clinical suspicion of preeclampsia or the HELLP (*h*emolysis, *e*levated *l*iver enzymes, and *l*ow *p*latelet count) syndrome.[33] An sFlt-1:PlGF ratio of 38 was the optimal cutoff in distinguishing between women in whom preeclampsia would develop and those in whom it would not develop in the next week. In a validation cohort of 550 women, a ratio of 0.38 or lower had a negative predictive value of 99.3% (95% confidence interval [CI], 97.9 to 99.9).

Despite the clear negative predictive value of an sFlt-1:PlGF ratio of less than 38 for preeclampsia diagnosis in the subsequent week, the clinical utility of this fact in managing pregnant women remains unclear. Thus at present, serum screening for preeclampsia risk is not recommended.[9]

Prevention

If an accurate predictor of preeclampsia could be identified, the next logical step would be the application of a preventive or ameliorative treatment. Unfortunately, attempts to identify an effective treatment have proven equally difficult. Given the recognized association between vascular endothelial dysfunction and preeclampsia, prostaglandin inhibitors have been proposed as a candidate for prophylaxis or treatment. Numerous trials[34] have been conducted with low-dose aspirin, based on the idea that the ability of aspirin to irreversibly inhibit production of the vasoconstrictive prostaglandin thromboxane would promote greater activity of

prostacyclin, a vasodilatory prostaglandin. This ability of aspirin would help to maintain patency in the maternal placental vascular bed and limit or prevent the evolution of preeclampsia.

In a recent systematic review of 34 randomized controlled trials of women at risk for recurrent preeclampsia, low-dose aspirin started at 16 weeks or earlier was associated with a significant reduction in preeclampsia (9.3% treated vs. 21.3% control) and IUGR (7% treated vs. 16.3% control), whereas aspirin started after 16 weeks was not.[35] Low-dose aspirin started at 16 weeks or earlier also was associated with a reduction in severe preeclampsia (0.7% treated vs. 15.0% control) and preterm birth (3.5% treated vs. 16.9% control). Of note, all studies for which aspirin had been started at 16 weeks or earlier included women identified to be at moderate or high risk for preeclampsia.

Calcium supplementation has been proposed as a preventive treatment on the basis of the known vasodilatory effect of calcium and impressive results in earlier, small studies.[36] Similarly, it has been suggested that antioxidants may have a role in preeclampsia prevention, but the only available trial to date showed mixed results, with improvements in biochemical indices in women receiving vitamins C and E, although perinatal outcomes were not different in treated and untreated groups.[37] Of concern was the finding that women in whom preeclampsia developed despite vitamin therapy had markedly worsened preeclampsia than controls in whom the disease developed.

A recent Cochrane systematic review[38] found that calcium supplementation commencing before conception may make little or no difference to the risk of preeclampsia (69/296 versus 82/283, risk ratio [RR] 0.80, 95% CI 0.61 to 1.06). For preeclampsia or pregnancy loss or stillbirth (or both) at any gestational age, calcium may slightly reduce the risk of this composite outcome; however, the 95% CI met the line of no effect (RR 0.82, 95% CI 0.66 to 1.00). Supplementation may make little or no difference to the severe maternal morbidity and mortality index, stillbirth, or cesarean, but the existing data neither confirm nor refute the value of early-pregnancy calcium supplementation.

Antepartum Management

Given the current inability to predict or prevent preeclampsia in the majority of cases, clinicians should actively manage established disease to minimize maternal and fetal morbidity. The recognition that preeclampsia has a form with severe features is of great value in escalating management and minimizing morbidity (Box 10.3).

• BOX 10.3 Preeclampsia With Severe Features

- Systolic blood pressure of 160 mm Hg or more, or diastolic blood pressure of 110 mm Hg or more on two occasions at least 4 hours apart (unless antihypertensive therapy is initiated before this time)
- Thrombocytopenia (platelet count < 100,000 × 109/L)
- Impaired liver function not accounted for by alternative diagnoses and as indicated by abnormally elevated blood concentrations of liver enzymes (to more than twice the upper limit of normal concentrations), or by severe persistent right upper quadrant or epigastric pain unresponsive to medications
- Renal insufficiency (serum creatinine concentration > 1.1 mg/dL or a doubling of the serum creatinine concentration in the absence of other renal disease)
- Pulmonary edema
- New-onset headache unresponsive to medication and not accounted for by alternative diagnoses
- Visual disturbances
- Eclampsia

From Gestational hypertension and preeclampsia. ACOG Practice Bulletin, No. 222. American College of Obstetricians and Gynecologists *Obstet Gynecol.* 2020;135(6):e237–e260.

Mild disease is generally managed expectantly until 37 weeks with frequent fetal and maternal biophysical assessments or until severe features are identified, whereupon prompt delivery is indicated regardless of gestational age.

Patients with preeclampsia should undergo regular laboratory evaluation for severe features, including urine collection for protein/creatinine ratio, complete blood count with platelet measurements, serum uric acid, blood urea nitrogen, creatinine levels, and liver transaminases. Fetal size and interval growth should be determined ultrasonographically at least monthly. Although no longer a criterion for severe preeclampsia, the diagnosis of intrauterine growth restriction with estimated fetal weight less than the 10th percentile is a sign of fetal jeopardy.

At 37 weeks of gestation, patients with mild disease should be delivered because prolonging pregnancy further increases the risks of maternal and fetal morbidity. Patients at earlier gestational ages should be closely monitored with sequential clinical and laboratory evaluations. Fetal well-being should be evaluated once or twice weekly by means of nonstress tests or biophysical profiles. Because antihypertensive therapy may mask worsening preeclampsia, oral antihypertensive therapy should be avoided or held constant during expectant management.

However, severe hypertension (>160/110 mm Hg) requires prompt intervention with rapid-acting antihypertensive agents if stroke and placental abruption are to be avoided. Intravenous hydralazine is well established as a first-line drug for this purpose, although there is a growing experience with intravenous labetalol and oral nifedipine (Table 10.4).[39] The aim of treatment is to lower blood pressure into the mild preeclampsia range (<160/100 mm Hg) to reduce the risk of maternal stroke and cardiovascular complications. Parenteral magnesium sulfate to prevent eclampsia should be administered in all cases of severe disease.

Severe features of preeclampsia can also present with atypical central nervous system abnormalities including headache, visual deficits, confusion, seizures, and in the most severe cases, intracranial hemorrhage. This constellation of findings, when demonstrated on brain magnetic resonance imaging, has been termed the *posterior reversible encephalopathy syndrome* (PRES). Neuroimaging typically demonstrates cerebral edema in the parietal and occipital lobes. The long-term sequelae of PRES after preeclampsia/eclampsia and other PRES-related conditions are inadequately understood.[40]

More commonly, patients show evidence of intravascular microangiopathy leading to the hemolysis, elevated liver enzymes, and low platelets of HELLP syndrome. The full-blown clinical syndrome of HELLP carries a significant maternal risk related to coagulopathy and hepatic dysfunction, even hepatic rupture.[41] Most patients with HELLP never experience catastrophic features

TABLE 10.4 Drugs for Acute Treatment of Hypertension in Severe Preeclampsia

Drug	Dosage
Hydralazine	5 mg IV or IM, then 5–10 mg every 20–40 min as required, to a total of 30 mg or Constant intravenous infusion 0.5–10 mg/h
Labetalol	10–20 mg IV, then 20–80 mg every 20–30 min to a maximum of 300 mg or Constant intravenous infusion 1–2 mg/min
Nifedipine	10–20 mg PO, repeat in 30 min, then 10–20 mg every 2–6 h

of the syndrome because delivery is initiated before their condition deteriorates to a critical level.

Preeclampsia and Fetal Risk

While prompt delivery in cases of severe preeclampsia optimizes both maternal and newborn outcome, this practice increases the incidence of prematurity and its attendant complications. IUGR is not uncommon in severe preeclampsia, and there may be evidence of progressive deterioration in fetal well-being, with progressive placental dysfunction. Infants delivered before 36 weeks' gestation will benefit from as little as 8 hours of antenatal steroid therapy.[42] The incidence of respiratory distress syndrome is lower in infants of mothers with preeclampsia than in those of age-matched controls without antenatal steroid exposure.[43] Venkatesh et al. performed an analysis from the US Consortium on Safe Labor Study of deliveries <34 weeks with preeclampsia with severe features.[44] Among 2217 deliveries assessed, 50% had a maternal or neonatal comorbidity, namely chronic hypertension (30%), pregestational diabetes (8%), gestational diabetes (8%), twin gestation (10%), and fetal growth restriction (7%). Pregnancy complicated by fetal growth restriction (adjusted risk difference: 12.2%, 95% CI: 5.48 to 19.03) was more likely to result in adverse neonatal outcome but not with other comorbid conditions.

Intrapartum Management

Patients with preeclampsia and severe features should be delivered expeditiously once the maternal condition is stabilized. In most cases, induction of labor can be undertaken, reserving cesarean section for obstetric indications such as breech presentation, placenta previa, and concerning fetal status. The only exception to prompt delivery may be a case of severe preeclampsia limited to proteinuria and intermittently severe hypertension but remote from term (<28 weeks' gestation). Such patients may be managed conservatively within a high-risk center while antenatal corticosteroids are administered.[45] Patients with severe preeclampsia at less than 24 weeks' gestation should be offered termination of the pregnancy.

All women with preeclampsia and severe features should receive magnesium sulfate as seizure prophylaxis (Box 10.4). Current guidelines do not require magnesium sulfate for those with gestational hypertension/preeclampsia lacking severe findings. The safety and therapeutic superiority of magnesium sulfate over other agents (e.g., diazepam) have been validated in multiple randomized trials.[46]

Intrapartum blood pressure should be maintained in the mild preeclampsia range (<160/105 mm Hg) using intravenous antihypertensive agents (labetalol, hydralazine, or nifedipine). Careful attention to fluid balance, especially avoiding overload, should be maintained. After delivery, the preeclamptic process typically begins to resolve rapidly.

• BOX 10.4 Magnesium Sulfate Therapy for Prevention of Eclampsia

- Bolus 4–6 g IV over 20 min
- Continuous infusion 1–2 g/h
- Follow magnesium levels every 6–8 h to maintain serum level 4–6 mEq/L
- Continue infusion for 24 h after delivery or 24 h after seizure

Eclampsia

Eclampsia, by definition a "severe feature," is the occurrence of generalized tonic-clonic seizures in association with preeclampsia and affects approximately 1 in 2500 deliveries in the United States. Eclampsia is much more common in developing countries, affecting as many as 1% of parturients. Up to 10% of maternal deaths in the United States are due to eclampsia.[47]

Most cases of eclampsia occur immediately prior to or within 24 hours of delivery. Almost half of seizures occur before the patient's admission to labor and delivery, approximately 30% are intrapartum, and the remainder are postpartum. There is a considerable drop in the risk of eclampsia by 48 hours postpartum, with seizures occurring in less than 3% of women beyond that time. Most patients have antecedent symptoms that are suggestive of preeclampsia, although in some cases eclampsia may occur without warning. If eclampsia is left untreated, repetitive seizures become more frequent and of longer duration, and ultimately status eclampticus may develop. Maternal and fetal mortality are as high as 50% in severe cases, especially if the seizures occur while the patient is remote from medical care.

Randomized controlled trials have demonstrated the clear superiority of magnesium sulfate for the treatment of eclampsia over all other anticonvulsants.[48–50] Intravenous magnesium sulfate is given as a 4-g bolus over 5 minutes followed by a maintenance infusion of 1 to 2 g/h for 24 to 48 hours after delivery. Subsequent seizures can be treated with further bolus injections. In refractory cases, second-line treatment with other anticonvulsants may be required. Rarely, the patient may require pharmacologic paralysis and mechanical ventilation.

Delivery after an eclamptic seizure should take place expeditiously but in a controlled, careful manner. There is little value in performing an emergency cesarean section in response to a seizure.[51] Stabilization and optimization of both maternal and fetal status are important prerequisites for delivery procedures. Once magnesium sulfate has been administered and maternal vital signs controlled, vaginal delivery is preferable in most cases. Potential indications for cesarean include a significantly unfavorable cervix, evidence of ongoing fetal compromise, or inability to achieve acceptable blood pressure control. Infants born to mothers after eclampsia require careful observation after birth.

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11

Intrauterine Drug Exposure: Fetal and Postnatal Effects

GERRI R. BAER, RACHANA SINGH, AND JONATHAN M. DAVIS

KEY POINTS

- Substance use remains at epidemic proportions, both nationally and globally.
- Increasing legalization of marijuana has resulted in new concerns for fetal and infant exposure, with limited understanding of the short- and long-term effects in this population.
- The recent focus of perinatal substance use has grown to include widely prescribed substances, including opioids and other psychotropic drugs.
- The importance of establishing and maintaining consistent protocols in managing neonatal abstinence syndrome (NAS) has been clearly demonstrated.
- The choice to breastfeed is challenging and healthcare professionals have difficulties making recommendations for specific mother-infant dyads. The known benefits of breastfeeding must be weighed against any potential risks to the infant, most of which are not well understood.
- A comprehensive postnatal discharge care plan for the mother-infant dyad should be instituted to provide ongoing support and to improve health and psychosocial outcomes.

Introduction

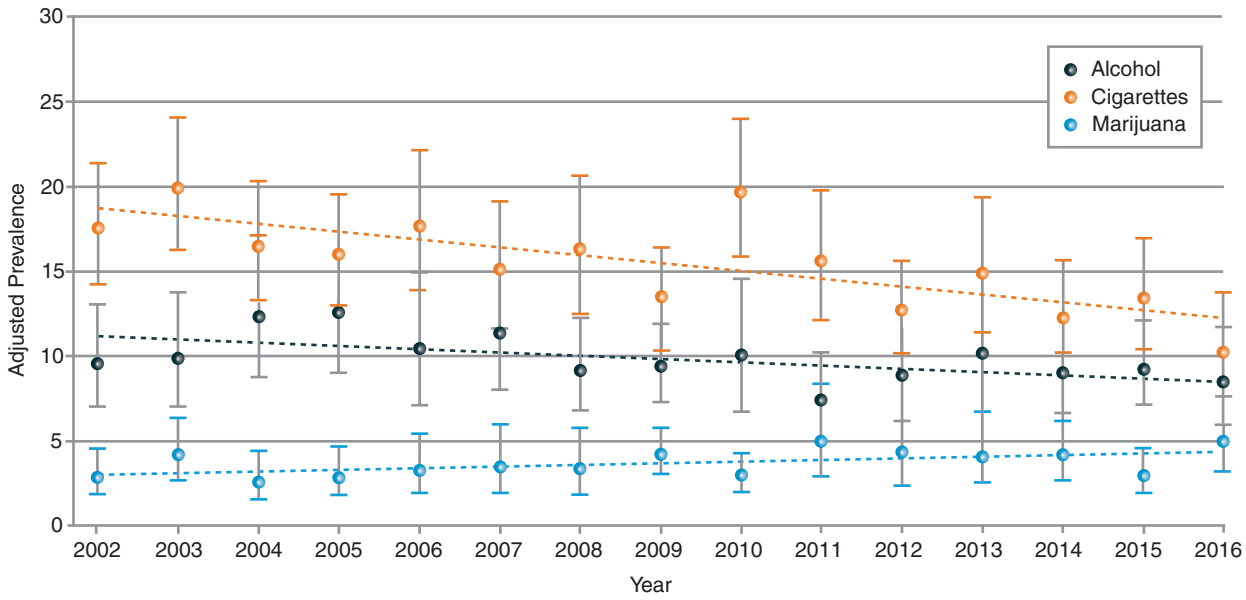
Substance use and misuse during pregnancy has been recognized as a problem for more than a century, but the problem has reached epidemic proportions in the United States in the past two decades. Prenatal exposure to psychotropic substances, both licit (e.g., alcohol, nicotine, opioids, cannabis) and illicit (e.g., heroin, cocaine, methamphetamines), is associated with significant maternal, fetal, and neonatal complications. These include poor fetal growth, preterm birth, placental abruption, stillbirth, fetal malformations, neonatal neurobehavioral dysfunction, and sudden infant death syndrome (SIDS).[1] Although substance use disorders (SUD) occur in all socioeconomic classes, illicit drug use is observed more frequently in populations with poor access to prenatal care, untreated medical conditions, poverty, food insecurity, stress, and psychiatric disorders. Confounding makes it difficult to distinguish effects of drug exposure from socioeconomic and environmental factors. This chapter addresses (1) epidemiology of perinatal substance use, (2) known effects of specific intrauterine exposures on the fetus and neonate, (3) maternal comorbidities and their effects on the neonate, (4) identification of pregnancies and infants considered to be at high risk, (5) smanagement of drug-exposed neonates, and (6) the long-term effects of intrauterine substance exposures. The discussion focuses on substances that are known to be associated with significant perinatal and neonatal morbidity.

Epidemiology of Perinatal Substance Exposure

Among people aged 12 or older in 2019 in the United States, 60.1% (165.4 million people) used tobacco, alcohol, or an illicit drug in the previous month.[2] This includes 50.8% (139.7 million) who drank alcohol, 21.1% (58.1 million) who used a tobacco product, and 13.0% (35.8 million) who used an illicit drug. Data on drug use during pregnancy in 2019 indicate that many pregnant women had a SUD with 120,000 (5.8%) using illicit drugs, 198,000 (9.6%) tobacco products, 197,000 (9.5%) alcohol, and 112,000 (5.4%) cannabis.[3] The prevalence of opioid use disorder (OUD) at delivery significantly increased from 1.5 per 1000 in 1999 to 6.5 per 1000 delivery hospitalizations in 2014.[4] While alcohol and cigarette use during pregnancy has declined over the past several years, the use of cannabis products and e-cigarettes has actually increased (Fig. 11.1).[5] This is largely due to individuals believing that these products are safer and not harmful to the fetus.

Determining accurate prevalence rates of perinatal substance use is extremely difficult. Under-reporting of substance use by pregnant persons, unreliable drug use survey and detection methods, polysubstance use, and negative stigma are just some of the challenges. In particular, SUDs are known to be more common in adolescents and adults with psychiatric disorders (e.g., anxiety disorders, major depressive and other mood disorders) that also require treatment. Thus, it is not uncommon for pregnant persons to receive multiple psychotropic drugs simultaneously, often without adequate safety data being available.

Updated resources for perinatal substance exposure and the impact on the mother-infant dyad are available online. The Substance Abuse and Mental Health Services Administration (SAMHSA) conducts an annual U.S. National Survey on Drug Use and Health (NSDUH) and also has a Center for Behavioral Health Statistics and Quality (codified after passage of the



• **Fig. 11.1** Alcohol, cigarettes, and cannabis use in pregnancy (2002–2016). Derived from a 2019 publication, this figure demonstrates contemporary trends in the use of alcohol, tobacco, and cannabis during pregnancy. (From Agrawal A, Rogers CE, Lessov-Schlaggar CN, et al. Alcohol, cigarette, and cannabis use between 2002 and 2016 in pregnant women from a nationally representative sample. *JAMA Pediatr*. 2019;173[1]:95–96.)

21st Century Cures Act) that tracks substance use closely. The Pregnancy Risk Assessment Monitoring System (PRAMS) provides comprehensive information on substance exposure before, during, and after pregnancy. PRAMS is designed to monitor maternal behaviors and experiences among persons who deliver live born infants, covering approximately 81% of all births. PRAMS surveillance data have historically demonstrated wide geographic, age-related, and racial/ethnic variation in tobacco, alcohol, and illicit drug use.

Health Policy

Pregnant persons who use opioids or other substances without a prescription are considered to have SUD, and the dyad is at much higher risk without treatment. Martin et al. found that only 9.3% of reproductive age women with a SUD needing treatment were able to access it.[6] Pregnant and parenting persons had similar or poorer access to treatment than the general population. Black and Latina pregnant and parenting persons were less likely to receive necessary treatment than white populations.

Health policy interventions for SUD during pregnancy should focus on treating SUD as an illness and not a criminal offense. This is crucial since up to 55,000 pregnant persons are incarcerated in the United States each year, many for crimes related to their SUD.[7] Incarceration is known to exacerbate mental illness, increase psychological distress, promote financial instability, and harm the parent-child bond. The need for appropriate treatment programs is growing as the number of pregnant persons with SUD increases. The effectiveness of these treatments is well established, with multiple studies demonstrating decreased morbidity and mortality in the mother and infant.[8] Current healthcare policy should focus on providing appropriate care for pregnant persons with SUD, including medical management and psychosocial support, and stigmatization of individuals with SUD must change. Schiff et al. examined existing public attitudes toward pregnant women with SUD with 85% of 1227 respondents agreeing that a pregnant person with SUD was "responsible for their opioid use" and "addiction was caused by poor choices, putting the baby in danger."[9] It is also important to recognize that illicit drugs may not be more harmful to the fetus than licit drugs. Future efforts need to include better education on the significant impact of these substances on the fetus, preferably initiating treatment before pregnancy occurs.

There are promising legislative efforts addressing the opioid epidemic and the problem of NAS.

- The Protecting Our Infants Act of 2015 aims to reduce the number of neonates exposed to antenatal opioids developing NAS. This act requires the development of recommendations, programs, strategies, data, and research relevant to prenatal opioid use.
- The Child Abuse Prevention and Treatment Act (CAPTA) provides funds to improve states' child protective services.
- The Comprehensive Addiction and Recovery Act of 2016 (CARA) identified limited physical capacity to care for neonates with NAS; coordination of care for mothers and neonates with NAS; and gaps in research and data on NAS.

Finally, private groups such as the National Advocates for Pregnant Women combine legal advocacy, education, and organizing to defend and protect pregnant persons with a variety of conditions including alcohol use disorder and OUD.[10] It is essential for health care providers to treat pregnant women with SUD in a respectful and non judgmental manner in order to build therapeutic relationships which will ultimately benefit the parent-infant dyad.

Perinatal Exposure to Specific Substances

Alcohol

Introduction

Alcohol consumption during pregnancy can cause a range of disorders, known as the fetal alcohol spectrum disorders (FASDs). FASDs include fetal alcohol syndrome (FAS), alcohol-related neurodevelopmental disorder (ARND), alcohol-related birth defects

(ARBDs), partial FAS, and neurobehavioral disorder associated with prenatal alcohol exposure (ND-PAE). Intrauterine alcohol exposure also is associated with growth restriction that persists postnatally.[11] The classic features of FAS were initially described by a French pediatrician in 1968 who observed a common pattern of birth anomalies in children born to alcoholic mothers.[12] In the 1970s, two U.S. reports were published describing similar features in children born to women consuming alcohol while pregnant.[13,14] This recognition led to the U.S. federal law placing warning labels on all alcoholic beverage containers regarding the increased risk of alcohol-related birth defects. In 2005, the Surgeon General reissued an advisory for people who are or might become pregnant, urging abstinence from alcohol to eliminate the risk for FASD.

Data from the NSDUH in 2015–2018 indicated that nearly 10% of pregnant respondents had admitted to alcohol consumption and 4.5% to binge drinking within the past 30 days.[15] Prevalence differed, with greater consumption and binge drinking in the first trimester compared to the second and third trimesters. Overall, nearly 20% of pregnant respondents reported drinking any alcohol and using tobacco within the past 12 months. The objective set by Healthy People 2030 is to increase abstinence from alcohol among pregnant persons to 92.2%.[16] The rate of FAS in the United States has been estimated to vary from 0.5 to 2 cases per 1000 live births, but its true prevalence is unknown and is likely underdiagnosed worldwide.[17–21]

Pediatricians often do not recognize FAS in the neonatal period and do not always inquire about alcohol exposure during pregnancy.[22] There are prenatal alcohol exposure screening instruments that are appropriate for use in pregnant persons as well as expert guidelines to clarify the diagnosis of FASD and aid in the earlier recognition and referral of affected infants and children.[18,23,24]

Diagnosis and Classification

The Institute of Medicine defined FASD in 1996 as an umbrella term for a range of sequelae of prenatal alcohol exposure.[25] A scientific task force led by the Centers for Disease Control (CDC) confirmed and refined the diagnostic criteria in 2004. FASD includes FAS as well as additional classifications for patients with variations on the classic FAS presentation. A diagnosis of FAS requires evidence of: (1) all three facial abnormalities (see description below), (2) growth deficits, and (3) central nervous system (CNS) abnormalities (structural, neurological, and/or functional).

Additional classifications include:

- Partial FAS (pFAS)—some, but not all of the physiologic features of FAS,
- ARND, where there are no facial deformities but features of CNS injury,
- ARBD, presenting primarily with physical malformations (cardiac, renal, bone, visual, and/or hearing),
- ND-PAE.[17]

A diagnosis of FASD is made using maternal history, the infant's physical findings, and neurobehavioral testing results. Although biomarkers such as fatty acid ethyl ester levels in meconium have been evaluated for use in diagnosis of prenatal alcohol exposure, no laboratory tests are available and validated for clinical use to quantify the extent of fetal alcohol exposure.[26] There are also no clinical methods for validating maternal self-reporting of alcohol use, quantifying the level of fetal exposure, or predicting future disability after fetal exposure.

Pharmacology and Biologic Actions

Alcohol is a mood-altering substance that enhances the effects of the inhibitory neurotransmitter γ-aminobutyric acid while reducing the effects of the excitatory neurotransmitter glutamate. This results in dose-dependent depressant or sedative effects on the CNS. Alcoholic beverages contain ethanol, which is metabolized in the liver to acetaldehyde by alcohol dehydrogenase (ADH). Acetaldehyde is then metabolized to acetate by aldehyde dehydrogenase and eventually eliminated as water and CO_2. A small amount of oxidation in the liver is catalyzed by the cytochrome P450 enzyme CYP2E1.

Ethanol readily diffuses across the placenta and can be rapidly detected in the fetus. The placenta expresses an isoform of ADH that has a low affinity for ethanol and a reduced metabolic rate. The fetal liver expresses CYP2E1 by mid-gestation, but at lower levels and less metabolic capacity than adult liver. Despite the presence of fetal and placental metabolic activity, fetal ethanol clearance largely relies on maternal metabolism. Toxic effects of alcohol during pregnancy appear to be related to the peak and circulating alcohol concentrations.[27] Undernutrition and alcohol exposure may interact to cause greater fetal toxicity, due to slower maternal metabolism.[27]

Fetal and Neonatal Effects

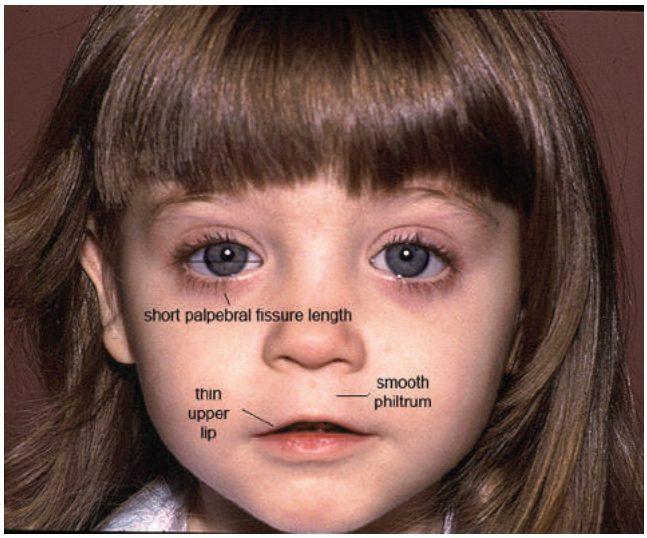
It is unclear how much alcohol exposure is necessary to cause fetal teratogenicity and even high consumption levels do not always result in the birth of a child with FASD.[28] The adverse effects of alcohol on the fetus are related to gestational age (GA), duration of exposure, amount of alcohol consumed, pattern of consumption (e.g., binge drinking), maternal peak blood alcohol concentrations, and maternal alcohol metabolism.[29] Studies show that maternal blood alcohol levels are affected by body size and genetic disposition, with poor maternal nutritional status a significant risk factor for FASD.[27,30,31] A variety of additional risk factors increase susceptibility to FASD, including increased maternal age, poverty, and socioeconomic status.[28,32,33]

Growth Restriction

Fetal growth restriction (FGR) is one of the most consistent sequelae of prenatal alcohol exposure. The diagnostic criteria for FAS require birth weight and/or length to be below the 10th percentile (adjusted for age, sex, gestational age, and race or ethnicity).[34] Restricted growth begins in utero and continues postnatally, with high levels of alcohol exposure causing greater impact on head and body growth. Fetal and childhood growth deficits appear to be multifactorial, with etiologies including abnormal placentation, abnormal vascular development, and impaired production of insulin-like growth factors that are involved in somatic growth and placental function.[35,36] Growth deficiency may persist through childhood, particularly in those children with the highest levels of intrauterine exposure.[37,38]

Dysmorphology

Key features of FAS include characteristic facial dysmorphology, including a smooth philtrum (using University of Washington Lip-Philtrum Guide), a thin vermillion border, and small palpebral fissures.[17,34] Characteristic features also may include midface hypoplasia, broad flat nasal bridge, and thin upper lip (Fig. 11.2). Patients with FAS also have a higher risk of microcephaly, micrognathia, and cleft palate. Genetic syndromes must be excluded because some of their dysmorphic features can overlap with FAS. The facial features and growth restriction may become



• **Fig. 11.2** Child with three diagnostic facial features of fetal alcohol syndrome. (From Williams JF, Smith VC. AAP committee on substance abuse. Fetal alcohol spectrum disorders. *Pediatrics*. 2015;136[5]:e1395–e1406; copyright 2022 Susan (Astley) Hemingway PhD, University of Washington.)

less distinctive during adolescence and puberty.[39] While skeletal anomalies, abnormal hand creases, and ophthalmologic, renal, and cardiac anomalies[40] have been described in children with FAS, they are less frequent than the facial dysmorphology.[41]

Central Nervous System Abnormalities

Children with FASD are at higher risk of seizure disorders as well as significant cognitive, motor, and neurobehavioral problems.[42] A host of CNS abnormalities are associated with FASD. Table 11.1 lists some of the more common findings.[20,42]

Long-Term Effects

FASDs are associated with lifelong social, behavior, intellectual, and psychological difficulties, and deficits in reading, spelling, and math are common.[43] In a cohort of 76 children and young adults with FASD, high rates of attention deficit/hyperactivity disorder (ADHD; 62%), oppositional defiant disorder (43%), and developmental coordination disorders (41%) were noted. Visual and/or ocular abnormalities were also found in 86% of the children. Of the adults evaluated, 70% had ADHD, 52% had an anxiety disorder, 42% had experienced a depressive episode, and 24% had engaged in self-injurious behavior.[44] Adolescents and young adults who were exposed prenatally to alcohol are at an increased risk for earlier alcohol use and subsequent abuse.[45,46]

Researchers using the Canadian National FASD Database reported on a sample of 726 adolescents and adults (mean age 19.8 years [range 12 to 60]) who had prenatal alcohol exposure. Impairments in adaptive behavior, social skills, executive functioning, academic achievement, and attention were seen in approximately 2/3 of the sample, and 40% had an intelligence quotient less than 70. Difficulties included independent living needs (63%), substance misuse (46%), employment problems (37%), legal problems (30%), and housing problems (21%), with 81% experiencing at least one such difficulty at the time of assessment.[47] Earlier recognition and intervention for children with FAS and its variants may help mitigate eventual adulthood disabilities and help to prepare affected adolescents and young adults for independent living.[17,18]

Cigarette Smoking, Electronic Cigarettes

Introduction

Nicotine in the form of cigarettes (including electronic cigarettes—ECIGS and electronic nicotine delivery systems—ENDS), smokeless tobacco, and nicotine replacement patches, is the most commonly used substance during pregnancy which negatively affects perinatal outcomes. Cigarette smoking in the United States has decreased significantly over the past 25 years, due to effective public health strategies. Unfortunately, these efforts have not been as effective for pregnant persons. Since the 2019 NSDUH survey revealed that 9.6% of pregnant persons used tobacco, targets set by the Healthy People 2020 initiative to decrease tobacco use to less than 2% of pregnant people have not been reached.[2,16] These targets are becoming even more tenuous with the use of ECIGS steadily increasing during pregnancy, likely because they are believed to be a safer alternative than conventional cigarette smoking.[48] Pregnant persons who use nicotine products are more likely to use opioids, alcohol, cocaine, amphetamines, and marijuana.[2] Cigarette smoking is especially prevalent among pregnant persons with OUD (85% to 95%) and smoking cessation programs should improve both maternal and neonatal outcomes.

Pharmacology and Biological Actions

Cigarette smoke contains a mixture of approximately 4000 compounds (93 of which are considered toxic), including nicotine and carbon monoxide. Nicotine stimulates the reward center of the brain through activation of nicotinic acetylcholine receptors. It readily crosses the placenta and concentrates in fetal blood and amniotic fluid, where levels can significantly exceed maternal blood concentrations.[49] The maternal serum concentration of cotinine (the primary metabolite of nicotine) is used to quantitate smoking and fetal exposure. Cotinine has a half-life of 15 to 20 hours with serum levels 10-fold higher than nicotine.[50]

Smoking during pregnancy can impact fetal growth and development; nicotine and its metabolites are potent vasoconstrictors which decrease uterine and placental blood flow.[51,52] Carbon monoxide crosses the placenta, forming carboxyhemoglobin in the fetus and resulting in significant hypoxemia.[51] Serum erythropoietin levels are significantly higher in cord blood from neonates who were exposed to cigarette smoke, a finding likely reflecting fetal hypoxia.[53] Nicotine may also act as a developmental toxin targeting the placenta as well as the brain and other organ systems in the fetus.[51] Since the amount of nicotine delivered by ECIGS is similar to conventional cigarettes, potential toxicity would be expected to be similar.[48]

Long-Term Effects of Perinatal Exposure to Cigarettes and Electronic Cigarettes

Maternal smoking has been shown to increase the risk of stillbirth, miscarriage, and preterm delivery. Liu et al. studied over 25 million pregnancies in the U.S. National Vital Statistics System from 2011 to 2018.[54] They demonstrated that smoking during the first or the second trimester of pregnancy (even 1 to 2 cigarettes per day) was associated with an increased risk of preterm birth, which was in part due to increased placental abruption and placenta previa.[54–56] Cigarette smoking is also well recognized to cause FGR in a dose-dependent manner.[51] Reductions in weight, fat mass, and other anthropometric measurements occur through impaired placental blood flow, tissue

TABLE 11.1 Central Nervous System Abnormalities Associated With Fetal Alcohol Spectrum Disorders

Abnormality	Organ or Function	Specific Examples
Sensory	Visual	Strabismus Refractive errors Impaired oculomotor control Microphthalmia Coloboma Optic nerve hypoplasia Retinal dysplasia Retinal vascular tortuosity
	Auditory	Hearing loss—sensorineural, conductive, central Impaired auditory processing
Neurological	Gross motor	Delayed walking Slowed motor reaction timing
	Fine motor	Visual-motor integration Poor graphomotor skills
	Coordination	Gait ataxia Impaired static postural control
	Sensory integration and processing	Auditory and visual processing dysfunction
Neuropsychological	Cognitive	Intellectual impairment Executive function Learning and memory Attention deficits Decreased processing speed Speech and language Reading and spelling Numerical processing/estimation
	Behavioral/emotional	Problem behaviors—externalizing and internalizing Attention deficit hyperactivity disorder Impulse control problems Aggression Anxiety Depression
	Social/adaptive	Decreased interpersonal skills and social competence Decreased social problem solving Poor social judgment
	Sleep	
Neuropathological	Imaging	Reduced frontal, temporal, parietal lobe size Reduced gray and white matter volumes Corpus callosum hypoplasia Cerebellar hypoplasia Hippocampal hypoplasia Basal ganglia and thalamic hypoplasia Schizencephaly Polymicrogyria Vascular anomalies Heterotopias Perivascular space dilation Pituitary hypoplasia Ventriculomegaly Cavum septum pellucidum Simplified gyral pattern Brainstem abnormalities

hypoxia, and alterations in protein metabolism and enzyme activity in the placenta. When these children are followed to 3 years of age, body weights are increased, consistent with early childhood obesity.[57] Although nicotine replacement therapy (NRT) and ENDS do not show clear evidence of effectiveness and safety in pregnant women, those who stopped smoking before or during early pregnancy had appropriate fetal and childhood growth.

Fetal exposure to cigarette smoke can impact lung development, putting preterm neonates at increased risk of bronchopulmonary dysplasia (BPD) and longer-term pulmonary dysfunction termed chronic pulmonary insufficiency of prematurity (CPIP).[58,59] Term neonates are at increased risk of diminished lung function, altered central and peripheral respiratory chemoreception (e.g., SIDS), and asthma during childhood.[60] Possible mechanisms also include dysregulated cytokine production, oxidative stress, and the direct effects of nicotine on lung receptors resulting in altered lung development. Genetic (upregulation of asthma susceptibility genes) and epigenetic (alterations in DNA methylation) factors have been identified that may influence the risk for long-term lung disease. Pregnant persons who smoke during pregnancy often continue to smoke postnatally, exposing their children to secondhand smoke exposure.[60] This can further increase the risk of morbidity and mortality throughout childhood.

Finally, alterations in developmental patterning of DNA methylation and gene expression can be detected in the fetal brain following antenatal exposure to cigarette smoke. This can result in reduced mature neuronal content, fetal brain growth, and smaller volume of cortical gray matter.[61] This may explain the increased incidence of behavioral problems and impaired executive function that can be seen later in childhood.

Cannabis and/or Cannabidiol

Introduction

Cannabis is a potent psychoactive agent used commonly during pregnancy.[62] Between 2016 and 2019, the NSDUH reported a rate of cannabis use in pregnant persons aged 15 to 44 years ranged from 4.9% to 7.1%, with frequent use in 1.5% to 3.1%.[3] Another analysis found increased cannabis use occurring despite cigarette and alcohol use in pregnancy declining.[5] The majority of cannabis use is recreational and probably underreported due to recall bias.[2,63] Surveys of pregnant persons and non pregnant persons indicate that 70% believe that there is slight or no risk of harm from using cannabis.[64] The increased potency of cannabis products is of particular concern. Chandra et al. reported that mean Δ^9-tetrahydrocannabinol (Δ^9-THC; the active component of the endocannabinoid system) concentrations have increased from 8.9% in 2008 to 17.1% in 2017 and the mean Δ^9-THC: Cannabidiol (CBD) ratios from 23 to 104.[65] The greatest reported use occurs in the first trimester, often before the person is aware of being pregnant.

Pharmacology and Biological Actions

Endocannabinoids and phytocannabinoids activate cannabinoid receptors in the endocannabinoid system. CB1 is primarily found in the central nervous system and CB2 in immune cells and the retina.[66] Cannabinoid signaling is necessary for normal placental implantation and receptors are expressed early in the fetal brain, particularly in white matter and areas with high cellular proliferation.[67] During pregnancy, cannabis readily crosses the placenta and the fetal blood-brain brain barrier since it is highly lipophilic, altering normal endocannabinoid signaling, synaptogenesis, development of neuronal interconnections, and developing neurotransmitter systems.[68] All of these effects can potentially influence embryogenesis and fetal development by disrupting normal angiogenesis, increasing cellular apoptosis, and reducing cellular migration and DNA replication.[69]

Cannabis use during the postpartum period can increase neonatal exposure through breast milk and secondhand exposure.[70–72] THC may remain in breast milk up to 6 weeks after ingestion and up to eight times the maternal serum level.[73,74] Recent studies have suggested that cannabis use during pregnancy is associated with placental abruption, preterm birth, fetal growth restriction, admission to a neonatal intensive care unit, and lower 5-minute Apgar scores which can significantly impact both short- and longer-term outcomes.[75]

Long-Term Effects of Perinatal Cannabis Exposure

Lee and colleagues evaluated cardiovascular structure and function in rat pups born to pregnant dams exposed to Δ^9-THC. At birth, Δ^9-THC exposed pups were noted to have more FGR, lower cardiac output, and cardiac remodeling (increased in cardiac collagen content).[76] This cardiac dysfunction could persist throughout the lifespan and contribute to long-term cardiovascular morbidity and mortality. These findings in conjunction with potential epigenetic and biochemical alterations in the placenta and fetal brain highlight the potential impact on short- and long-term health outcomes.[77,78] Additional studies focusing on epigenetics and long-term outcomes associated with cannabis use in pregnancy are urgently needed to better understand the impact of fetal exposure on the life long risk of cardio-metabolic disorders.[79,80]

The longitudinal Adolescent Brain and Cognitive Development (ABCD) Study recruited 11,875 children aged 9 to 11 years between 2016 and 2018. Compared to unexposed children and those exposed in utero prior to knowledge of the pregnancy, children exposed to cannabis during gestation had higher rates of psychopathology. Cannabis-exposed children had lower cognitive scores, birth weight, and brain volumes and increased sleep problems, but these associations weakened after adjusting for confounding factors.[81] Other studies have suggested that antenatal cannabis exposure is associated with increased impulsivity, hyperactivity, delinquent behavior, memory dysfunction, and decreased intelligence quotient (IQ) scores in children.[82] While more definitive evidence is needed, these recent studies indicate that cannabis use should be avoided during pregnancy and lactation.

Opioids (Including Prescription Drugs)

Introduction

Opioids may be used in pregnancy either for illicit or recreational use, or for therapeutic reasons such as treatment of OUD or chronic pain. Pregnant persons are not a uniform group and their perinatal outcomes are variable. Approaches to the care of the dyad should be tailored according to the clinical scenario such as chronic pain, illicit use, or medication-assisted treatment (MAT) for OUD.[83]

Pharmacology and Biologic Actions

All opioids bind to opiate receptors, including natural derivatives from opium poppy (e.g., morphine, narcotine, codeine, thebaine, papaverine, narceine) or synthetically manufactured opioids. Semisynthetic and synthetic derivatives include heroin, fentanyl, hydromorphone, methadone, buprenorphine, and others.[84] Specific opioid receptors (μ, δ, κ) have been identified in the CNS and bowel that are activated by endogenous opioids (e.g., endorphins, enkephalins).[85,86] As modulators of the sympathoadrenal system, endogenous opioids are important during periods of

stress with receptor activation producing analgesia and alterations in multiple organ systems. Synthetic opioids have similar physiologic and adverse effects.[83] The chronic use of opioids can result in tolerance, physiologic dependence, and addiction. Tolerance leads to a shortened duration of the action followed by the need for higher doses to obtain the same clinical effect.[87]

Fetal and Neonatal Effects

Studies to evaluate the impact of early fetal opioid exposure on the incidence of congenital anomalies have yielded variable results.[88–90] Although there may be a marginally increased risk, the benefits of a healthier pregnancy with prescribed opioids (i.e., MAT) greatly outweigh any potential risks associated with uncontrolled substance use. Obstetric complications of untreated OUD may include spontaneous abortion, preterm delivery, placental abruption, chorioamnionitis, and placental insufficiency.[83] Antenatal illicit opioid exposure is associated with a smaller head circumference (with increased risk of white matter lesions), higher rates of meconium-stained amniotic fluid, perinatal asphyxia, lower Apgar scores, and an increased incidence of infections such as syphilis, HCV, and HIV at birth.[91–93] The etiology of these complications is multifactorial with poor prenatal care, ongoing illicit opioid use, poor nutrition, infections, and polydrug use likely contributing.

Long-Term Effects of Perinatal Opioid Exposure

Key knowledge gaps exist regarding longer-term neurocognitive and mental health outcomes after antenatal exposure to opioids. While numerous studies suggest that exposure during fetal development may produce lifelong alterations in brain development, many have significant limitations such as: (1) not delineating antenatal/postnatal drug exposure (single or polydrug); (2) using large datasets with coded diagnoses that may not be accurate and variables that may be statistically significant, but not clinically relevant; (3) not describing if prenatal toxicology testing was performed; and (4) poorly matched comparison groups. All the confounding variables may be impossible to control for in a single follow-up study and all of these factors need to be considered when interpreting long-term studies.[94]

Significant neurobehavioral differences have been found between neonates prenatally exposed to methadone who either did or did not require pharmacotherapy for NAS, demonstrating variable impact on clinical outcomes.[95] Emerging data also suggest an increased risk for behavioral and/or developmental disorders in infants born to mothers with OUD when followed to school age.[96,97] While one study found that infants exposed to methadone or buprenorphine had normal measures of cognition, sensory processing, and behavior, other investigators have shown normal development for opioid-exposed infants during the first 2 years of life after data were controlled for a variety of socioeconomic and environmental influences.[94,98,99]

Cocaine

Introduction

Cocaine and its metabolites readily cross the placenta and enter the amniotic fluid and fetus.[100] The amniotic fluid may serve as a reservoir for cocaine and its metabolites and prolong exposure to vasoactive compounds. The confounding effects of multiple other drugs (e.g., tobacco, alcohol), nutritional deficits, and poor prenatal care complicate the establishment of causal relationships between antenatal cocaine exposure, intrauterine growth, and subsequent neurobehavioral development.[101]

Pharmacology and Biologic Actions

Cocaine is a highly psychoactive stimulant with a long history of abuse. Two forms of cocaine are commonly used—cocaine hydrochloride and cocaine base (crack). Cocaine hydrochloride is used orally, intranasally, or intravenously with intravenous users more likely to have a history of heroin use. Crack is the most widely available form of freebase and when smoked, it readily enters the bloodstream to produce levels similar to intravenous administration. Cocaine rapidly reaches the blood stream and the brain to produce its psychological effects. The pharmacologic actions of cocaine include inhibition of postsynaptic reuptake of norepinephrine, dopamine, and serotonin neurotransmitters by sympathetic nerve terminals.[102] Tryptophan uptake is similarly inhibited, altering serotonin pathways with resultant effects on sleep. In adults, cocaine has been associated with cerebral hemorrhage, hyperthermia, cardiac arrest, cardiac arrhythmias, myocardial infarction, intestinal ischemia, and seizures. Chronic use is associated with anorexia, nutritional problems, and paranoid psychosis.[103]

Fetal and Neonatal Effects

Adverse perinatal outcomes associated with cocaine use are primarily due to vasoconstrictive properties of cocaine on maternal blood vessels in the uterus and placenta.[104,105] Cocaine increases maternal mean arterial blood pressure, decreases uterine blood flow, raises fetal systemic blood pressure, and causes fetal hypoxemia in an ovine model.[106] These effects contribute to the higher risks of stillbirth, spontaneous abortion, placental abruption, preterm delivery, and FGR seen in cocaine-exposed infants.[107,108] Cocaine is hypothesized to reduce fetal growth via constriction of uteroplacental vessels with consequent decreased fetal substrate and oxygen delivery. Neonates exposed to cocaine in utero have lower birthweight and length and smaller head circumference.[101,108] In the Maternal Lifestyle Study, cocaine-exposed neonates had lower birthweight (151 g), length (0.71 cm), and head circumference (0.43 cm) at 40 weeks' gestation.[109]

To date, no well-defined cocaine-associated malformation syndrome has been identified and the teratogenic potential of cocaine remains controversial. Early reports had suggested that cocaine-exposed neonates had a higher rate of limb reduction anomalies, heart defects, ocular anomalies, intestinal atresia or infarction, and other vascular disruption sequences. However, more recent data has failed to demonstrate higher rates of congenital anomalies among cocaine-exposed infants.[107]

Subtle neurobehavioral abnormalities have been reported using the NICU Network Neurobehavioral Scale (NNNS) in the month after birth. Cocaine-exposed neonates manifest a range of neurobehavioral abnormalities that are present at birth and wane as the cocaine and metabolites are cleared from plasma. Neonates may present with signs of central and autonomic dysfunction such as hypertonicity, irritability, tremulousness, and abnormal crying, sleep, and feeding patterns.[110,111] The intoxicant effects of cocaine that have been reported in two controlled studies include tachycardia, tachypnea, apnea, and elevations in cardiac output, stroke volume, mean arterial blood pressure, and cerebral artery flow velocity which typically resolve by day 2 of age.[112,113] Cocaine-exposed neonates may also have

abnormal electroencephalograms or clinical seizures which may be the result of direct toxicity or delayed and/or impaired neuronal myelination.[114–116]

Long-Term Effects of Prenatal Cocaine Exposure

Evidence from preclinical and clinical studies, including adults who had prenatal cocaine exposure, suggests that there is persistence of behavioral and cognitive deficits secondary to multiple cellular and molecular mechanisms.[116] The neurodevelopmental problems among children exposed to cocaine may occur from direct drug effects during gestation or from postnatal environmental influences, or both.[117] Using family case management as an intervention for cocaine-exposed infants resulted in improved verbal development at 36 months in those children who remained with their birth mothers.[118] Cocaine-exposed infants in a prospective longitudinal cohort (with a masked comparison group) were studied at an urban teaching hospital and were twice as likely to have significant cognitive but not motor delays at 2 years of age.[119] Language development appears to be affected into adolescence in a dose-dependent manner.[120]

Prenatal cocaine exposure is associated with increased externalizing behaviors and a doubling of the risk for SUD in adolescence, even after controlling for other prenatal drug exposure and environmental influences.[121,122] In mid-adolescence, girls who were exposed have impaired executive functioning compared to unexposed peers and exposed boys.[123] Both direct and indirect impact of prenatal cocaine exposure have been detected in long-term follow-up, including emotional regulation problems, arrest history, and conduct disorder.[104] Early screening and appropriate intervention in adolescents with mental health and behavioral problems can improve outcomes.

A study of volumetric magnetic resonance imaging (MRI) in thirty-five 12-year-old children exposed to cocaine in utero found smaller total parenchymal volumes, lower cortical gray matter volumes, and smaller head circumferences (HC) compared to controls.[124] Akyuz and colleagues conducted a longitudinal cohort study in 11 children with antenatal cocaine exposure and 10 controls (6 with noncocaine polysubstance exposure and 4 with no reported substance exposure) and found decreased HC in cocaine-exposed infants and trends toward reduced cerebral cortical volumes that persisted into the teenage years.[125]

Amphetamines

Introduction

The use of amphetamines, including illicit and prescription amphetamines, has increased worldwide in the past two decades. Methamphetamine (also known as "speed," "ice," "crystal meth," or "crank") is abused most often because it can be produced cheaply using over-the-counter decongestants and cough medicines. Amphetamines also include ecstasy (3,4-methylenedioxy methamphetamine, or MDMA) and prescription amphetamine stimulants that may be misused or abused, especially among adolescents and persons of childbearing age.[126] In a large national sample of hospital deliveries between 2004 and 2015, 1.7/1000 deliveries included a diagnosis related to amphetamine use. The rate of amphetamine-related diagnoses was trending higher and by 2015 affected 2.4/1000 birth.[127]

Pharmacology and Biologic Actions

Methamphetamine (or "crystal") is increasingly abused because it readily dissolves in water for injection, inhalation, or rectal administration.[126] The amphetamine isomers are CNS stimulants that provide an intense euphoria and increased energy. Amphetamines were initially marketed for the treatment of obesity and narcolepsy and continue to be used for the treatment of ADHD. Amphetamines are classified as schedule II drugs, similar to cocaine and narcotics.

Like cocaine, amphetamines are sympathomimetics and potentiate the actions of norepinephrine, dopamine, and serotonin. In contrast to cocaine, amphetamines appear to exert their CNS effects primarily by enhancing the release of neurotransmitters from presynaptic neurons. Amphetamines also can block reuptake of released neurotransmitters and can exert a weaker direct stimulatory action on postsynaptic catecholamine receptors.

The clinical effects and toxicity of these agents are difficult to distinguish from those of cocaine. While the psychotropic effects of cocaine are of short duration (5 to 45 minutes), the effects of amphetamines can last from 2 to 12 hours. Amphetamine withdrawal is characterized by prolonged periods of hypersomnia, depression, and intense, often violent paranoid psychosis.

Obstetrical and Fetal Effects, Including Fetal Growth Restriction

Amphetamines cross the placenta and are detectable in umbilical cord, placenta, and amniotic fluid.[128] Animal studies suggest that the increased rate of FGR, prematurity, and fetal demise derive from increases in maternal and fetal blood pressure and uterine vascular resistance.[129] These changes restrict oxygen and nutrient delivery to the fetus and may be further complicated by diminished maternal nutrient intake from the anorectic effect of amphetamine. Methamphetamine exposure has direct and indirect effects on the fetus, many of which are confounded by comorbidities, other drugs, smoking, poverty, and poor prenatal care.[126] A meta-analysis of eight studies showed statistically significant decreases in gestational age, birth weight, head circumference, body length, and Apgar scores in exposed neonates.[130] A population-based study using California's mother-infant linked datasets also showed that methamphetamine use significantly increased the odds of preterm delivery as well as gestational hypertension and preeclampsia, abruption, intrauterine fetal demise, neonatal death, and infant death (controlled for maternal age, race/ethnicity, insurance status, prenatal care, education, parity, chronic hypertension, drug and alcohol exposure, and diabetes).[131]

The Infant Development, Environment, and Lifestyle (IDEAL) study, published in 2006, followed 84 methamphetamine-exposed and 1534 unexposed infants (confirmed by screening for meconium). Both groups included alcohol, tobacco, and marijuana use but excluded opioids, phencyclidine (PCP), and lysergic acid diethylamide (LSD). Cocaine, tobacco, alcohol, and marijuana use were also more frequent in methamphetamine users. Methamphetamine-exposed infants were 3.5-fold more likely to be small for gestational age after adjusting for confounders such as low socioeconomic status, GA, and tobacco exposure.[132] Methamphetamine is neurotoxic to the developing brain, and prenatal exposure is associated with smaller subcortical brain volumes, including putamen, globus pallidus, and hippocampus.[133,134]

Neonatal and Infant Neurobehavioral Effects

Although the IDEAL study reported no increase in dysmorphism or other congenital anomalies, there was a higher rate of NICU admission.[127] Amphetamine exposure was associated with poor suck and less likelihood of breast feeding, but no specific withdrawal

syndrome requiring treatment has been noted.[135] Prenatal methamphetamine exposure is linked to abnormal NNNS scores and underarousal, low tone, poorer quality of movement, and increased stress.[136] It is important to note that by 1 month of age, stress and arousal were significantly improved in exposed neonates.[137]

Long-Term Effects

Height trajectories are lower in methamphetamine-exposed infants during the first 3 years of life.[138] In a Swedish cohort evaluated at age 14 to 15 years, amphetamine-exposed boys were taller and heavier and girls were smaller and lighter than national standards.[139] Although by age 3 the IDEAL cohort had no differences in receptive or expressive language, gross motor skills, or mental development between exposed and unexposed children, at age 5 differences in externalizing behavioral problems, attention processing, and risk of ADHD were noted. By including an adversity index score over the first 3 years, the investigators found that the effects of methamphetamine on neurobehavior were primarily due to early adversity, caregiver stress, and/or psychological symptoms.[135,140]

Prenatal Medication Exposures That May Be Associated With Neonatal Withdrawal

Pregnant persons may require medication to treat preexisting medical conditions, including neurological or psychiatric conditions. In a prospective U.S. cohort including nearly 10,000 nulliparous pregnant persons (2010–2013), 73.4% took at least one medication (excluding vitamin or mineral supplements) during pregnancy with 55% taking a medication in the first trimester.[1] The most commonly prescribed were antiemetics or gastrointestinal medications (34.3%), antibiotics (25.5%), and analgesics (23.7%). Antidepressants were taken in 6.1% of the sample and antipsychotics in 0.5%.[141]

Selective Serotonin Reuptake Inhibitors

The prevalence of major depressive disorder (MDD) during pregnancy has been estimated to be 14% to 23%.[142] Untreated depression during pregnancy is associated with increased odds of preterm birth and low birth weight.[143] While a 2003 study indicated that 13.4% of pregnant persons were prescribed antidepressant medications, primarily selective serotonin reuptake inhibitors (SSRIs) (the most commonly prescribed antidepressants), the more recent nuMoM2b study found that 6.1% of pregnant persons were treated with antidepressants between 2010 and 2013.[141,142] It is unclear whether this is a true decrease in prescribing or differences in the populations sampled, but the increased recognition of potential effects of SSRIs on the neonate may have influenced prescribing patterns.

SSRIs selectively inhibit reuptake of serotonin in presynaptic nerve terminals and increase the synaptic concentrations of serotonin. They are better tolerated and have fewer side effects than tricyclic antidepressants because they do not affect other neuroreceptors. Animal studies have shown that serotonin appears as a neurotransmitter in the early fetal brain, but its role in development is not clearly delineated. Although fluoxetine and its active metabolite cross the placenta and enter the fetal brain, the impact on brain development is not clearly understood.[144]

Studies of SSRI use in pregnancy have not shown patterns of fetal malformations with first trimester use, except for congenital heart defects.[143] Fetal losses, lower birth weights, and preterm birth appear to be more common in pregnancies exposed to antidepressants, but confounding has prevented full understanding of causality.[144,145] After intrauterine exposure, neonates are more likely to require NICU admission and are at risk for a self-limited syndrome of poor neonatal adaptation that may include tachypnea, irritability, jitteriness, hypertonia, sleep disturbance, temperature instability, poor feeding, hypoglycemia, and/or seizures.[146] There is a small absolute risk but increased relative risk of persistent pulmonary hypertension of the newborn (PPHN) associated with SSRI exposure in later pregnancy.[147]

Benzodiazepines

Insurance claims data from 2015 in the United States indicated that benzodiazepines were prescribed for 2.9% of pregnant persons, which was increased from 1.3% in 2007.[148] Worldwide prevalence of benzodiazepine use during pregnancy was found to be 1.9%, with the highest prevalence (3.1%) during the third trimester.[149] Benzodiazepine exposure in early pregnancy is associated with an increased risk of spontaneous abortion.[150]

Gamma-aminobutyric acid (GABA) is the primary inhibitory neurotransmitter in the brain. Benzodiazepines bind to the GABA type A receptor, increasing its affinity for GABA. Relaxation, fatigue, lethargy, and sedation are short-term effects. Tolerance and dependence result from long-term use of benzodiazepines and withdrawal may occur.[151] There are multiple oral benzodiazepines available in the United States with varying pharmacokinetic properties.

Benzodiazepines readily cross the placenta. A 2019 meta-analysis found that prenatal benzodiazepine use alone is not associated with an increased risk of congenital anomalies, but used concurrently with antidepressants, there was an increased risk of congenital malformations (OR 1.4; 95% CI, 1.09 to 1.8).[152] Signs of benzodiazepine withdrawal overlap with those of neonatal opioid withdrawal and benzodiazepine co-exposure increases the severity of NAS.[153]

Gabapentin

Gabapentin is indicated for management of postherpetic neuralgia in adults and for treatment of partial onset seizures in children and adults. It is widely prescribed off-label for multiple indications, including neuropathic pain, bipolar illness, hyperemesis gravidarum, and restless leg syndrome.[154] Co-prescription of gabapentin with opioids is common and it is used to potentiate the effects of opioids and other CNS depressants. [155,156] Umbilical cord tissue sent for drug screening has shown a positivity rate of 2% for gabapentin, with 70% of the gabapentin-positive specimens testing positive for at least one other drug.[157]

Gabapentin is structurally related to GABA but does not appear to act via the GABA receptor. It is a CNS depressant that binds to voltage-gated calcium channels, inhibiting release of neurotransmitters. It recently has been found to modify the action of NMDA-sensitive glutamate receptors as well as other proteins that mediate its effects.[158]

In a prospective cohort, gabapentin was not associated with increased rates of fetal malformations, but exposed neonates had a higher rate of preterm delivery, low birth weight, and NICU admission.[159] Neonates born after co-exposure to opioids and gabapentin appear to have more severe NAS. A recent case series of 19 co-exposed neonates described a specific pattern of withdrawal including tongue thrusting, back arching, wandering eye movements, and continuous movements of the extremities. Treatment with gabapentin and subsequent tapering improved signs of withdrawal.[160]

Stimulant Therapy for Attention Deficit Hyperactivity Disorder (ADHD)

The use of stimulant medications for the treatment of ADHD (such as methylphenidate and amphetamines) in pregnant persons (both adolescent and into adulthood) is uncommon but has increased in the past two decades.[161,162]

Although most data do not suggest a serious risk, associations have been noted between early pregnancy ADHD medication use (unspecified medications) and gastroschisis, omphalocele, transverse limb deficiency, and cardiac malformations (specifically associated with methylphenidate).[161,163,164] No substantive data are published that describe abnormal neonatal or childhood neurodevelopment secondary to intrauterine exposure to ADHD medications.[163]

Screening Pregnant Persons for Substance Use Disorder

Maternal and neonatal outcomes of any pregnancy depend on multiple factors including parental health, socioeconomic factors, family infrastructure, community support, genetic and metabolic factors, treatment medications, infant feeding type, parental education, and the degree of parental involvement in infant care. Early identification of potential negative exposures and appropriate management in the prenatal period can improve the outcomes for the dyad. Thus, early identification and treatment of maternal OUD and associated comorbidities with an empathic and comprehensive approach are imperative.[165] However, stigma and legal concerns make pregnant women less likely to initiate prenatal care and/or to access treatment for their SUD.[166–168]

The American College of Obstetricians and Gynecologists (ACOG) recommends early universal screening for substance use during all pregnancies, preferably at the first prenatal visit.[83] Nonuniversal screening based on previous pregnancy outcomes, adherence to care, socioeconomic status, and race or ethnicity is unreliable and creates further mistrust between the caregiver and the patient.[169] A number of screening tools have been developed including 4Ps, NIDA Drug Use Screening Tool: NIDA-Modified Assist, and CRAFFT—Substance Abuse Screen for Adolescents and Young Adults.[170–172] The 4Ps questionnaire focuses on Parents, Partner, Past, and Present as related to problems with alcohol and/or drugs (including prescription drugs). If there is a positive response to any of these tools, further relevant inquiries, toxicology testing, and referral for treatment are needed. Therefore a history of drug and alcohol use should be routinely included in the initial contact with every pregnant patient. The person taking the history should be prepared to offer preliminary counseling on risk reduction and referrals for treatment programs, as available and feasible in the given geographic locations.

Although screening works well for persons who seek prenatal care, it is not as useful for pregnant persons who do not seek prenatal care. For these patients, it is more helpful to identify risk indicators for perinatal substance abuse at the time of delivery. Use of one of the screening questionnaires (in addition to urine toxicology testing) increases the likelihood of identifying at-risk pregnancies and neonates, allowing for earlier referral for treatment or specialized interventions.[83] While urine toxicology testing with the patient's consent may assist in pregnancy management, caution must be exercised due to occurrence of false positive and false negative results.

Pregnancy Management

Establishing comprehensive and consistent preconception, antenatal, postnatal care, and counselling programs for families affected by SUD is paramount (Fig. 11.3). Making the dyad an active participant in their care enables them to achieve optimal health status and positive outcomes.[165,173] Screening procedures and counseling should always include the following topics:

Preconception

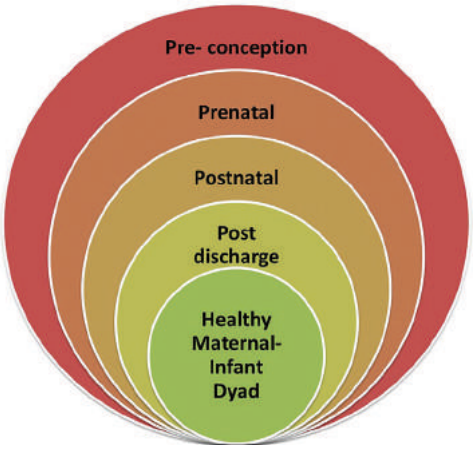
- Many pregnancies in persons with SUD are unintended (though not undesired) and discussions with healthcare providers may proactively assist with reproductive plans to achieve healthy pregnancy outcomes.[174,175] These discussions should be incorporated into all routine healthcare visits.

Antepartum

- Attention should focus on routine pregnancy counseling per ACOG guidelines, including smoking cessation (tobacco, cannabinoids) as indicated.
- Initial screening for hepatitis, HIV, syphilis, tuberculosis (if not part of routine prenatal care), and ongoing screening for other sexually transmitted infections
- Referral to drug dependency/addiction treatment programs, if appropriate
- Mental health counseling, if warranted
- Discussion of possible drug effects on the fetus and neonate, potential for the development of NAS/neonatal opioid withdrawal syndrome (NOWS)
- A multidisciplinary prenatal consulting team including obstetrics, psychiatry, addiction medicine, neonatology/pediatrics, pain services/anesthesiology, and social work to prepare families for potential outcomes
- Discussion of breastfeeding related to alcohol and drug use
- Establishing connections with community resources (e.g., early intervention) to help provide peer and family support which is crucial to successful transitioning home

Intrapartum

- Effects of recent drug use on labor and fetal well-being
- Pain management



• **Fig. 11.3** Comprehensive prenatal and postnatal care for the dyad. Paradigm for developing comprehensive clinical practice guidelines for holistic care provision to pregnant people with the goal of achieving a healthy maternal-infant dyad. (Rachana Singh, MD, personal communication, 2021.)

- Intrapartum prophylaxis for HIV and herpes simplex virus infections
- Need for social services involvement

Postpartum

- Breastfeeding (related to both drug use and infection)
- Contraception and pregnancy prevention
- Support for initiation or continuation in a drug treatment program
- Screening, counseling, and referral for postpartum depression, exacerbation of mental health disorders, OUD relapse, and/or overdose
- Notification of Child and Family Services
- Referral to community support resources (e.g., early intervention) and peer support programs

Human Immunodeficiency Virus and Other Viral Infections

Nationwide, intravenous drug users are a high-risk group for HIV and hepatitis C virus (HCV) infections. In the United States from 2015 to 2019, HIV diagnoses were stable among people who inject drugs (PWID) overall. In 2018, PWID accounted for 7% (2508) of the 36,801 new HIV diagnoses. Men who inject drugs accounted for 4% of the new HIV diagnoses, while women who inject drugs accounted for 3% of new HIV diagnoses.[176] The rate of vertical transmission has decreased significantly due to implementation of antenatal HIV testing and treatment during pregnancy, labor, and in the neonatal period.[177] However, most vertical transmission occurs during the intrapartum period and there is a risk of transmission through breastfeeding.[178] The AAP Committee on Infectious Diseases recommends rapid HIV testing of any mother whose HIV status is not known, with appropriate consent as required by local law.[178] Many states have now adopted a policy promoting testing with an "opt out" approach, rather than requiring consent for testing.[179]

HCV infection is also spread by exposure to infected blood and can be acquired in the perinatal period. The most common route for HCV infection is vertical transmission. The 2018 AAP Red Book indicates that seroprevalence of HCV during pregnancy has been estimated at 1% to 2% and the risk of perinatal transmission 5% to 6% from pregnant persons who are HCV-RNA positive at the time of delivery.[180] Maternal co-infection with HIV has been associated with increased risk of perinatal transmission of HCV. Although antibodies to HCV and HCV RNA have been detected in colostrum, the risk of HCV transmission is similar in breastfed and formula fed infants. Breastfeeding is currently allowed, with specific education needed about breast/nipple health.

Medication-Assisted Treatment for Opioid Use Disorder During Pregnancy

OUD is defined as a pattern of opioid use involving tolerance, cravings, inability to control use, and ongoing use despite negative consequences. Opioid agonists prevent signs of withdrawal and reduce the risk of relapse and obstetrical complications (e.g., preterm delivery). Improved perinatal outcomes are believed to be the result of a stable intrauterine environment uncomplicated by periods of intoxication and withdrawal, as well as less stress and better nutrition during pregnancy.

Population-based data from Massachusetts (2003–2007) demonstrated that one third of pregnant persons with OUD did not receive treatment prior to delivery.[181] Methadone and buprenorphine are the mainstays of treatment for OUD during pregnancy.[83] Substantial differences between the two in key maternal and neonatal outcomes have not been established in randomized controlled trials.[182] Small studies have suggested that treatment with buprenorphine may be associated with better fetal activity and heart rate variability than methadone.[8] An observational study that evaluated singleton neonates born at term and diagnosed with NAS after delivery found that prenatal exposure to buprenorphine was associated with a shorter length of hospital stay than methadone (21 vs. 24 days).[183] An analysis of Massachusetts Medicaid data (adjusted for confounding) found a similar decreased length of stay for neonates prenatally exposed to buprenorphine compared to methadone, as well as reductions in preterm birth and low birth weight.[184]

Methadone

Methadone is a long-acting mu-opioid agonist. Benefits of maternal methadone maintenance include the prevention of opioid withdrawal in the mother, improved health and growth of the fetus, and decreases in the use of illicit drugs and the potential for perinatal infections. For the greatest benefits, methadone maintenance treatment should be part of a comprehensive approach including addiction treatment specialists, counseling, nutritional education, and other medical and psychosocial support services.[83]

The current recommendation for methadone initiation in pregnancy is to titrate the methadone dose until the person is asymptomatic and adjust as needed to avoid signs of withdrawal.[83] Alterations in methadone dosing may be required during pregnancy (especially in the third trimester) because of physiologic and pharmacokinetic changes. Rapid metabolism may require split dosing (rather than once daily) in some pregnant persons to control withdrawal.[83,185] Disadvantages of methadone include drug-drug interactions and potential QTc prolongation. Several investigators have found that the incidence and severity of NAS, birthweight, length of pregnancy, and the duration of NAS treatment do not correlate with maternal methadone dosage.[186]

Buprenorphine

Buprenorphine is a partial μ-agonist and κ-antagonist with approximately 50% oral bioavailability due to extensive first-pass metabolism.[187] Its advantages over methadone include fewer drug-drug interactions, fewer dose adjustments during pregnancy, and options for outpatient treatment without daily visits. Disadvantages include the lack of long-term data on infant outcomes, potential risk of precipitated withdrawal with induction, and increased diversion. Buprenorphine treatment can be considered for patients who can administer the drug safely without the structure and multidisciplinary support of a daily methadone treatment program.[83]

The Maternal Opioid Treatment: Human Experimental Research (MOTHER) trial was a randomized, controlled trial comparing buprenorphine with methadone for treatment of OUD during pregnancy. There were more dropouts in the buprenorphine arm compared to methadone (33% vs. 18%), potentially related to dissatisfaction with treatment effects. The investigators found that although the neonates exposed to buprenorphine and methadone were treated for NAS at the same rate, neonates exposed to

buprenorphine received significantly lower total amounts of morphine and had a shorter hospital length of stay.[188] Follow-up of children born in the MOTHER trial showed comparable growth, development, and behavior at 3 years of age.[98]

Buprenorphine Plus Naloxone

Buprenorphine can be prescribed as monotherapy or in a combination product containing naloxone. There are insufficient data to recommend this combination in pregnancy, although small observational studies have not raised significant safety concerns.

Medically Supervised Withdrawal

The standard of care for pregnant persons with OUD is opioid agonist pharmacotherapy. Detoxification during pregnancy is associated with higher relapse rates and poorer outcomes.[189] Historically there have been concerns about fetal deaths, but a recent retrospective analysis of 301 pregnant persons described several scenarios of detoxification and recorded no fetal deaths in the second or third trimester. There were four fetal deaths among the 28 persons detoxified during the first trimester.[190] Significant concerns remain about the safety of this practice and its potential impact on fetal brain development.

Neonatal Management After Gestational Substance Exposure

Introduction

It is important for practitioners to understand the difference between a substance-exposed neonate and one with NAS. (Note: For the purposes of this chapter, NAS is considered a general description of withdrawal from prenatal exposure to opioids, other substances, or opioids plus other substances that produces a broad range of signs of withdrawal in the neonate. An alternative designation, NOWS can be considered a subset of NAS when the withdrawal is believed to result primarily from prenatal opioid exposure.)

Identification of substance-exposed neonates usually occurs by:

- A documented history of maternal substance use during pregnancy or
- Laboratory confirmation of maternal use of selected controlled substances within the 30 days preceding delivery or
- Positive toxicology screen (urine, meconium, umbilical cord, hair) in the neonate

Identification of infants with NAS occurs by:

- Confirmed neonatal substance exposure,
- A Finnegan/modified Finnegan score ≥8, and/or
- A diagnosis of NAS entered in the infant's medical record by a medical practitioner

The lack of a standardized definition of NAS, both for clinical and research purposes, has been an ongoing issue as the number of infants with NAS continues to increase.[191,192] To address this, 20 clinical experts caring for substance-exposed mother-infant dyads explored including some key evidence-based clinical elements defining opioid withdrawal in the neonate and leading to a diagnosis of NAS. This panel concluded the following were required for diagnosis: (1) in utero exposure (by history or toxicology) to opioids with or without the presence of other psychotropic substances, and (2) the presence of at least two of the most common clinical signs characteristic of withdrawal (excessive/continuous crying, fragmented sleep, tremors, increased muscle tone, and gastrointestinal dysfunction).[193]

A better understanding of maternal and neonatal risk factors is essential to optimize management (Table 11.2). Neonatal toxicology testing with a comprehensive panel including fentanyl and gabapentin should be considered. Testing neonates for in utero drug exposures is complicated by variations in neonatal liver metabolic activity, screening methodologies, and immunoassay cutoffs.[194] For urine testing, there is a poor correlation between maternal and neonatal testing and the earliest urination may be missed. While some drugs such as cocaine may be present for 4 to 5 days, cannabis may persist for weeks. The utility of neonatal urine drug testing is limited because most substances are detectable only with recent use, and alcohol is nearly impossible to detect. Meconium drug testing (at term) reflects substance exposure during the second half of gestation, has a high sensitivity for opioids and cocaine, and can detect more drugs than urine testing. Other neonatal drug tests include hair (high sensitivity for cocaine, amphetamines, opioids) and umbilical cord segments. These tests are becoming more widely available and detect earlier exposure, with results similar to meconium testing.[195] However, both meconium and umbilical cord testing can be negative for exposures in a significant percentage of neonates with a diagnosis of NAS.[196] If indicated by neonatal or maternal risk indicators, toxicology testing should be performed on neonatal urine, meconium, and/or umbilical cord segments as soon as possible after birth.

Although at higher risk for medical complications, most substance-exposed neonates do not require intensive care. However, caring for infants with moderate to severe NAS does require additional resources and adds to healthcare costs. A detailed physical examination on admission should include a gestational age assessment, anthropometrics, and evidence of any congenital malformations and/or dysmorphisms. Neonates whose mothers had limited prenatal care require screening for HIV, hepatitis B, HCV, and syphilis. In some states, rapid testing of the neonate for HIV is required by law if the mother refuses to be tested so that appropriate neonatal treatment can be started before 12 hours of age.

TABLE 11.2 Risk Indicators for Gestational Substance Exposure

Maternal	Neonatal
No or late prenatal care	Jittery, with normal serum electrolyte levels
Precipitous labor	Marked irritability with excessive crying
Placental abruption	NEC in otherwise healthy term/near-term infant
Repeated spontaneous abortions	Neurobehavioral abnormalities
Hypertensive episodes	Significant hypotonia or hypertonia
Severe mood swings	Unexplained IUGR
Previous unexplained fetal demise	Unexplained poor suck and feeding
Myocardial infarction or stroke	Unexplained seizures or apneic spells
	Clinical signs of narcotic withdrawal

IUGR, Intrauterine growth restriction; *NEC*, necrotizing enterocolitis.

Breastfeeding and Drug Exposure

Multiple organizations support breastfeeding to provide optimal neonatal nutrition and to promote bonding and attachment.[83,197,198] Breastfeeding is generally supported for women with SUD, especially those who have had a confirmed period of abstinence (from street drugs) prior to delivery. Similarly, women who are dependent upon opioids for chronic pain or other conditions are encouraged to breastfeed if they are medically able. Concentrations of methadone and buprenorphine in human milk are low and there is increasing evidence supporting a reduction in the severity and duration of NAS when mothers breastfeed.[199]

For women with SUD, the choice to breastfeed is often challenging. In addition, most healthcare professionals have not been adequately educated and are equally challenged in making recommendations, especially when addressing racial disparities that may be seen in this population.[200] Wachman et al. reported that only 24% of 276 opioid-exposed neonates had any breast milk during their hospital stay with 60% stopping after an average of 6 days.[201] There are many issues to consider including lactation and pharmacology issues, coexisting risk factors or conditions, polydrug use/abuse, and infection. In neonates, numerous factors may affect drug clearance, accumulation, and/or toxicity. The most current/comprehensive information regarding drug transfer into human milk, bioavailability, and potential toxicity are available at NIH's Drugs and Lactation Database (LactMed).[202] For many drugs, this information is limited to data from animal studies or anecdotal case reports. For neonates whose mothers engage in ongoing use of illicit drugs, the risks of breastfeeding often outweigh any potential benefits. In addition, use of illicit drugs is associated with a higher risk for drug overdose, so breastfeeding is not currently recommended in women with active illicit drug use at the time of delivery.[203]

Alcohol levels in human milk generally parallel maternal blood alcohol levels. Heavy alcohol use during lactation has been associated with decreased milk production via interference with the milk ejection reflex. Studies evaluating the effects of maternal alcohol consumption during breastfeeding on the infant have yielded mixed results, including mild effects on infant sleep. Women are generally advised to wait 90 to 120 minutes after consuming alcohol before breastfeeding.[197] Maternal smoking is not an absolute contraindication to breastfeeding, although there are measurable levels of nicotine and cotinine in breast milk. However, lactating women are encouraged to stop smoking (if they can) as exposure to nicotine increases the severity and duration of NAS for infants with in utero opioid exposure.[204] Finally, cannabis may remain in breast milk for up to 6 weeks after use and can reach up to eight times the maternal serum level. [73,74] Most medical organizations highlight the importance of eliciting a history of cannabis use during prenatal care visits and recommend counseling the mother to avoid the drug during pregnancy and breastfeeding.

Neonatal Abstinence Syndrome

The incidence of neonatal opioid withdrawal has been steadily increasing in the United States. From 2000 to 2014, there was a sevenfold increase in the number of neonates with documented NAS which accounted for $2.5 billion in medical costs.[205] Exposed infants may be hospitalized for 5 to 7 days to monitor for signs of NAS, incurring significant hospital charges.[206] The signs of NAS usually manifest within 72 hours of birth, with severity that can vary significantly depending on drug(s) exposure. A number of maternal, neonatal, and environmental factors are known to contribute to this variable expression.

Clinical Findings and Biomarkers

NAS results from fetal exposure to licit (prescription) and/or illicit opioids. Neonates exposed in utero to opioids develop physical tolerance and dependence through placental transfer of the drug. The abrupt cessation of the drug transfer at the time of birth causes a drug withdrawal syndrome characterized by dysregulation of both the autonomic and central nervous systems. Clinical signs are summarized in Table 11.3. If untreated, these signs can progress to seizures and become life-threatening.[207] These signs are most often related to gestational opioid exposure but are relatively non specific, with the differential diagnosis including infection, meningitis, hypoglycemia, hypocalcemia, hyponatremia, intracranial hemorrhage, seizures, and stroke.[111]

The incidence and severity can be influenced by other psychotropic agents such as antidepressants (SSRIs), anxiolytics (benzodiazepines), atypical agents such as gabapentin, nicotine, and other substances.[111,153,157,160] With almost 30% to 40% of women with OUD also taking a variety of other psychotropic substances, the signs of opioid withdrawal may overlap with "atypical withdrawal" from gabapentin, SSRIs, amphetamines, nicotine, and cannabis.[208] These agents have different mechanisms of action, pharmacokinetics, pharmacodynamics, receptor activation, and neurotransmitter production with drug-drug interactions potentially impacting the severity of NAS.

The timing of withdrawal from specific drug exposures can often be anticipated. While heroin withdrawal usually occurs within 24 hours of birth, methadone has a longer half-life and withdrawal typically begins approximately 48 to 72 hours after birth. The incidence of NAS in neonates born to pregnant persons using opioids is steadily increasing, with 30% to 60%

TABLE 11.3 Clinical Signs of Neonatal Abstinence Syndrome

Central nervous system dysfunction	Excoriation (from frantic movement) Hyperactive reflexes Increased muscle tone Irritability, excessive crying, high-pitched cry Jitteriness, tremulousness Myoclonic jerks Seizures Sleep disturbance
Autonomic dysfunction	Excessive sweating Frequent yawning Hyperthermia
Respiratory problems	Nasal stuffiness, sneezing Tachypnea
Gastrointestinal and feeding disturbances	Inadequate oral intake Diarrhea (loose, watery, frequent stools) Excessive sucking Hyperphagia Regurgitation

Adapted from Patrick SW, Barfield WD, Poindexter BB. Neonatal opioid withdrawal syndrome. *Pediatrics*. 2020;146(5). e2020029074.

receiving pharmacologic treatment and inpatient stays averaging 2 to 3 weeks.[192] Although preterm birth is a known complication of maternal SUD, severe NAS is unusual in preterm neonates (potentially due to shorter exposure to opioids and fewer opioid receptors in the brain). Some signs of NAS (e.g., feeding problems, tachypnea) overlap with typical findings in preterm neonates. Allocco et al. reported that preterm neonates were less likely to be scored for signs of neurologic and/or autonomic dysfunction compared to term neonates.[209]

An important aspect of monitoring for NAS is the method of assessment and the scores assigned. Many tools have been developed to determine the severity of NAS and need for treatment (Table 11.4).[210] The Finnegan Neonatal Abstinence Scoring Tool (FNAST) has been the most widely used since its development over 40 years ago. When first developed, the FNAST was a research tool which provided some degree of uniformity in monitoring NAS severity. Even though training programs were introduced to increase inter rater reliability, the FNAST remains subjective and somewhat nonspecific.[211] This includes elevated scores for non-opioid drug exposure (e.g., nicotine, benzodiazepines) seen in almost 30% to 40% of affected pregnancies. Several modified versions of the FNAST have been reported, reducing items for scoring from 21 to 8 to 10 items, with revised thresholds for treatment. For example, Devlin et al. studied 424 term opioid-exposed neonates and found that 8 items of the FNAST (sleeps ≤3 hours after feeds, any tremors, increased muscle tone, temperature ≥37.2°C, respiratory rate >60, excessive suck, poor feeding, regurgitation) were independently associated with pharmacologic treatment.[212] External validation was conducted with thresholds of four on the simplified scale correlating with FNAST thresholds of eight. These data suggest that eight signs of NAS may be sufficient to assess whether a neonate meets criteria for pharmacologic therapy.

The Lipsitz score was also developed and is simpler to use, with a score greater than 4 indicating significant signs of withdrawal.[213] The emergence of a new approach to NAS monitoring and treatment—Eat, Sleep and Console (ESC)—has gained increasing popularity. This tool consists of 3 items including eating at least 1 ounce/feed or breastfeeding well, sleeping undisturbed for ≥1 hour, and being able to be consoled when irritable within 10 minutes.[214] When comparing results and determining protocols, it is notable that a single validated objective tool is still not universally accepted or utilized. In an effort to provide more objective assessments for NAS, new physiologic and neurobehavioral indictors of NAS severity are being examined. These include measurement of skin conductance reflecting autonomic regulation through sympathetic-mediated filling of sweat glands, changes in pupillary diameter, alterations in sleep patterns, and a computer-based cry analysis system.[215–218]

Another novel concept being studied focuses on serial measurements of biochemical markers of stress, inflammation, and oxidation in neonates with NAS. Rodrigues and colleagues measured salivary cortisol levels and reported: (1) significantly higher cortisol levels in neonates who required treatment within the first week of life, (2) significant decreases 72 hours after birth

TABLE 11.4 Assessment Tools for Neonatal Abstinence Syndrome

Tool	Number of Subjects	Number of Item Scales	Validated	Threshold for Treatment
Finnegan et al., FNAST, 1975	55	31	No	8, 10, 12
Lipsitz et al., NDWSS, 1975	41*	11	No	4
Green et al., NNWI, 1983	50	18	No	5
Zahorodny et al., NWI, 1998	80	12	No	8
Oji-Mmuo et al., Skin conductance, 2015	14	N/A	No	N/A
McGuire et al., Shortened M-FNAST 2013	171	7	No	>8
Gomez Pomar et al., sFNAS, 2017	367	10	Yes	6,10
Grossman et al., ESC, 2018	50	3	No	1
Kocherlakota et al., NAS, 2020	45	9	No	5
Devlin, et al., sFNAS, 2020	424	8	Yes	4, 5
Chervoneva et al., sMNAS-9, 2020	775	9	Yes	5, 7

*Only 8 infants were opioid exposed.

for neonates not requiring pharmacotherapy, and (3) no circadian rhythm demonstrated for either group.[219] Animal studies have demonstrated that opioid exposure may be associated with increased oxidative stress and disruptions to glutamate and glutathione homeostasis that could be measured at the time of birth and modified by antioxidants such as *N*-acetylcysteine (NAC).[220] These studies and many more support the need for better biomarkers that are more definitively associated with the severity of NAS and correlate with short- and long-term neurodevelopmental outcomes.[221] The role of additional testing such as EEG and MRI is still being explored. Neuroimaging data from in utero opioid-exposed neonates demonstrate structural as well as functional brain alterations that may persist into adolescence and young adulthood.[93,222,223]

Finally, genetic differences and epigenetic changes in opioid-exposed neonates may place them at higher risk of developing severe NAS. Studies in neonates with antenatal opioid exposure have found an association (in both mothers and their neonates) of specific single nucleotide polymorphisms (SNPs) in the OPRM1, COMT, and PNOC genes with a shorter length of hospital stay and less need for treatment.[224] Increased DNA methylation of opioid receptor gene OPRM1 may be associated with increased NAS severity.[225] In the future it may be possible to link comprehensive clinical and demographic data to genetic and epigenetic information to allow for more targeted management of neonates at risk for NAS.

Management

The goal of NAS management is to maintain physiologic stability and avoid complications such as failure to thrive, inability to sleep, poor feeding, and/or seizures. Strategies to begin treatment, escalate treatment, and to wean treatment once started should be influenced by the neonate's age, comorbidities, other conditions leading to abnormal behavior, and constant evaluation of the clinical elements observed in the relevant scoring system.[226] Standard medical practice is to combine both developmental and behavioral methods with pharmacologic interventions as necessary to control signs of opioid withdrawal. Recent work has confirmed that use of a standard protocol for management of NAS decreases length of hospital stay and use of multidrug therapy.[165,227]

Nonpharmocologic Treatment

Although treatment for opioid withdrawal should always begin with nonpharmacologic measures, pharmacologic management should be adopted when medically necessary.[228] In 2016 and again in 2020, the American Academy of Pediatrics Committee on Fetus and Newborn released a clinical guideline for the care of neonates with NAS. Nonpharmacologic bundles included swaddling, rocking, cuddling, frequent feedings, and skin-to-skin contact as the first line of treatment.[111,165,226,228] Work done by several perinatal quality collaboratives provides additional evidence supporting the use of these nonpharmacologic bundles in minimizing the need for pharmacologic treatment and reducing the length of hospital stay.[227,229] Nonpharmacologic care, especially swaddling and cuddling, requires prolonged involvement of parents or other personnel to soothe the infant. However, not all parents can "room in" and provide this care (especially 24 hours/day). In these situations, trained volunteers can be very helpful. Location of care is equally important and care should ideally be provided to these infants in a non-NICU environment, especially when parental rooming-in is feasible.[165,207] Rooming-in also allows mothers to directly care for their neonate, initiate breast feeding, enhance their parenting skills in a safe environment (facilitating parental retention of custody), and judiciously utilize hospital resources.[165]

Pharmacologic Treatment

Many neonates exhibiting signs of NAS will receive pharmacologic treatment.[230] The mainstay of pharmacologic treatment for opioid withdrawal is opioid replacement, either alone or in combination with other medications.[111,226] Each of the opioid replacement therapies and adjunctive therapies has unique pharmacokinetic properties in neonates, which may vary significantly from that of older children and adults and may vary significantly between individuals.[231] Medication is titrated for each neonate according to the severity of the signs of withdrawal as assessed by various scoring systems. Various threshold scores have been reported for initiating pharmacologic treatment, with common approaches of FNAST scores 8 to 12, or a positive score on 1 component of the ESC scoring tool.[207] NIH-supported trials comparing short- and longer-term outcomes in opioid-exposed neonates managed by the ESC versus the more traditional FNAST approach are ongoing.

Morphine and methadone are the most common initial drugs of choice for NAS, with phenobarbital or clonidine as second-line therapies if clinical signs continue to worsen despite opioid replacement.[226] A small, randomized, controlled trial comparing morphine to phenobarbital for primary treatment of NAS suggested that opioids are superior at decreasing treatment duration and lowering NAS scores.[232] A typical starting dose of morphine is 0.03 to 0.05 mg/kg given orally every 3 to 4 hours. This dose can be increased in increments of 0.03 to 0.1 mg until the signs are controlled.[111] Methadone is increasingly being used as a first-line agent for treatment of NAS. Methadone has a long duration of action and can be administered by either the oral or parenteral route.[231] The initial recommended methadone dose is 0.05 to 0.1 mg/kg followed by 0.025 to 0.05 mg/kg every 8 to 12 hours until signs are controlled. In a recent randomized clinical trial, methadone as compared to morphine was associated with decreased length of stay by 14% and length of treatment by 16%.[233] Study participants' neurobehavioral and developmental outcomes at 18 months were similar in the morphine and methadone arms.[234]

Sublingual buprenorphine also appears safe and effective for short-term outcomes when used to treat NAS. It has a low intrinsic activity which results in less physiological effects than seen with other complete μ-agonists.[235] Because there is no appropriate marketed formulation for neonates, an extemporaneous formulation containing 30% ethanol was developed to conduct clinical trials.[236] In neonates with NAS, pharmacokinetic data are available to inform model-based dosing strategies.[237] Kraft and colleagues conducted a single-site randomized, double-blind, double-dummy controlled trial and demonstrated that in neonates with significant NAS, buprenorphine was associated with a shortened treatment duration compared with morphine as first-line therapy.[238].

Phenobarbital is a commonly used second-line therapy, especially when maternal multidrug use is suspected.[231] However, phenobarbital does not reduce most physiologic signs of opioid withdrawal such as diarrhea and seizures. Czynski and colleagues demonstrated that the use of phenobarbital was associated with a variety of neurobehavioral problems when infants with NAS were assessed at 18 months of age.[234] It is unclear if this was due to an effect of phenobarbital (and the specific formulation) or related to more severe NAS that required second-line pharmacologic treatment. The doses of phenobarbital that have been used are 5 to 20 mg/kg in the first 24 hours, followed by 2 to 4 mg/kg every

12 hours; however, the optimal dosing and therapeutic blood level of phenobarbital for treatment of NAS are not known. A National Institutes of Health (NIH)-funded cohort study that compared phenobarbital versus clonidine as a second-line therapy found that the phenobarbital-treated infants had fewer morphine treatment days and shorter length of stay. However, many infants were discharged home on phenobarbital therapy, which may result in a longer total pharmacologic treatment time, and raises questions about long-term neurodevelopment and safety.[239]

Clonidine is an α_2-adrenergic receptor antagonist used in treating opioid withdrawal in older children and adults. In 2009, a randomized, controlled trial showed that adding clonidine to an opioid for the treatment of NAS significantly reduced the number of treatment failures, the median length of opioid therapy, and the number of neonates requiring high-dose opioids.[240] There were reportedly no significant cardiovascular or other adverse events related to clonidine use.[239,240] In 2015, another randomized, controlled pilot study compared clonidine to morphine as a first-line, single-drug therapy for NAS and found that patients receiving morphine required significantly longer duration of treatment. In addition, measurement of NNNS at 1 week and 2 to 4 weeks of age improved significantly with clonidine, but not with morphine. One-year follow-up did not show significant differences in motor, cognitive, or language scores between treatment groups.[241]

Once the drug(s) have been titrated to control the manifestations of NAS, tapering of the dose should begin 12 to 24 hours later. A common method is to decrease the opioid dose by 10%–20% of the highest dose, with continued assessments to ensure the neonate tolerates the decrease. Signs of opioid withdrawal may increase during the medication tapering, but the goal of weaning is to allow the neonate to acclimate to a lower dose of medication while ensuring that they are able to maintain physiologic stability. Whether opioid replacement can safely be weaned more rapidly in neonates is currently under investigation. Clonidine must be carefully weaned in the hospital because of the risk of rebound autonomic activity with possible hypertension and tachycardia; recent studies have proposed stepwise clonidine weans.[241]

Challenges With Polysubstance Exposure and Nonopioid Withdrawal

About 30% to 40% of pregnant women with OUD are taking more than one psychotropic drug which may increase NAS severity.[242,243] Intrauterine polysubstance exposure could be due to prescribed medications as well as illicit drugs. Psychiatric comorbidities are common in pregnant persons with OUD, and awareness of fetal exposures can help identify infants at risk for complex withdrawal syndromes to allow a tailored management approach. (See Prenatal Medication Exposures That May Be Associated with Neonatal Withdrawa for additional information.)

Postdischarge Infant Follow-Up

Well-designed postdischarge medical and neurodevelopmental follow-up for all neonates with prenatal substance exposures is essential. Since family support is critical for success, postdischarge community support programs should be readily available with referrals in place prior to discharge. If the infant is being discharged home on any medications (phenobarbital), then parents, caregivers, and primary care providers should be given clear, precise written instructions about administration and storage of medications and a proposed weaning schedule. There should be provision for regular postdischarge follow-up visits with the primary care providers to ensure monitoring of medication weaning, following up HIV and HCV testing, and longitudinal growth monitoring. An early intervention (EI) referral should be made prior to discharge and routine neurodevelopmental screenings should be performed at each well child visit as opioid-exposed infants are significantly more likely to have neurodevelopmental impairments in early childhood.[96,243] A significant number of neonates with NAS have long-term neuromotor, cognitive, and/or behavioral morbidities, including insecure and disorganized attachment, impaired speech and language development, aggressive behavior, peer conflicts, hyperactivity, inattention, anxiety, depression, and substance abuse.[244] Even though early identification and intervention has been shown to improve outcome, fewer than half of the eligible infants with NAS enroll in EI services.[245] Efforts to improve EI referral rates during the birth hospitalization, particularly among infants discharged into foster care, and close follow-up for infants with shorter hospital stays are needed to enhance the developmental supports for infants with NAS.

Conclusions

While significant concerns exist with illicit maternal substance use during pregnancy related to observed perinatal outcomes, the number of exposed infants pales in comparison to the established health and developmental risks associated with licit substances such as tobacco and alcohol as well as diversion of prescription drugs. The greatest impact of continued illicit substance use may be the risks of neglect, maltreatment, and disruptions in the home environment. Health policy must be directed at reducing all these complex factors associated with perinatal substance use and providing support for families and children with in utero substance exposure. Most importantly, providing support to the mother-infant dyad following birth (when the risk of relapse is highest) with comprehensive psychosocial services is essential to improving maternal and child health outcomes and reducing the stigma associated with perinatal substance use.

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12 Assessment of Fetal Well-Being

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KEY POINTS

- Fetal assessment demands a view into the somewhat inaccessible intrauterine environment; the primary tools are ultrasound and fetal heart rate monitoring.
- It is important to make the distinction between intrapartum (during labor) and antepartum (before labor) fetal assessment; the latter is the focus of this chapter.
- Current debate centers on who should undergo sonographic examination and what type of evaluation these patients should have.
- Fetal assessment in the third trimester is centered on prediction and/or detection of fetal hypoxemia and academia.
- Antepartum fetal testing in high-risk pregnancies includes the nonstress test, amniotic fluid volume measurement, biophysical profile, and Doppler velocimetry.
- The primary difficulty with antepartum fetal testing is the as-yet unproven utility to improve outcome.

A primary objective of obstetric care is the assessment and prevention of adverse fetal and neonatal outcomes. Maternal care is an integral step toward this goal. Optimization of the maternal state, through monitoring and treatment of chronic conditions such as diabetes or hypertension or acute states like preeclampsia or preterm labor, is one important facet of care to achieve desirable perinatal outcomes. Monitoring and management of the fetus, although a more obvious step toward this goal, is somewhat less straightforward. Fetal assessment demands a view into the somewhat inaccessible intrauterine environment. The ability to gain access to this space to gauge the needs and health of the fetus improved dramatically with developments in technology and increased understanding of fetal physiology over the past several decades. As a result, perinatal morbidity and mortality decreased considerably over that time (Fig. 12.1).

In general, antepartum fetal assessment utilizes various techniques to assess fetal health and well-being in pregnancies that are at increased risk of fetal death due to preexisting maternal conditions (chronic hypertension) or pregnancy-related complications (fetal growth restriction [FGR]). Selecting appropriate patients at risk for adverse perinatal events can enhance the prediction of these events, although some tests may be appropriate even for a low-risk population. The assessment may allow for certain therapeutic options—often, timely delivery—to prevent fetal harm. The overall goal of these efforts is to reduce perinatal mortality, although the reduction of morbidities such as cerebral palsy or preventable birth injury is intertwined with this objective. In antenatal assessment in the third trimester, the prediction and detection of fetal acidemia and hypoxemia form a central principle underlying these efforts.

It is important to make the distinction between antepartum and intrapartum fetal assessment: the latter is specifically related to monitoring the fetus during labor. The nature of labor affords certain advantages (e.g., dilation allows blood samples from the fetus) and restrictions (the lack of fluid after rupture of membranes creates difficulties for ultrasound examination) that do not occur in the antenatal period. As a result, this chapter focuses only on events and assessments preceding labor.

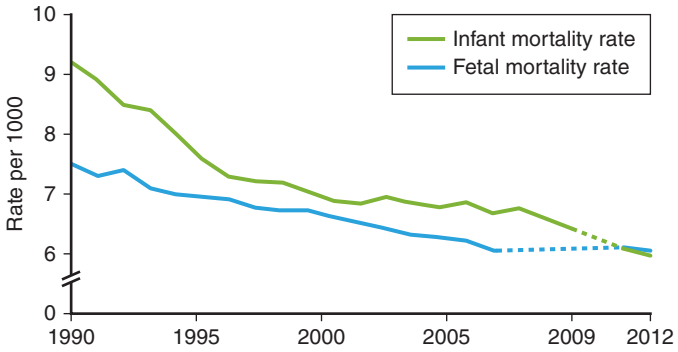
General Principles

Principles of Testing

Many of the tests used for antepartum fetal assessment are screening tests that will lead to further testing allowing for diagnosis and decision making; therefore it is important to note the principles guiding such tests. The outcome, principally perinatal morbidity and mortality, is a significant burden to both the individual and the overall healthcare system. The primary tools for assessment, ultrasound examination and fetal heart rate (FHR) monitoring, are generally easy, safe, and acceptable to patients. Screening has the potential to allow important and timely interventions, such as antenatal steroid administration or delivery. The predominant difficulty with fetal testing comes in the unproven utility of testing to improve outcomes. Furthermore, some tests, such as the nonstress test (NST), have high false-positive rates; therefore when used as a diagnostic test (e.g., to decide on delivery), they can lead to the overuse of interventions. The specificity and sensitivity of the tests vary, and the critical step to enhancing test performance is patient selection. The utility of fetal surveillance involves the judicious application of the tests in patients with specific risk profiles.

Fetal Physiology and Behavior

The first trimester (≤14 weeks' gestation) is mainly a time of system development and organogenesis. The hyperplastic enlargement during the first 11 weeks produces standard rates of growth, with deviation being rare. At the completion of the first trimester, the major organ systems have developed, allowing the opportunity during the second trimester to assess for anomalies in development.



• **Fig. 12.1** Fetal and Infant Mortality Rates in the United States, 1990–2012. Infant mortality rate is the number of infant deaths per 1000 live births. Fetal mortality rate is the number of fetal deaths of 20 weeks' gestation or later per 1000 live births and fetal deaths. (From CDC/NCHS, National Vital Statistics System.)

The second and third trimesters involve the maturation of these systems. Fetal assessment, primarily performed in the third trimester, is concerned with the prediction or detection of fetal hypoxemia and acidemia. Integration of the fetal neurologic and cardiovascular systems as they relate to acid-base status is the cornerstone of this assessment. By the beginning of the third trimester, there is usually adequate maturation present in the neurologic and cardiovascular systems to enable meaningful fetal assessment. We are thus able to monitor the manifestations of hypoxemia and acidemia as shown by neurologic and cardiovascular changes. In pregnancies at extremely high risk for adverse perinatal outcome or stillbirth, a fetal assessment may be performed in the second trimester, as early as 23 to 24 weeks' gestation.

Technology

The technologic underpinning of fetal assessment is ultrasound. FHR monitoring during the antepartum period depends on a Doppler cardiogram; movements of the fetal heart, in particular the sounds of the valves, are detected by this monitor. The time between the beats is translated into a heart rate, which is then graphically represented on a chart over time. This process produces the FHR monitoring strip that becomes the NST or contraction stress test (CST).

Contemporary ultrasound imaging technology involves a wide array of features, including B-mode (basic imaging), M-mode (mapping the movement of structures over time), pulsed Doppler (demonstrating flow velocity in a particular area, such as a vessel), color Doppler (showing intensity and direction of flow through shades of red and blue), and power Doppler (a more sensitive form of colorized Doppler). Magnetic resonance imaging is often used to supplement ultrasound imaging, especially for imaging of the fetal brain.

Indications and Timing

Most fetal testing protocols involve a stepwise approach, and the first step is the selection of the appropriate patient. Suggested assessments for low-risk pregnancies include one ultrasound examination for dating and one for the basic anatomic survey. Prenatal risk assessments for chromosomal disorders, such as cell-free fetal DNA analysis, first-trimester risk assessment with maternal serum analysis and fetal nuchal translucency assessment, or the second-trimester maternal quadruple serum screen, are additional options (see Chapter 26, Prenatal Diagnosis and Counseling, for additional discussion of these tests). Whereas up to 30% of perinatal morbidity may occur in low-risk patients, routine fetal testing beyond that described previously in a low-risk pregnancy is an ineffective use of resources.

High-risk pregnancies are those at greater peril for perinatal morbidity and mortality. These pregnancies often have more justification for targeted or detailed anatomic ultrasound examinations and for regular assessment of fetal growth or FHR. Common conditions requiring increased fetal surveillance are shown in Box 12.1.

Pregnancy dating should be confirmed at the earliest possible moment, and fetal anatomic screening is best accomplished in the second trimester, specifically at 18 to 20 weeks' gestation, when visualization of the anatomic features is adequate. However, standards for the timing of antepartum fetal assessment to survey for fetal compromise do not exist. Certainly, assessment with NST or biophysical profiles (BPP) would have little utility before viability (approximately 23 to 24 weeks' gestation). Guidelines for initiating fetal testing for specific indications are largely based on the risk of fetal loss at a particular gestational age (GA). Most fetal testing algorithms begin antepartum fetal testing no earlier than 32 weeks' gestation. However, the most at-risk pregnancies may be monitored at earlier GAs when delivery would be considered for fetal benefit.

• BOX 12.1 Common Indications for Antepartum Fetal Assessment: High-Risk Pregnancies

Fetal
- Abnormal fetal testing, fetal distress
- Decreased fetal movement
- Fetal growth restriction
- Monochorionic multiple gestation
- Oligohydramnios

Maternal–Fetal
- Placental abruption (abruptio placenta)
- Alloimmunization
- Late term or postterm pregnancy
- Gestational hypertension or preeclampsia
- Gestational diabetes
- History of fetal death

Maternal
- Advanced maternal age (>40 years old)
- Cyanotic cardiac disease (severe)
- Hypertension, chronic
- Morbid obesity
- Pulmonary disease (severe)
- Chronic renal disease
- Systemic lupus erythematosus
- Hyperthyroidism

Fetal Assessment in Low-Risk Pregnancies

Ultrasound: Pregnancy Dating

The *estimated date of delivery* is defined at the beginning of pregnancy based on the best available information, including menstrual history, ultrasound data, and assisted reproduction technology. The median duration of a singleton pregnancy is

280 days (40 weeks) from the first day of the last menstrual period or 266 days (38 weeks) from the time of ovulation. *Term* is defined as 37 to 41 + 6 weeks' (259 to 294 days) gestation. This definition is further broken down into two subgroups: early term (37 weeks 0 days to 38 weeks 6 days) and late term (41 weeks 0 days to 41 weeks 6 days). Preterm is defined as delivery prior to 37 weeks. This definition is further broken down into the late preterm subgroup (34 weeks 0 days to 36 weeks 6 days). Postterm pregnancies are defined as delivery at 42 weeks and beyond (Box 12.2). Given that the preterm and postterm periods are associated with increased risks to the fetus and newborn, pregnancy dating provides an approximate expectation for the completion of the pregnancy and serves as a basis for the efficient and appropriate use of fetal surveillance, testing, and treatment. Accurate pregnancy dating by ultrasound has been associated with reduced diagnoses of growth restriction,[1] reduced use of tocolysis for preterm labor,[2] and a reduced need to intervene in postterm pregnancies.[3,4] Additionally, early ultrasound assessment is associated with an increased diagnosis of multifetal pregnancies.[4]

In a spontaneous pregnancy in a woman with regular cycles and normal menstrual periods, the last menstrual period is often an accurate way of dating a pregnancy. Menstrual dating is less accurate in women who are taking oral contraceptives, were recently pregnant, or have irregular periods or intermenstrual bleeding. In these cases, and others in whom there is uncertainty, ultrasound dating in the first trimester is accurate and effective. A fetal pole may be seen beginning at 6 weeks' gestation, and the fetal heartbeat should be visualized by 6 to 7 weeks' gestation.[5] In the first trimester, measurement of the crown-rump length is accurate to within 5 to 7 days; therefore this measurement should take priority in dating a pregnancy when the timing of the last menstrual period suggests a GA outside this range of variation.[6–9] A first-trimester ultrasound examination is indicated to confirm an intrauterine pregnancy (i.e., exclude ectopic pregnancy), confirm fetal viability, document fetal number, and estimate GA. During this ultrasound, the maternal pelvis and ovaries may be assessed to look for the presence of abnormalities, including uterine fibroids, müllerian anomalies, and ovarian masses, such as a dermoid cyst. In the second trimester, ultrasound dating is less accurate (discrepancy from 7 to more than 14 days) but can nonetheless be helpful. Measurement of the biparietal diameter (BPD) of the fetal head, the most accurate parameter, can be accurate to within 7 to 10 days.[10,11] The BPD is also a parameter of choice because it is less affected by chromosomal anomalies, in particular Down syndrome.[12] Usually in the second or third trimesters, several biometric measurements—such as BPD, head circumference, abdominal circumference (AC), and femur length—are recorded, and a computerized algorithm can generate an estimated GA. Additional fetal measurements, including other fetal long bones (i.e., humerus, ulna, and tibia) and transverse cerebellar diameter may also assist with estimating fetal GA.[13,14]

Ultrasound: Second and Third Trimesters

Perinatal ultrasound examination in the second and third trimesters can be classified broadly into three types: the basic or standard examination, the specialized (detailed) examination, and the limited examination. The standard or basic examination includes the determination of the fetal number, fetal viability, fetal position, fetal biometry, placental location, amniotic fluid volume, the presence or absence of a maternal pelvic mass, and the presence of gross fetal malformations (Box 12.3).[15,16] Most pregnancies can be evaluated adequately by this basic examination. If the patient's history, physical examination, or basic ultrasound examination suggests the presence of a fetal malformation, a specialized or detailed examination should be performed by a sonographer who is skilled in fetal evaluation. During a detailed ultrasound, which is best performed at 18 to 20 weeks' gestation, fetal structures are examined in detail to identify and characterize any fetal malformation. In addition to identifying structural abnormalities, a specialized ultrasound examination can identify sonographic markers of fetal aneuploidy. In some situations, a limited examination may

• BOX 12.2 Definition of Gestational Age Categories

Term Pregnancy	37 + 0 to 41 + 6 weeks
Early	37 + 0 to 38 + 6 weeks
Late	41 + 0 to 41 + 6 weeks
Preterm Pregnancy	<37 weeks
Late	34 + 0 to 36 + 6 weeks

• BOX 12.3 Images and Information Obtained During a Standard Second- and Third-Trimester Ultrasound

Fetal cardiac activity
Fetal number including chorionicity and amnionicity
Fetal position
Fetal biometry
Placental location and umbilical cord insertion
Amniotic fluid volume
Assessment of maternal adnexa, uterus, and cervix
Fetal anatomy
Head, face, neck
- Lateral cerebral ventricles
- Choroid plexus
- Midline falx
- Cavum septi pellucidi
- Cerebellum
- Cistern magna
- Upper lip

Chest
- Heart
- Four-chamber view
- Left ventricular outflow tract
- Right ventricular outflow tract
- Three-vessel view and 3-vessel trachea view

Abdomen
- Stomach
- Kidneys
- Urinary bladder
- Umbilical cord insertion site into the fetal abdomen
- Umbilical cord vessel number

Spine
- Cervical, thoracic, lumbar, and sacral spine

Extremities
- Legs and arms
- Hands and feet

Sex
- In multiple gestations and when medically indicated

be appropriate to answer a specific clinical question (such as fetal viability, amniotic fluid volume, fetal presentation, placental location, or cervical length) or to provide sonographic guidance for an invasive procedure (such as amniocentesis).

Current debate centers on who should undergo sonographic examination and what type of evaluation these patients should have. Advocates of routine sonography cite several advantages of universal ultrasound evaluation, including more accurate dating of pregnancy and earlier and more accurate diagnosis of multiple gestation, structural malformations, and fetal aneuploidy (discussed later in Ultrasound section). Opponents of routine sonographic examination argue that it is an expensive screening test ($100 to $250 for a standard examination) and that the cost is not justified by published research, which suggests that routine ultrasound examinations do not significantly change perinatal outcome.[2,17,18] However, subsequent cost-benefit analyses regarding the routine use of ultrasound in low-risk pregnancies have concluded both cost savings,[19] if the ultrasound is performed in a tertiary care setting, and net loss, if the ultrasound is performed by less experienced providers. Ultrasound performed in a country with socialized medicine proved cost saving.[20] Thus it may be the more-tempered approach to ultrasound screening with the utilization of highly skilled sonographers and sonologists that allows the routine ultrasound screening of pregnancies to be both effective and economical. Nonmedical use of obstetric ultrasound is not supported by either the American College of Obstetricians and Gynecologists or the American Institute of Ultrasound in Medicine for many reasons, including the potential for false reassurance at the time of the ultrasound, as well as no formal process for follow-up if an abnormality is identified.[21]

Second-trimester ultrasound examination may be indicated for various reasons, including uncertain dating, a uterine size larger or smaller than expected for the estimated GA, vaginal bleeding, suspected multiple gestation, a medical disorder that can affect fetal growth and development (e.g., diabetes, hypertension, collagen vascular disorder), a family history of an inherited genetic abnormality, a suspected fetal malformation or growth disturbance, or suspected fetal death.[15,16] In the United States, most patients undergo a standard examination at 18 to 20 weeks' gestation to screen for structural defects. An understanding of normal fetal physiology is critical to the diagnosis of fetal structural anomalies. For example, extra-abdominal herniation of the midgut into the umbilical cord occurs normally in the fetus at 8 to 12 weeks' gestation and can be misdiagnosed as an abdominal wall defect. The placental location should be documented with the bladder empty because overdistension of the maternal bladder or a lower uterine contraction can give a false impression of placenta previa. If placenta previa is identified at 18 to 22 weeks' gestation, serial ultrasound examinations should be performed to follow placental location. The presence of placenta previa at 15 to 17 weeks has about a 12% risk of being present at term, while presence later in the second trimester (24 to 27 weeks) confers a 49% risk of persistence at term.[22] The umbilical cord should also be imaged, including the number of vessels and its fetal and placental insertion.

The indications for third-trimester ultrasound examination are similar to that for second-trimester ultrasounds. Fetal anatomy survey examinations and estimates of fetal weight become less accurate as GA increases, especially in obese women or in pregnancies complicated by oligohydramnios. However, fetal biometry and an anatomic survey should still be performed, because certain fetal anomalies, such as achondroplasia, may become evident for the first time later in gestation.

Fetal Movement Counting

Fetal movement (quickening) is typically perceived by the mother at 16 to 22 weeks' gestation. Fetal hypoxemia is typically associated with a reduction in fetal activity; the fetus is essentially conserving energy and oxygen for vital activities. A typical procedure for fetal movement counting consists of having the patient record the interval taken to feel 10 fetal movements, usually after a meal when the fetus is more active. If 10 movements are not detected in 1 hour, further testing is often recommended. The data supporting fetal movement counting are mixed. A large UK and Ireland cluster-randomized trial involving more than 400,000 pregnancies demonstrated no benefit,[23] and a Cochrane analysis found insufficient evidence to support this technique to prevent stillbirth.[24] However, a Norwegian study found a significant reduction in the number of stillbirths without a subsequent increase in the number of hospital visits for decreased fetal movement when a standardized approach to patient education regarding the recommended approach to decreased fetal movement[25] was implemented. Fetal movement counting represents a low-technology screening test that can be applied easily to all pregnancies. Although its effectiveness in improving perinatal outcomes is debatable, it can be used as a cost-effective first-line strategy.

Fetal Assessment in High-Risk Pregnancies

Cardiotocography

Cardiotocography is the visual representation of FHR and uterine contractions. FHR has been recognized as an important indicator of fetal status since the 19th century, with Lejumeau Kergaradec of Switzerland being credited with the first accounts of direct fetal auscultation and the uterine souffle in 1821. FHR monitoring is based on the principle that the fetal neurologic system, through its afferent and efferent networks, serves as a key mediator to demonstrate fetal well-being. Oxygenation, acidemia, and other vital functions are monitored by peripheral chemoreceptors and baroreceptors, which provide input on fetal status through afferent neurologic networks to the central nervous system (CNS). This information is processed by the CNS, and signals are conducted through efferent networks to produce peripheral changes, particularly to the heart via direct parasympathetic vagal neurons, direct sympathetic signals, or indirect sympathetic stimulation of catecholamine release. In this way, fetal cardiac activity can be seen as a surrogate for fetal oxygenation and acid-base status.

For many years, assessment of the FHR was limited to the fetoscope, a direct stethoscope attributed to Adolphe Pinard in 1876. In 1957, Orvan Hess and Ed Hon at Yale University (New Haven, CT) introduced electronic FHR monitoring as a window into the status of the fetus.[26] This technology relied on direct monitoring through a scalp electrode; only years later would Doppler technology allow cardiac signals to be detected noninvasively. FHR monitoring became a tool for fetal assessment, as it was recognized that certain FHR patterns were associated with fetal compromise and poor fetal outcomes.

The basic elements of an FHR strip are baseline, variability, accelerations, and decelerations. A baseline of 110 to 160 beats/min is normal. Variability is determined by the irregular fluctuations in amplitude and frequency in the baseline, and variability of fewer than 6 beats/min is often abnormal. Accelerations are classified as visually apparent abrupt increases that peak at

15 beats/min or more above the baseline and last 15 seconds or longer. Fetal movements often coincide with FHR accelerations. Finally, decelerations, often classified as early, variable, or late, are decreases in the FHR that have specific pathologic and physiologic associations. Although primarily focused on intrapartum monitoring, the 2008 National Institute of Child Health and Human Development workshop report on fetal monitoring provides an excellent summary of the nomenclature and interpretation involved (Table 12.1).[27]

Nonstress Test

A normal result of an NST is defined as a 20-minute FHR tracing that contains two heart rate accelerations lasting 15 seconds or longer that peak 15 beats or more above the baseline: often this is called a *reactive NST* (Fig. 12.2). Modifications are made in reference to GA. NSTs for fetuses at less than 32 weeks' gestation are often considered reactive if the acceleration is 10 beats/min or more above the baseline and lasts for at least 10 seconds.[28,29] Furthermore, to account for the periodicity of 20- to 30-minute sleep cycles in the fetus, an NST that is not reactive over the first 20 minutes may be continued for an additional 20 to 40 minutes. A nonreactive NST or an NST with specific abnormalities (e.g., high or low baseline, decelerations) should be followed by a BPP. It is important to note that some abnormal states, such as a fetal CNS abnormality or maternal drug ingestion, may contribute to a nonreactive NST. In these cases, ultrasound examination may provide appropriate information to determine the diagnosis or required management.

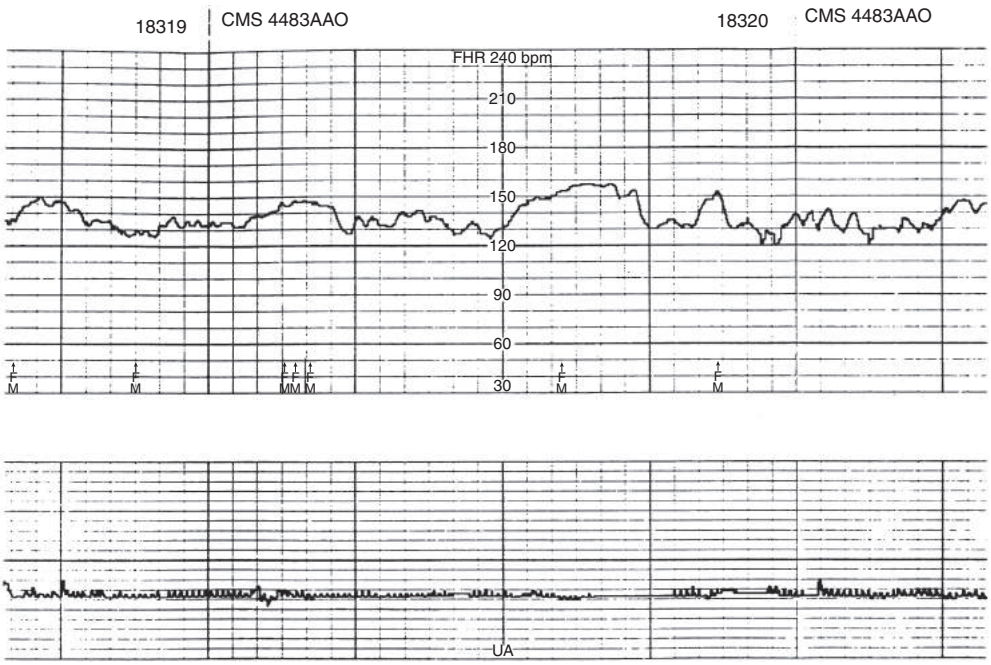
Falsely normal NSTs occur at a rate of 3 to 5 per 1000 tests, although this does not account for a baseline rate of unpreventable fetal deaths.[30] The difficulty with the NST really lies in its lack of specificity for fetal death or compromise; the false-positive rate may be as high as 50%.[30]

The rather modest false-negative rate is likely because of the NST being a measurement of short-term hypoxemia. Indeed, longer-term fetal status can be measured through amniotic fluid assessment, because the amniotic fluid is correlated with fetal urinary output, which is a surrogate for renal perfusion. When combined with an assessment of amniotic fluid level, the false-negative rate of the NST is reduced to 0.8 per 1000, although a 60% false-positive rate remains.[31] Thus when the amniotic fluid level is combined with the NST—sometimes known as the *modified BPP*—the risk of fetal death is reduced to negligible levels in high-risk populations.[32] For these reasons, the modified BPP is a modality of choice for monitoring high-risk pregnancy.

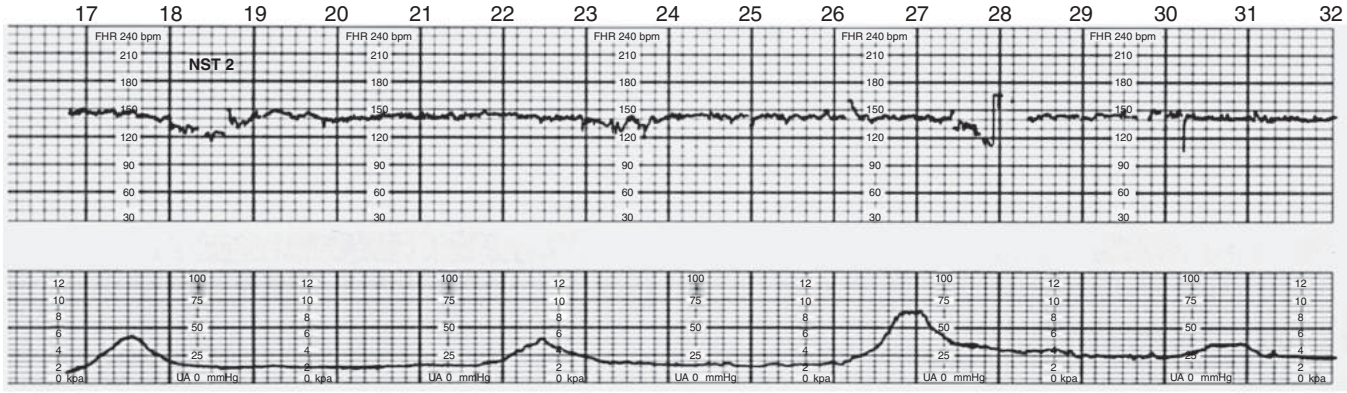
TABLE 12.1 Interpretation of Antepartum Cardiotocography

Term	Characteristic	Description
Baseline	Definition	Mean FHR, rounded to increments of 5 beats/min (e.g., 140, 145); need baseline duration of ≥2 min during a 10-min segment, between periodic or episodic changes, to determine baseline
	Bradycardia	<110 beats/min for >10 min
	Tachycardia	>160 beats/min for >10 min
Variability	Definition	Fluctuations of the baseline heart rate; measured from peak to trough
	Absent	Undetectable
	Minimal	Undetectable to ≤5 beats/min
	Moderate	6–25 beats/min
	Marked	>25 beats/min
Acceleration	Definition	Abrupt increase ≥15 beats/min lasting ≥15 s
	Prolonged	≥2 min and <10 min (≥10 min is a baseline change)
Deceleration	Definition	Decreases in the FHR
	Variable	Abrupt decrease onset to nadir <30 s; decrease ≥15 beats/min lasting ≥5 s to <2 min
	Early	Gradual decrease onset to nadir ≥30 s with contraction
	Late	Gradual decrease onset to nadir ≥30 s; nadir of deceleration occurring after peak of contraction
	Prolonged	Decrease ≥15 beats/min lasting ≥2 min, but <10 min (≥10 min is a baseline change)
	Recurrent	Occur with ≥50% of uterine contractions in any 20-min window
	Intermittent	Occur with <50% of uterine contractions in any 20-min window
Contractions	Considerations	Frequency, duration, intensity, and relaxation
	Normal	≤5 contractions per 10 min averaged over a 30-min window
	Tachysystole	>5 contractions per 10 min averaged over a 30-min window; should always be qualified as to the presence or absence of associated FHR decelerations

FHR, Fetal heart rate.



• **Fig. 12.2** Normal Nonstress Test. Note two fetal heart rate accelerations exceeding 15 beats/min and lasting at least 15 seconds during the monitoring period. *FHR*, Fetal heart rate.



• **Fig. 12.3** Contraction Stress Test. The fetal heart rate is plotted above the uterine contraction signal. Note the late decelerations after the contractions; this is a positive, or abnormal, test result. *FHR*, Fetal heart rate; *NST*, nonstress test.

Contraction Stress Test

The CST assesses the FHR response in the presence of contractions. This test improves on the specificity and sensitivity of the NST by assessing the fetal response to stress. In fact, the CST preceded the NST, although the NST became more favorable because of fewer contraindications, ease of administration, and reduced time and supervision necessary. Compared with the NST, there is a much lower incidence of falsely normal tests (0.4 per 1000), representing an eightfold reduction in the risk of fetal loss in one study.[33]

Contractions are stimulated by the administration of intravenous oxytocin or through maternal nipple stimulation. Of course, the CST is contraindicated in patients in whom contractions should not be provoked, such as threatened preterm delivery or preterm premature rupture of membranes, prior classical cesarean delivery, or placenta previa. A minimum of three contractions over a 10-minute period of continuous FHR assessment are necessary for a satisfactory test interpretation. An unsatisfactory test should be followed by continued testing with a modification of the mode of contraction stimulation. A negative (i.e., normal) test result demonstrates no late decelerations, whereas a positive test result shows late decelerations after 50% or more of contractions (Fig. 12.3). A positive test result requires immediate further testing or evaluation, if not delivery. An equivocal test demonstrates late decelerations with less than 50% of contractions and requires further testing or monitoring. A test that encompasses a hyperstimulatory contraction pattern (e.g., five contractions within 10 minutes or contractions lasting longer than 90 seconds) is also considered equivocal and requires further testing.

Ultrasound

Although routine sonography for low-risk pregnant women is controversial, few would disagree that the benefits far outweigh the costs for high-risk patients. Given the higher risk for fetal complications such as anatomic anomalies or growth disturbances,

a specialized examination is performed between 18 and 20 weeks' gestation in most high-risk pregnancies.

Additional ultrasound modalities are also available, including fetal echocardiography, three-dimensional (3D) sonography, and Doppler. Cardiac anomalies are the most common major congenital defects encountered in the antepartum period. A four-chamber view of the heart at the time of fetal anatomy survey at 18 to 20 weeks' gestation will detect only 50% of congenital cardiac anomalies, although the detection rate can be increased to approximately 65% to 90% if the four-chamber and outflow tract or outlet tract and three vessels with trachea views are adequately visualized,[34] but this still leaves 10% to 35% of all congenital cardiac anomalies undiagnosed. Factors that affect the sensitivity include the type of practice (university-based vs. community-based), operator training and experience, GA at the time of the scan, maternal weight, fetal position, and type of defect present.[35] For this reason, fetal echocardiography should be performed by a skilled and experienced sonologist at 20 to 22 weeks' gestation in all pregnancies at high risk of a fetal cardiac anomaly; this includes pregnancies complicated by pregestational diabetes mellitus, a personal or family history of congenital cardiac disease (regardless of the nature of the lesion or whether it has been repaired), maternal use of potential cardiac teratogens (e.g., lithium and valproic acid),[36] monochorionic twins, and pregnancies conceived by in vitro fertilization, but not if the pregnancy was conceived using clomiphene citrate or ovarian stimulation or intrauterine insemination alone.[37]

Compared with standard two-dimensional (2D) ultrasound, 3D ultrasound (or four-dimensional [4D] if fetal movements are included) allows for visualization of fetal structures in all three dimensions concurrently for the improved characterization of complex fetal structural anomalies and for storage of scanned images with 3D reconstruction at a later date or remote location (telemedicine). Unlike 2D ultrasound, 3D images are greatly influenced by fetal movements and are subject to more interference from structures such as fetal limbs, umbilical cord, and placental tissue. Because of movement interference, visualization of the fetal heart with 3D ultrasound is suboptimal.

In addition to the rapid acquisition of images that can be later reconstructed and manipulated, 3D ultrasound has other potential advantages:

- Surface rendering mode can provide clearer images of many soft tissue structures. Such images can improve the diagnosis of certain fetal malformations, especially craniofacial anomalies (cleft lip and palate, micrognathia, ear anomaly, facial dysmorphism, club foot, finger and toe anomalies), intracranial lesions, spinal anomalies, ventral wall defects, and fetal tumors.
- 3D ultrasound may be useful in early pregnancy by providing more accurate measurements of the gestational sac, yolk sac, and crown-rump length. It may also allow for a more accurate midsagittal view of the fetus for measuring nuchal translucency.
- 3D ultrasound can also be used to measure tissue volume. Preliminary data suggest that the assessment of cervical volume may predict the risk of cervical insufficiency,[38] and measurement of placental volume in the first trimester may predict fetuses at risk of FGR.[39]

Despite these advantages and the fact that 3D ultrasound has been available since the early 1990s, it has yet to live up to its promises. Although 3D ultrasound is unlikely to replace standard 2D imaging in the near future, it is a valuable complementary modality in obstetric imaging. Areas amenable to 3D evaluation include fetal facial anomalies, neural tube defects, fetal tumors, and skeletal malformations.

Growth Assessment

Normal fetal growth is a critical component of a healthy pregnancy and the subsequent long-term health of the child. A systematic method of physical examination of the gravid abdomen was first described by Leopold and Sporlin.[40] Although this examination has several limitations, particularly in the setting of maternal obesity, multiple pregnancy, uterine fibroids, or polyhydramnios, it is safe, low cost, and well tolerated and may add valuable information to assist in antepartum management. Palpation is divided into four separate Leopold maneuvers. Each maneuver is designed to identify specific fetal landmarks or to reveal a specific relationship between the fetus and mother. For example, the first maneuver involves measuring fundal height. The uterus can be palpated above the pelvic brim at approximately 12 weeks' gestation. Thereafter, fundal height should increase by approximately 1 cm/week, reaching the level of the umbilicus at 20 to 22 weeks' gestation. Between 20 and 32 weeks' gestation, the fundal height (in centimeters, from the superior edge of the pubic symphysis) is approximately equal to the GA (in weeks) in healthy women of average weight with an appropriately grown fetus. However, there is a wide range of normal fundal height measurements. One study has shown a 6-cm difference between the 10th and 90th percentiles at each week of gestation after 20 weeks.[41] Moreover, fundal height is maximal at approximately 36 weeks' gestation, at which time the fetus drops into the pelvis in preparation for labor, and the fundal height decreases. For these reasons, reliance on fundal height measurements alone will fail to identify more than 50% of fetuses with FGR.[42] Serial fundal height measurements by an experienced obstetric care provider are more accurate than a single measurement and will lead to an improved diagnosis of FGR, with reported sensitivities as high as 86%.[41] However, a more recent systematic review failed to conclude that serial fundal height measurements were superior to abdominal palpation alone due to a lack of randomized controlled trials.[43]

If the clinical examination is not consistent with the stated GA, an ultrasound examination is indicated to confirm GA and to establish a more objective measure of fetal growth. Ultrasound examination may also identify an alternative explanation for the discrepancy, such as multiple pregnancy, polyhydramnios, oligohydramnios, fetal demise, or uterine fibroids.

For many years, obstetric sonography has used fetal biometry to define fetal size by weight estimation, although this approach has a number of key limitations. For example, regression equations used to create weight estimation formulas are derived primarily from cross-sectional data that rely on infants delivering within an arbitrary period of time after the ultrasound examination, and they assume that body proportions (i.e., fat, muscle, bone) are the same for all fetuses. Moreover, growth curves for healthy infants from 24 to 37 weeks' gestation rely on data collected from pregnancies delivered preterm, which should not be regarded as normal pregnancies and are likely to be complicated by some element of uteroplacental insufficiency, regardless of whether the delivery was spontaneous or iatrogenic. Despite these limitations, if the GA is well validated, the prevailing data suggest that prenatal ultrasound can be used to verify an alteration in fetal growth in 80% of cases and exclude abnormal growth in 90% of cases.[44]

Sonographic estimates of fetal weight are commonly derived from mathematical formulas that use a combination of fetal measurements, especially the BPD, AC, and femur length.[45] Whereas the BPD may be the most accurate indicator of GA in the second or third trimesters, fetuses gain weight in their abdomen, making the AC the single most important measurement for fetal size. The

AC is thus given more weight in these formulas. Unfortunately, the AC is also the most difficult measurement to acquire, and a small difference in the AC measurement will result in a large difference in the estimated fetal weight (EFW). The accuracy of the EFW depends on a number of variables, including GA (in absolute terms, EFW is more accurate in preterm or FGR fetuses than in term or macrosomic fetuses), operator experience, maternal body habitus, and amniotic fluid volume (measurements are more difficult to acquire if the amniotic fluid volume is low). Although objective, sonographic EFW estimations are not particularly accurate and have an error of 15% to 20%, even in experienced hands.[46] Indeed, a sonographic EFW at term is no more accurate than a clinical estimate of fetal weight by an experienced obstetric care provider or the mother's estimate of fetal weight if she has delivered before.[47] Sonographic estimates of fetal weight must therefore be evaluated within the context of the clinical situation and balanced against the clinical estimate of fetal weight. Serial sonographic evaluations of fetal weight are more useful than a single measurement in diagnosing abnormal fetal growth. The ideal interval to evaluate fetal growth is every 3 to 4 weeks, with a minimum 10-day to 14-day interval necessary to see significant differences. Because of the inherent error in fetal biometric measurements, more frequent ultrasound determinations of EFW may be misleading. Similarly, the use of population-specific growth curves, if available, will improve the ability of the obstetric care provider to identify abnormal fetal growth. For example, growth curves derived from a population that lives at a high altitude, where the fetus is exposed to lower oxygen tension, will be different from those derived from a population at sea level. Abnormal fetal growth can be classified as insufficient (i.e., FGR) or excessive (fetal macrosomia).

The definition of FGR has been a long-standing challenge for modern obstetrics. Distinguishing the healthy, constitutionally small fetus, defined as an EFW below the 10th percentile for a given week of gestation, from the nutritionally deprived, truly growth-restricted fetus has been particularly difficult. Fetuses with an EFW less than the 10th percentile are not necessarily pathologically growth restricted. Conversely, an EFW greater than the 10th percentile does not mean that an individual fetus has achieved its growth potential, and such fetuses may still be at risk of perinatal mortality and morbidity. As such, the most widely utilized definition of FGR is EFW less than the 10th percentile for GA in a well-dated pregnancy.[48,49] More restrictive definitions of FGR (EFW less than the 5th or 3rd percentiles) can identify fetuses at higher risk for pathologic growth restriction as opposed to constitutionally small fetuses. An AC below the 10th percentile can also be used to define FGR.[50] Additional findings suggestive of fetal compromise like oligohydramnios or abnormal umbilical artery Doppler velocimetry increase the likelihood of pathologic FGR.

FGR can be classified into maternal, fetal, or placental etiologies. These distinct pathophysiologic mechanisms associated with FGR may occur in isolation or collectively but result in the common final pathway of suboptimal fetal growth and compromised fetal nutrition. Fetuses affected by poor antepartum growth may be further categorized as having symmetric or asymmetric FGR. In cases of symmetric FGR, both the fetal head size and body weight are reduced, indicating a global insult that probably occurred early in gestation. Symmetric FGR may reflect an inherent fetal abnormality (e.g., fetal chromosomal abnormality, inherited metabolic disorder, early congenital infection) or long-standing severe placental insufficiency caused by an underlying maternal disease (e.g., maternal hypertension, long-standing pregestational diabetes, or a significant collagen vascular disorder). Asymmetric FGR is characterized by suboptimal body growth with preserved head growth. It is more commonly observed in the third trimester and is thought to result from a later pathologic event, such as chronic placental abruption leading to uteroplacental insufficiency, in an otherwise uncomplicated pregnancy and healthy fetus.

Currently, patients with risk factors for FGR, those who develop obstetric complications, and those identified with lagging symphysial fundal height measurements are subsequently screened with ultrasound to assess fetal growth. This screening algorithm is in place in the United States, the UK, and various other countries; however, it is known to be imprecise and many fetuses at risk for growth restriction as well as perinatal mortality are not identified with this system. For this reason, a prospective cohort study evaluated screening for FGR with a universal third-trimester ultrasound to assess fetal growth.[51] The authors found that standard screening for FGR identified 20% of small for gestational age (SGA) infants, and implementation of universal screening identified 57% of SGA infants. The increased sensitivity resulted in a decrease in specificity from 98% to 90%, resulting in an increased number of false-positive cases. Therefore the implementation of a universal screening program to identify fetuses at risk for growth restriction and increased perinatal mortality would have to take into consideration the issue of over-identification of possible at-risk fetuses and subsequent overtreatment.

Early and accurate diagnosis of FGR coupled with the appropriate intervention will lead to an improvement in perinatal outcome. If FGR is suggested clinically and by ultrasound examination, thorough evaluations of the mother and fetus are indicated. Referral to a maternal-fetal medicine specialist should be considered. Every effort should be made to identify the cause of FGR and to modify or eliminate contributing factors. Up to 20% of cases of severe FGR are associated with fetal chromosome abnormalities or congenital malformations, 25% to 30% are related to maternal conditions characterized by vascular disease, and a smaller proportion are the result of abnormal placentation. However, in a substantial number of cases (50% or more in some studies), the cause of the FGR will remain uncertain even after a thorough investigation.[52]

Fetal macrosomia is defined as an EFW (not birthweight) of 4500 g or greater, measured either clinically or by ultrasound, and is independent of GA, diabetic status, or actual birthweight.[21] Fetal macrosomia refers to a single cutoff EFW; this should be distinguished from the large for GA fetus, which is one in whom the EFW is greater than the 90th percentile for GA. By definition, 10% of all fetuses are large for GA at any given GA. Fetal macrosomia is associated with an increased risk of cesarean delivery, operative vaginal delivery, and birth injury to both the mother (including vaginal, perineal, and rectal trauma) and the fetus (orthopedic and neurologic injury).[21,53–56] Shoulder dystocia with resultant brachial plexus injury (Erb palsy) is a serious consequence of fetal macrosomia and is further increased in the setting of diabetes because of the increased diameter of the upper thorax and neck of those fetuses.

Fetal macrosomia can be determined clinically, by abdominal palpation using Leopold maneuvers, or by ultrasound examination; these two techniques appear to be equally accurate.[57] However, EFW measurements are less accurate in large (macrosomic) fetuses than in normally grown fetuses, and factors such as low amniotic fluid volume, advancing GA, maternal obesity, and the position of the fetus can compound these inaccuracies. Clinical examination has been shown to underestimate the birthweight by 0.5 kg

or more in almost 80% of fetuses with macrosomia.[58] For these reasons, the prediction of fetal macrosomia is not particularly accurate, with a false-positive rate of 35% and a false-negative rate of 10%.[57,58] A number of alternative sonographic measurements have therefore been proposed in an attempt to better identify the macrosomic fetus, including fetal AC alone, umbilical cord circumference, cheek-to-cheek diameter, and upper arm circumference; however, these measurements remain investigational and should not be used clinically.

Despite the inaccuracy in the prediction of fetal macrosomia, an EFW should be documented either by clinical estimation or ultrasound examination in all women at high risk for fetal macrosomia at approximately 38 weeks' gestation. Suspected fetal macrosomia is not an indication for induction of labor prior to 39 weeks of gestation, because the evidence is lacking that the benefits of reducing shoulder dystocia risk would outweigh the harms of early delivery.[21] However, if the EFW is excessive, elective cesarean delivery should be considered to prevent fetal and maternal birth trauma. Although controversy remains as to the precise EFW at which an elective cesarean delivery should be recommended, a suspected birthweight in excess of 4500 g in women with diabetes or 5000 g in women without diabetes is a reasonable threshold.[21,59]

Amniotic Fluid Assessment

Amniotic fluid plays a key role in the health and development of a growing fetus. Once considered an afterthought during the ultrasound examination of the fetus, evaluation of the amniotic fluid is now considered an integral part of ultrasound evaluation for fetal well-being. The amniotic fluid serves a number of important functions for the developing embryo and fetus. It provides cushioning against physical trauma; creates an environment free of restriction and or distortion, allowing for normal growth and development of the fetus; provides a thermally stable environment; allows the respiratory, gastrointestinal, and musculoskeletal tracts to develop normally; and helps to prevent infection.[60]

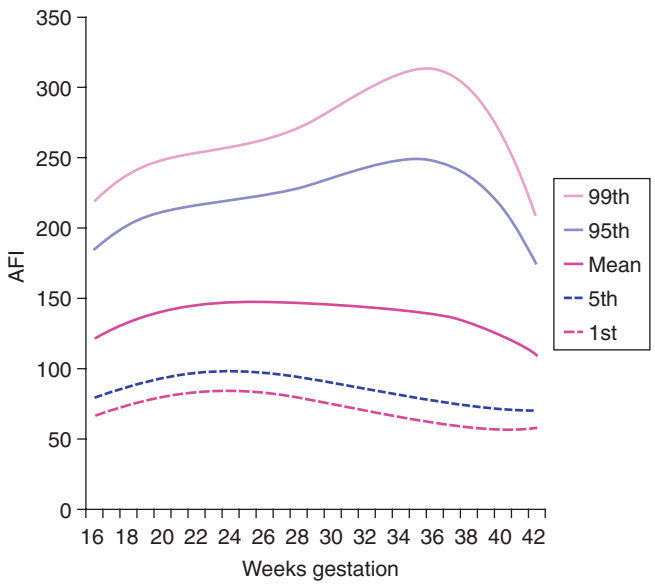
The chorioamnion acts as a porous membrane early in pregnancy, allowing the passage of water and solutes across the membrane; there is little contribution from the small embryo. As the pregnancy progresses into the late first trimester, diffusion of fluid across the fetal skin occurs, increasing the volume of amniotic fluid. In the second half of the pregnancy, the main sources of amniotic fluid come from fetal kidneys and lungs. The primary sources for removal of fluid are fetal swallowing and absorption into fetal blood perfusing the surface of the placenta. As more fluid is produced than is resorbed by the fetal–placental unit, the volume of amniotic fluid increases throughout the first 32 weeks of pregnancy (Fig. 12.4). The volume peaks at approximately 32 to 33 weeks' gestation, and at this GA, equal amounts of fluid are produced and resorbed. After term, the amniotic fluid declines at a rate of 8% per week.[61]

Because amniotic fluid plays a critical role in the normal development of a fetus, the assessment of amniotic volume is an essential component of the ultrasound evaluation for fetal well-being. Subjective estimates of the amniotic fluid volume have been validated, but two ultrasound measurements—amniotic fluid index (AFI) and maximum vertical pocket (MVP)—have been developed to quickly and accurately assess the quantity of amniotic fluid surrounding the fetus.

The AFI is a semiquantitative method for assessing the amniotic fluid volume with ultrasound. The gravid uterus is divided into four quadrants using the umbilicus, linea nigra, and external landmarks.[62] The deepest amniotic fluid pocket is measured in each quadrant with the ultrasound transducer perpendicular to the floor. The four measurements are added together, and the sum is regarded as the AFI. Pockets filled with the umbilical cord or fetal extremities should not be used for generating the AFI.[63] Researchers and clinicians have used a variety of measurements to define abnormalities in amniotic fluid volume. However, the normal range of the AFI most commonly used in clinical practice is greater than 5 to less than 24 cm of fluid. Pregnancies with an AFI of greater than 5 are described as having oligohydramnios, and pregnancies with measurements greater than 24 cm are described as having polyhydramnios.[64]

The MVP is another semiquantitative method for assessing fluid volume. The technique involves scanning the gravid uterus for the single deepest pocket of amniotic fluid that is free of the umbilical cord and fetal parts and, with the transducer perpendicular to the floor, measuring the pocket of fluid.[65] Oligohydramnios is defined as a single measurement of less than 2 cm. Polyhydramnios is defined as a single measurement greater than 8 cm. In contrast to AFI, this method can be used for both singleton and multiple gestation pregnancies. Currently, MVP is preferred to AFI for assessment of amniotic fluid volume and clinical decision-making not only because of its ease to perform but also because the use of MVP compared with AFI results in fewer obstetric interventions without a significant difference in perinatal outcome.[66]

Otherwise normal pregnancies affected by oligohydramnios are at increased risk for perinatal mortality as decreased amniotic fluid volume can be used as a proxy for declining uteroplacental perfusion and increasing placental dysfunction. Decisions to intervene in a pregnancy affected by oligohydramnios are based upon several factors such as GA, fetal condition, and maternal characteristics. First, rupture of membranes must be ruled out, as a low amniotic fluid volume in the setting of ruptured membranes is no longer predictive of poor placental perfusion. Expert opinion recommends delivery after 36 to 37 weeks' gestation in the setting of isolated and persistent oligohydramnios (MVP <2 cm) in an otherwise uncomplicated pregnancy.[67]



• **Fig. 12.4** Amniotic Fluid Index (in mm) Plotted Against Gestational Age. The curves represent percentiles. *AFI*, Amniotic fluid index.

Biophysical Profile

An NST alone might not be sufficient to confirm fetal well-being; in such cases, a BPP may be performed. The BPP refers to a sonographic scoring system performed over a 30- to 40-minute period designed to assess fetal well-being. The BPP was initially described for testing postterm fetuses but has since been validated for use in both term and preterm fetuses.[68–73] Notably, BPP is not validated for use in active labor. The five variables described in the original BPP were gross fetal body movements, fetal tone (i.e., flexion and extension of limbs), amniotic fluid volume, fetal breathing movements, and NST (summarized in Table 12.2).[74] More recently, however, BPP is interpreted without the NST.

The individual variables of the BPP become apparent in healthy fetuses in a predictable sequence: fetal tone appears at 7.5 to 8.5 weeks, fetal movement at 9 weeks, fetal breathing at 20 to 22 weeks, and FHR reactivity at 24 to 28 weeks' gestation. Similarly, in the setting of antepartum hypoxia, these characteristics typically disappear in the reverse order in which they appeared (i.e., FHR reactivity is lost first, followed by fetal breathing, fetal movements, and finally fetal tone).[72] The amniotic fluid volume, which is composed almost entirely of fetal urine in the second and third trimesters, is not influenced by acute fetal hypoxia or acute fetal CNS dysfunction. Rather, oligohydramnios (decreased amniotic fluid volume) in the latter half of pregnancy and in the absence of ruptured membranes is a reflection of chronic uteroplacental insufficiency, increased renal artery resistance leading to diminished urine output, or both;[75] it predisposes to umbilical cord compression, thus leading to intermittent fetal hypoxemia, meconium passage, or meconium aspiration. Adverse pregnancy outcome (including abnormal FHR tracing, low Apgar score, and neonatal intensive care unit admission) is more common when oligohydramnios is present.[75–78] Serial (weekly) screening of high-risk pregnancies for oligohydramnios is important because amniotic fluid can become drastically reduced within 24 to 48 hours.[79]

Although each of the five features of the BPP is scored equally (2 points if the variable is present or normal and 0 points if absent or abnormal, for a total of 10 points), they are not equally predictive of adverse pregnancy outcome. For example, amniotic fluid volume is the variable that correlates most strongly with adverse pregnancy events. The recommended management based on the BPP is summarized in Table 12.3.[74] A score of 8 to 10 out of 10 is regarded as reassuring; a score of 4 to 6 is suspicious and requires reevaluation, and a score of 0 to 2 suggests abnormal fetal testing—previously referred to as *fetal distress*.[65,68] Evidence of abnormal fetal testing or oligohydramnios in the setting of otherwise normal fetal testing should prompt evaluation for immediate delivery.[72,73]

It is important to recognize that administration of maternal systemic steroids to promote fetal maturity may result in fetal behavioral changes. Subsequent to systemic steroid administration, fetal body movements may decrease, FHR variability may decrease, and fetal breathing episodes may be less frequent.[80,81] These changes in fetal activity and FHR patterns are important to take into consideration when interpreting the various components of the fetal BPP.

TABLE 12.2 Fetal Biophysical Profile

Element	Criterion (2 Points for Each Element Satisfied)
Breathing	≥1 episode of breathing movements lasting 30 s
Movement	≥3 discrete body or limb movements
Tone	≥1 episode of active extension and flexion of limbs or trunk
Amniotic fluid	≥1 pocket of amniotic fluid measuring ≥2 cm in two perpendicular planes
NST	≥2 FHR accelerations lasting ≥15 s over 20 min

FHR, Fetal heart rate; *NST*, nonstress test.

TABLE 12.3 Interpretation and Management of Biophysical Profile

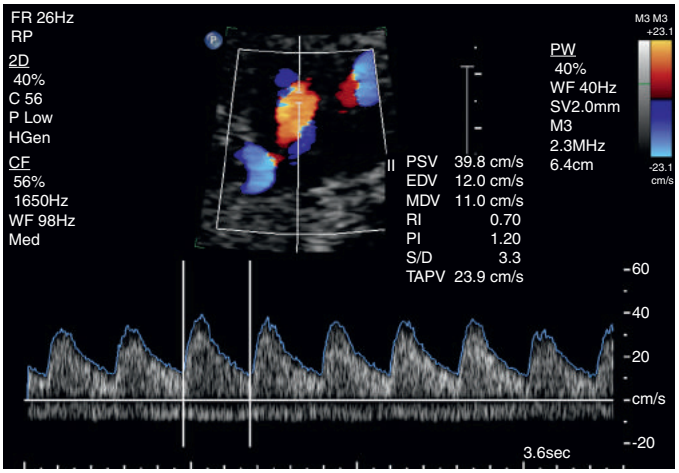
Score	Comment	Perinatal Morbidity or Mortality Within 1 Week (No Intervention)	Management
10/10	Normal	<1/1000	No intervention
8/8	Normal	—	No intervention
8/10 (abnormal NST)	Normal	—	No intervention
8/10 (abnormal amniotic fluid)	Suspect chronic fetal compromise, renal anomaly, or rupture of membranes	89/1000	Rule out renal abnormality or rupture of membranes; consider delivery or prolonged observation if dictated by gestational age
6/8 (other)	Equivocal, possible asphyxia	Variable	If fetus is mature, deliver; if immature, repeat test within 4–6 h
6/8 (abnormal amniotic fluid)	Suspect asphyxia	89/1000	Repeat 4–6 h; consider delivery
4/8	Suspect asphyxia	91/1000	If ≥36 weeks' gestation or documented pulmonary maturity, deliver immediately; if not, repeat within 4–6 h
2/8	High suspicion of asphyxia	125/1000	Immediate delivery
0/8	High suspicion of asphyxia	600/1000	Immediate delivery

NST, Nonstress test.
Adapted from Manning FA, Morrison I, Harman CR, et al. Fetal assessment based on fetal biophysical profile scoring: experience in 19,221 referred high-risk pregnancies. *Am J Obstet Gynecol.* 1987;157:880–884.

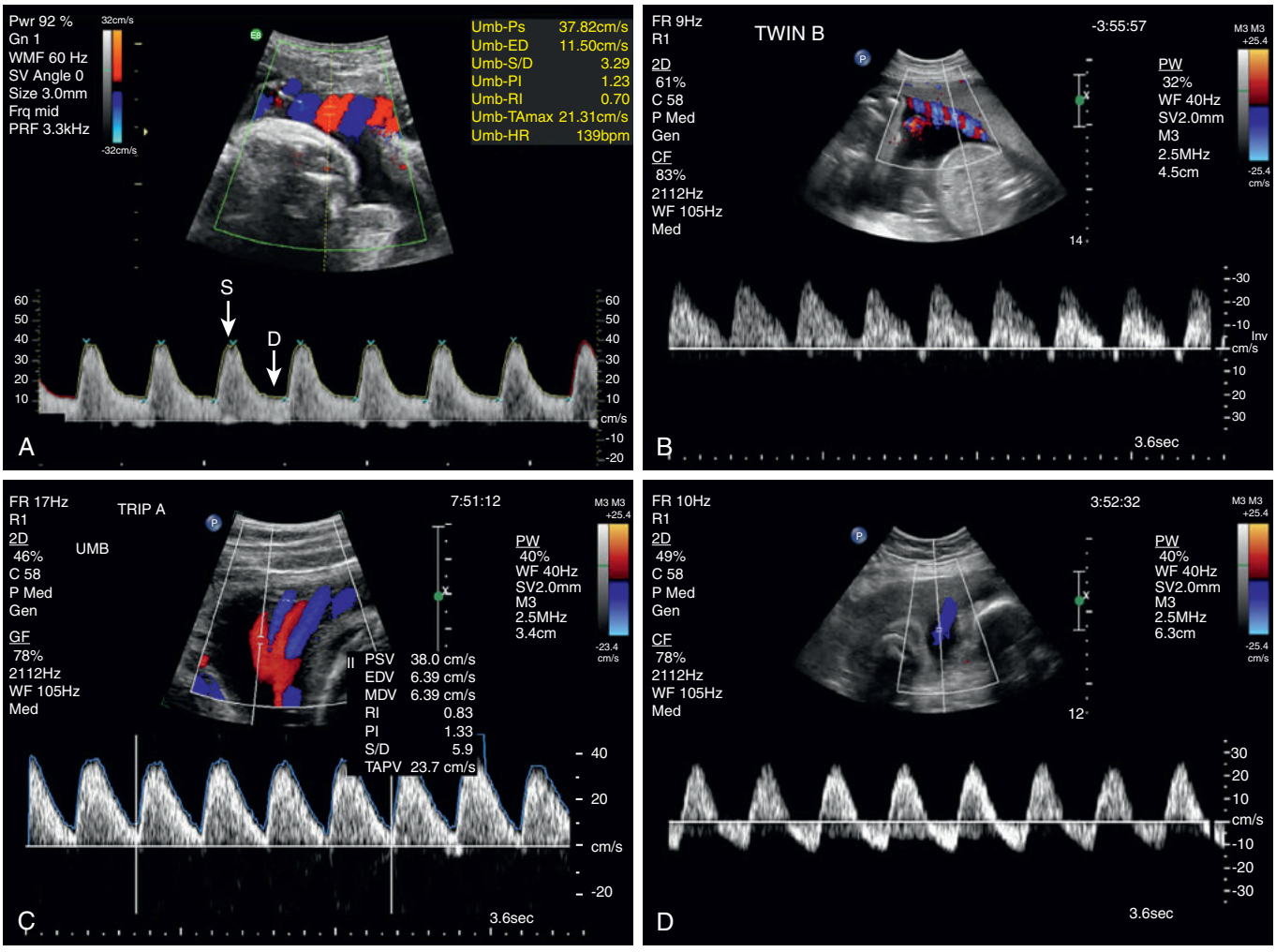
Doppler

Doppler velocimetry shows the direction and characteristics of blood flow, and it can be used to examine the maternal, uteroplacental, or fetal circulations. Because of placental capacitance, the umbilical artery is one of the few arteries that normally has forward diastolic flow, and it is one of the most frequently targeted vessels during pregnancy (Fig. 12.5). Umbilical artery Doppler velocimetry measurements reflect resistance to blood flow from the fetus to the placenta. Factors that affect placental resistance include GA, placental location, pregnancy complications (placental abruption, preeclampsia), and underlying maternal disease (chronic hypertension).

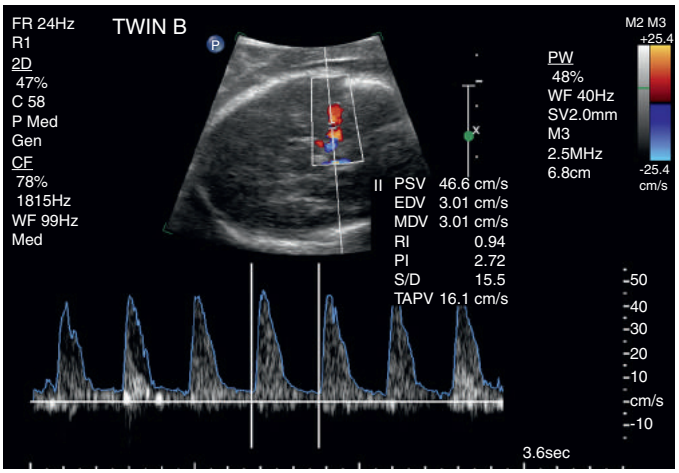
Doppler velocimetry of umbilical artery blood flow provides an indirect measure of placental function and fetal status.[82] Decreased diastolic flow with a resultant increase in systolic-to-diastolic ratio suggests increased placental vascular resistance and fetal compromise. Severely abnormal umbilical artery Doppler velocimetry (defined as absent or reversed diastolic flow) is an especially ominous observation and is associated with poor perinatal outcome, particularly in the setting of FGR (Fig. 12.6A–D).[83–88] The overall mortality rate for fetuses with absent or reversed flow may be



• **Fig. 12.5** Normal Fetal Umbilical Artery Doppler Recording. *EDV*, End diastolic velocity; *PSV*, peak systolic velocity; *S/D*, systolic/diastolic ratio.



• **Fig. 12.6** Uterine Artery Doppler to Assess Growth Restriction. Increasing levels of placental resistance as seen in the umbilical artery at the time of diastole (*D*) as it relates to systole (*S*). Normal end-diastolic flow (A), decreased end diastolic flow (B), absent end-diastolic flow (C), and reversed end-diastolic flow (D). *EDV*, End diastolic velocity; *PSV*, peak systolic velocity; *S/D*, systolic/diastolic ratio.



• **Fig. 12.7** Middle Cerebral Artery Peak Systolic Velocity for Fetal Anemia Assessment. *EDV*, End diastolic velocity; *PSV*, peak systolic velocity; *S/D*, systolic/diastolic ratio.

near 30%.[89] It should be noted that abnormal Doppler studies are often seen in cases of anatomic anomalies or chromosomal abnormalities, which should be noted when managing a case. A 2013 systematic review[90] found evidence that the use of umbilical artery Doppler ultrasound in high-risk pregnancies (FGR/suspected placental insufficiency) reduced the risk of perinatal deaths and resulted in fewer obstetric interventions.

The role of ductus venosus and middle cerebral artery (MCA) Doppler in the management of FGR pregnancies is not well defined. As such, urgent delivery should be considered in FGR pregnancies when the results of umbilical artery Doppler studies are severely abnormal, regardless of GA. However, it is unclear how to interpret these findings in the setting of a normally grown fetus. For these reasons, umbilical artery Doppler velocimetry should not be performed routinely on low-risk women. Appropriate indications include FGR, cord malformations, unexplained oligohydramnios, suspected or established preeclampsia, and possibly fetal cardiac anomalies. Umbilical artery Doppler velocimetry has not been shown to be useful in the evaluation of a variety of high-risk pregnancies, including diabetic and postterm pregnancies, primarily because of its high false-positive rate.[91–94]

As such, in the absence of FGR, obstetric management decisions are not usually made on the basis of Doppler velocimetry studies alone. An application that has proved extremely useful is the noninvasive evaluation of fetal anemia resulting from isoimmunization. When a fetus develops severe anemia, cardiac output increases and there is a decline in blood viscosity, resulting in an increase in MCA blood flow, which can be demonstrated by measuring the peak velocity using MCA Doppler velocimetry (Fig. 12.7).[95] This demonstration can help the perinatologist to better counsel such patients about the need for cordocentesis and fetal blood transfusion. Doppler studies of other vessels—including the uterine artery, fetal aorta, ductus venosus, and fetal carotid arteries—have contributed considerably to our knowledge of maternal-fetal physiology but as yet have resulted in few clinical applications.

Summary

There are a variety of testing modalities available to the obstetrician for assessing fetal well-being in the antepartum period, each with specific applications, advantages, and disadvantages. As such, it is difficult to apply generalized protocols to the assessment of the fetus. A stepwise approach entails applying the appropriate tests for low-risk patients and identifying those patients, from the results of those tests or from historical factors, for whom further testing is needed. Although many tests, including NST, fetal weight assessment, and uterine artery Doppler, may be somewhat nonspecific and may have misleading false-positive rates, combining those tests with others increases the specificity. Test results that raise concerns require further investigation or active management.

Suggested Readings

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13

Complicated Deliveries

KARA K. HOPPE AND BRAD BOSSE

KEY POINTS

- Cesarean section occurs in approximately one-third of all births in the United States, with substantial variation among hospitals that cannot be entirely accounted for by preexisting maternal or fetal comorbidities.
- Multidisciplinary team training can reduce infant morbidity after shoulder dystocia.
- Operative vaginal birth with either forceps or vacuum has declined to a multi-decade low despite the low frequency of infant complications directly attributable to this method of delivery.
- Regardless of mode of delivery, both the obstetrician and pediatrician must be aware that the infant in breech presentation requires careful attention upon birth for the presence of hip dislocation and traumatic morbidity (soft tissue trauma, fracture, facial nerve paralysis, and brachial plexus palsy).
- Twin gestations account for 3% of total births, however the twin birth rate decreased from 2018 (32.6/1000 births) to 2019 (32.1/1000 births). Twin gestations account for 17% of preterm births and approximately 25% of infants of low birth weight and very low birth weight.
- Vaginal birth after cesarean (VBAC) section should occur in delivery facilities capable of rapidly performing an emergent cesarean section because this improves the likelihood of minimizing adverse neonatal sequelae.

Overview

Historically, childbirth was often regarded as a perilous undertaking. However, over the past century in the United States, many advancements in perinatal medicine have improved modern obstetric care, such as widespread use of antibiotics, easy access to expedient cesarean delivery, and better understanding of the proper use of instruments, such as forceps and vacuum extraction.[1] Despite the improvements in obstetric care, and in contrast to the global trend in decreased maternal mortality, maternal mortality has been increasing in the United States (from 10 deaths per 100,000 livebirths in 1990 to a high of 18 deaths per 100,000 live births in 2014).[2] Indeed, adverse outcomes are generally uncommon in modern obstetrics and, unlike in the past, most labor and delivery concludes with a healthy mother and neonate. Nevertheless, complicated deliveries still exist, and knowledge of their conduct and sequelae is still required for the administration of proper maternal and infant care.

In this chapter we will address:

Brief overview of normal labor and delivery. A comprehensive discussion of labor and delivery is beyond the scope of this chapter, and the interested reader is directed to *Williams Obstetrics*, 25th ed., Chapter 22 on normal labor.[3]

The complicated vaginal delivery, with particular attention to neonatal outcomes.

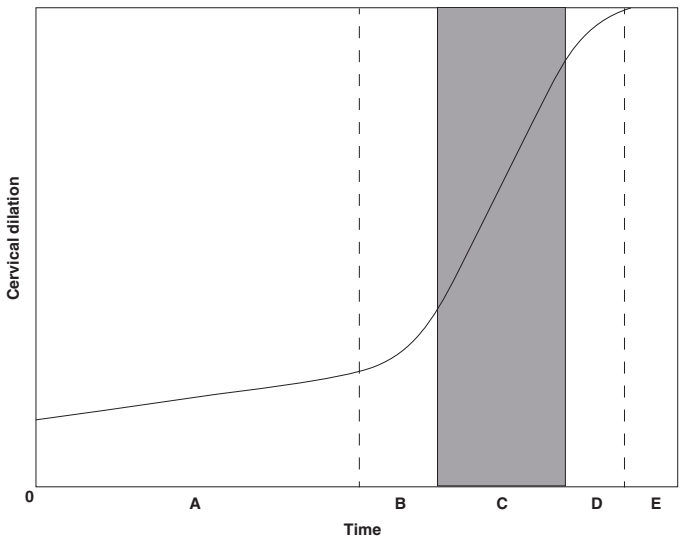
Cesarean delivery and vaginal birth after cesarean (VBAC) delivery and associated neonatal implications.

Vaginal Delivery

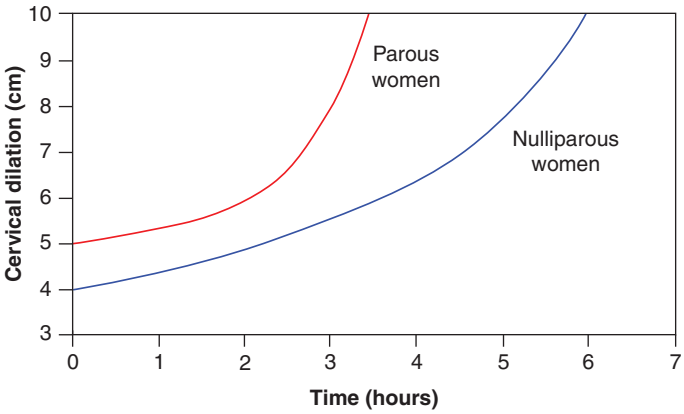
Labor begins with the onset of regular uterine contractions with concomitant cervical dilation and effacement. The first stage of labor is subdivided into a latent phase, the length of which is variable and can last for several hours, and an active phase. Historically, the active phase was defined as beginning when the cervix is dilated 4 cm and is marked by more rapid cervical dilation (Fig. 13.1).[4] The second stage begins with complete cervical dilation and terminates with expulsion of the fetus from the birth canal. The third stage of labor concludes with delivery of the placenta (Table 13.1).

In 2010, data from the Consortium on Safe Labor stimulated debate regarding whether the 60-year-old data of Friedman apply to currently laboring women.[5] Based on this research, the active phase became defined as beginning at 6 cm of cervical dilation (Fig. 13.2). In 2012, a summary was published by a joint committee including the Eunice Kennedy Shriver National Institute of Child Health and Human Development, Society for Maternal-Fetal Medicine, and American College of Obstetricians and Gynecologists (ACOG), regarding labor management guidelines largely based on the work of Zhang, and these guidelines have been largely adopted as the standard of care for labor management.[6]

Disorders of labor progress involve either protraction, with a slower rate of cervical dilation or fetal descent than expected, or arrest. Both disorders are addressed by operative delivery if they are unresponsive to active medical management; this can be performed abdominally through cesarean section or vaginally by obstetric forceps or vacuum extraction if the cervix is fully dilated and specific criteria are fulfilled (see later discussion on operative vaginal delivery). All these modalities can have neonatal and maternal adverse effects, and the choice of instrument or mode of delivery must always be selected when taking these potential morbidities into account.



• **Fig. 13.1** Friedman Labor Curve.



• **Fig. 13.2** Contemporary Labor Curve.

TABLE 13.1 Stages of Labor

Stage	
First (latent and active phase)	Onset of labor to complete (10 cm) cervical dilation
Second	Complete cervical dilation to fetal expulsion
Third	Fetal expulsion to placental expulsion

Cesarean Section

A cause-and-effect relationship between cesarean delivery and improved neonatal outcomes in the United States has never been demonstrated. Currently, almost one in three mothers gives birth by cesarean section in the United States. The rate of cesarean delivery in the United States, which peaked in 2009 at 32.9%, had declined to 31.7% in 2019.[7] Since 1985, the World Health Organization (WHO) had recommended cesarean section rates not exceed 10% to 15%. However, international data called the safety of this low projection into question, demonstrating that national cesarean section rates up to 19% were associated with lower maternal and neonatal mortality among WHO member states.[8] Recent recommendations from the US Department of Health and Human Services Healthy People 2030 have targeted a cesarean section rate of 23.6% for first-time pregnancies.

There are, however, wide variations in cesarean delivery rates among individual states within the United States[7] and among individual hospitals within a given state.[9] This cesarean section variability was shown to be 10-fold (7% to 70%) among birthing hospitals in the United States and varied from 17.5% to 31.8% among the 50 states. This suggests that factors other than pregnancy risk indicators may heavily influence the current cesarean delivery rate. Liability fears have been suggested as a leading cause of the variability in cesarean section rates. One study has lent validity to this concern, showing that higher cesarean section rates were associated with reduced risk of litigation.[10] The incidence of cesarean delivery on maternal request and its contribution to the overall increase in cesarean delivery rate are not well known, but it has been estimated that 2.5% of all births in the United States are due to maternal request for cesarean delivery. The ACOG has recommended that, in the absence of a maternal or fetal indication for cesarean delivery, vaginal delivery should be recommended. After counseling the patient about risks, benefits, and alternatives to cesarean delivery, if the patient decides to pursue an elective cesarean delivery, the delivery should not be performed before 39 weeks gestation. Women should also be informed that the risk of placental complications (which could lead to a gravid hysterectomy) will increase with each subsequent cesarean delivery.[11] This rise in cesarean delivery has been associated with a parallel drop in the vaginal operative delivery rate to less than 5%.

Cesarean section is usually performed through either a Pfannenstiel or vertical skin incision. The uterine incision is often made transversely in the lower uterine segment because it minimizes intraoperative blood loss and future risk of rupture during subsequent labor, compared with a vertical or classical incision. The risk of rupture in future labor is thought to be 0.5% to 0.9% for a low transverse incision.[12] However, risks of uterine rupture with previous classical incision have ranged from 1% to 12% in women undergoing a trial of labor.[13–15]

Cesarean delivery is also performed for disorders of protraction or arrest in the first stage of labor when conservative measures, such as oxytocin or amniotomy, fail to augment delivery or in the second stage when assisted or operative vaginal delivery is deemed unfeasible or unsafe.

A partial list of other accepted indications for cesarean delivery is as follows:

- Fetal malpresentation (e.g., shoulder or breech)
- Placenta previa
- Prior classical uterine incision
- Fetal status not reassuring, remote from vaginal delivery
- Higher-order multiple gestation (triplet or greater)
- Fetal contraindications to labor (alloimmune thrombocytopenia)
- Maternal contraindication to labor (e.g., history of rectal or perineal fistulas from inflammatory bowel disease, large lower-uterine segment, or cervical leiomyoma preventing vaginal delivery).
- Maternal choice after counseling regarding risks versus benefits.

Operative Vaginal Delivery: Obstetric Forceps and Vacuum Extraction

Description of the Obstetric Forceps

Obstetric forceps have been used to facilitate vaginal deliveries since 1500 BCE. More recently, their invention has been credited to Peter Chamberlen and his brother, both obstetricians from England. Designed originally as a means of extracting fetuses from women who were at high risk of dying during childbirth, forceps now are an alternative to cesarean delivery in women with a protracted second stage of labor. Originally, many of these instruments were furnished with hooks and other accessories of destruction, and they were intended to save the mother but not the fetus. Over the last 500 years, the modern instruments in current use have been through hundreds of modifications, safer techniques have been established, and the overriding goal now includes delivering an intact, living baby and a healthy mother.[16,17]

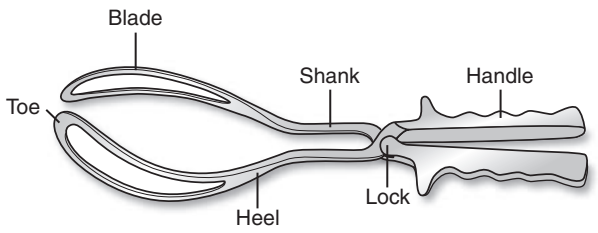
Current obstetric forceps were first devised for practical use in the 16th and 17th centuries and were perfected over the past 300 years into the models in current use. Although there are many variations on the standard blueprint, depending on the indication for its use, all obstetric forceps have a similar design.

Forceps are made of stainless steel and consist of two blades (each approximately 37.5 cm long, crossing each other), a lock at the site of crossing, and a handle, whereby the instrument is grasped by the obstetrician. The part of the forceps that grasps the fetal head is the blade; this is further divided into the heel, which is the part closest to the lock, and the toe, which is the most distal part of the blade. The blade can be either fenestrated—meaning the body of the blade is hollow—or solid to prevent fetal head compression. A further modification is pseudofenestration, in which a solid blade has a ridged edge, combining the advantages of easier applicability and reduced fetal trauma that a solid blade affords with the ease of traction of a fenestrated blade. Obstetric forceps also possess a rounded cephalic curve, which accommodates the fetal vertex, and a pelvic curve that mirrors the maternal pelvic curve (Fig. 13.3).

There are more than 60 different types of obstetric forceps described in the literature, but most of them are not used currently. The forceps used most often today are described in Table 13.2, along with their indications for use and the variations in anatomy, which distinguish one from the other.

Indications for Use of Obstetric Forceps

To an individual without obstetric training, the use of forceps can appear to be a dangerous and difficult undertaking, fraught with potential trauma for both the mother and fetus. It is true that the use of this instrument, if not performed carefully or appropriately, can have serious consequences. Nevertheless, with properly trained hands, and a proper appreciation of its use, forceps have traditionally been lifesaving for both mother and fetus.



• **Fig. 13.3** Simpson forceps: a standard obstetric forceps with features common to all such instruments.

TABLE 13.2 Types of Obstetric Forceps in Most Common Use

Type	Anatomic Modification	General Use
Classic		
Tucker–McClane	Solid blade	Nonmolded vertex
Simpson	Parallel shanks	Molded vertex or significant caput
Elliot	Convergent shanks	Nonmolded vertex
Laufe	Pseudofenestrated blade; divergent shanks	For preterm infants or EFW <2500 g
Rotational		
Kielland	English lock; absent pelvic curve	For rotation of fetal vertex ≥45°
Breech		
Piper	Long handles with no pelvic curvature	For after-coming head in breech vaginal delivery

EFW, Estimated fetal weight.

The criteria for the safe application of obstetric forceps are as follows:

- The cervix must be fully dilated.
- The position of the fetal vertex must be known. Forceps should not be applied when the fetal presentation is in doubt.

The fetal vertex must be engaged within the maternal pelvis. Often in difficult or challenging labors, significant caput can lead to the false impression that fetal station is lower than it actually is. For this reason, the obstetrician must be confident that the biparietal diameter has passed the pelvic inlet (engagement) as evidenced by the leading part of the fetal skull beyond the level of the ischial spines. In addition, when the presentation is occiput posterior, the leading point of the fetal skull may appear to be lower in the pelvis, although the biparietal diameter has not yet passed through the pelvic inlet, and can lead to an erroneous conclusion about fetal station. When the forceps are properly applied, the sagittal suture must be exactly midway between the blades, and the lambdoidal sutures should be equidistant from the edge of the blade. If these conditions are not met, the delivery with forceps should be reconsidered.

ACOG has revised its 1988 classification of the type of forceps delivery, according to the station of the fetal vertex before forceps application[17] which are as follows:

- Outlet forceps—the fetal vertex is visible at the labia without manually separating them, and the fetal skull has reached the pelvic floor.
- Low forceps—the leading point of the fetal skull is greater than 2 cm beyond the ischial spines.

- Mid forceps—the fetal head is engaged (0 to +1 station). The forceps should be applied only if cesarean delivery is not quickly or imminently possible with the fetus in distress, or there should be a high likelihood that the forceps operation will be successful.
- High forceps—the vertex is not engaged (leading part not at the level of the ischial spines or beyond). Under these circumstances, the forceps must never be applied.

The usual indications for use of the obstetric forceps are:

- Maternal exhaustion or inability to push (endotracheal intubation with sedation or paralysis, neuromuscular disease)
- Fetal heart tracing not reassuring
- Maternal contraindications to pushing (cardiopulmonary disease, cerebrovascular aneurysm)
- After-coming head in a vaginal breech delivery

Forceps and Potential Neonatal Morbidity

Forceps were used for hundreds of years without regard to fetal survival and were primarily needed to facilitate or terminate difficult labors for maternal benefit. Today, with the widespread availability of cesarean delivery, considerations turn to providing the best neonatal outcome possible; therefore, the difficult forceps deliveries of the past have largely been abandoned. Nevertheless, forceps can still play a role in modern obstetrics if judiciously used and in some circumstances can provide a safer alternative to cesarean delivery for both mother and baby.

The difficulty in interpreting the obstetric literature, in regard to neonatal morbidity incurred by forceps, is that the classification for type of forceps was revised by the ACOG in 1988; therefore, prior studies do not use the same clinical criteria used currently to select appropriate candidates for forceps use. Furthermore, residency training in operative vaginal delivery has dramatically decreased over the past 30 years, potentially increasing fetal risk. Consequently, for modern interpretation of adverse outcomes, one must look to studies performed after the 1988 ACOG revision of the classifications.

The incidence of operative vaginal delivery in the United States has declined to 3.1% of births, ranging by hospital from 1% to 23%.[7] Forceps deliveries have declined to 0.5%, while vacuum deliveries account for the majority of operative births (2.6%).[7] In a systematic review of randomized trials, failed operative delivery occurred in approximately 9% of forceps deliveries and 14% of vacuum deliveries (RR 0.65, 95% CI 0.45 to 0.94; seven trials, n-2419 deliveries).[18] In general, the failure rate increases as the station from which delivery is attempted decreases. The indication for forceps use varies widely by clinical situation, and the neonatal morbidity that can result from a "difficult pull" in a patient with a transverse arrest with marked fetal asynclitism may be different from the quick delivery of a 2600 g fetus whose mother is unable to push, even if both deliveries are by low forceps. Asynclitism refers to the position of a baby in the uterus such that the head of the fetus is presenting first and is tilted to the shoulder, causing the fetal head to no longer be in line with the birth canal.

There are few randomized prospective trials specifically addressing the issue of neonatal morbidity arising from forceps operations, and many of those are several decades old. In addition, there appears to be a substantial difference regarding frequency of operative delivery and outcomes between the United States and other English-speaking countries (UK and Australia),[19] which is likely related to higher rates of litigation in the United States.

The primary outcome that birth attendants wish to avoid when considering operative vaginal birth is birth trauma to the baby, primarily, and secondarily to the mother. A large population-based study in the United States describing over 11 million births showed that birth trauma (facial nerve injury, cephalhematoma, intracranial hemorrhage, need for mechanical ventilation) was more likely with operative vaginal birth than spontaneous birth and even higher if sequential (vacuum and forceps) operative attempts had been made.[20] When comparing forceps, vacuum, and spontaneous vaginal delivery, the rates of neonatal death were 5.0, 4.7, and 3.7/10,000 deliveries; assisted ventilation less than 30 minutes was 250, 295, and 147/10,000 deliveries, birth injury was 75.1, 109.1, and 21.4/10,000 deliveries, and neonatal seizures were 250, 293, and 147/10,000 deliveries.[20] A large population-based study in California, however, found no differences in outcome with forceps versus vacuum except for a higher incidence of facial nerve palsy with forceps delivery.[21] The absolute frequencies of birth trauma in these two large series were very similar and, although the rate of birth trauma was higher than for spontaneous vaginal birth, the overall frequencies were quite low. Table 13.3 shows the incidence of intracranial injury by delivery mode.[21]

The hazard of making such comparisons is that in most instances the decision facing the birth attendant is not operative delivery versus spontaneous birth but operative birth versus cesarean delivery. In this regard, the data from Towner provided some important information. When comparing fetal outcomes after successful operative vaginal birth, unsuccessful operative vaginal birth, and cesarean section done for labor disorder without attempt at operative vaginal birth, the latter two were similar. The primary finding of this study was that there appeared to be an irreducible number of fetal injuries associated with labor rather than the method of delivery.

The clinical dilemma that has probably changed the frequency of operative delivery in the United States also comes from data presented by Towner and Ciotti (2007)[21] as well as others.[22,23] The dilemma is what to do in the instances in which operative vaginal birth fails. Towner showed that fetal injury was higher in sequential vaginal birth, even if successful, than with successful single operative attempts. Gardella et al. (2001)[22] found the same thing and expended the excess morbidity to the mother as well. However, Towner found that the excess morbidity to the baby after failed vaginal birth was not reduced by cesarean section. Another study looking at outcomes of failed operative delivery

TABLE 13.3 Risk of Neonatal Intracranial Hemorrhage[a] According to Type of Delivery

Mode of Delivery	Incidence of Intracranial Injury
Vacuum	1 in 860
Forceps	1 in 664
Combined vacuum and forceps	1 in 256
Cesarean, in labor	1 in 907
Cesarean, not in labor	1 in 2750
Spontaneous vaginal delivery	1 in 1900

[a]Intracranial hemorrhage was defined as subdural, cerebral, intraventricular, or subarachnoid. Adapted from Towner D, Castro MA, Eby-Wilkens E, et al. Effect of mode of delivery in nulliparous women on intracranial injury. *N Engl J Med.* 1999;341:1709–1714.

also found worse neonatal outcomes, but these were nearly all in infants who had fetal heart rate abnormalities listed as either the primary or secondary reason for operative delivery.[24] The knowledge of morbidity associated with failed vaginal births by most obstetricians in the United States has appeared to have changed behavior regarding forceps attempts. In the second stage of labor, if efforts to achieve spontaneous vaginal birth are unsuccessful, cesarean delivery is increasingly offered rather than attempted vaginal birth with the risk of failure and increased fetal morbidity. In many hospitals in the United States, the percentage of labor-related cesarean sections done in the second stage of labor exceeds 40%.

Vacuum Delivery: Indications, Uses, and Comparison With Forceps Procedures

Operative vaginal delivery for the indications listed previously can also be performed by the vacuum extractor. The situations that indicate the use of the vacuum and the requirements that must be fulfilled for its correct use are identical to those for the obstetric forceps. Care must be taken in its application to ensure that an adequate seal with the fetal head has been created and that no maternal soft tissue is trapped between the suction device and the fetus. Traction is then applied to the fetal head in the line of the birth canal to assist delivery. It is also cautioned that rocking movements or torque to the cup should not be used. The premature infant (<34 weeks) is a relative contraindication to vacuum application. It is generally advised that no more than three detachments occur before attempts at vacuum extraction are abandoned.[1]

In a laboratory experiment, Duchon et al. (1988)[25] compared the maximum force at suggested vacuum pressures (550 to 600 mm Hg) prior to detachment for different types of vacuum devices. They found that the average force of traction exerted before detachment ranged from 18 to 20 kg. This result is interesting to bear in mind when one considers the older data of Wylie, who estimated the average tractive force required for delivery of infants was 15.95 kg for a primigravida and 11.33 kg for a multipara (Fig. 13.4).[26,27]

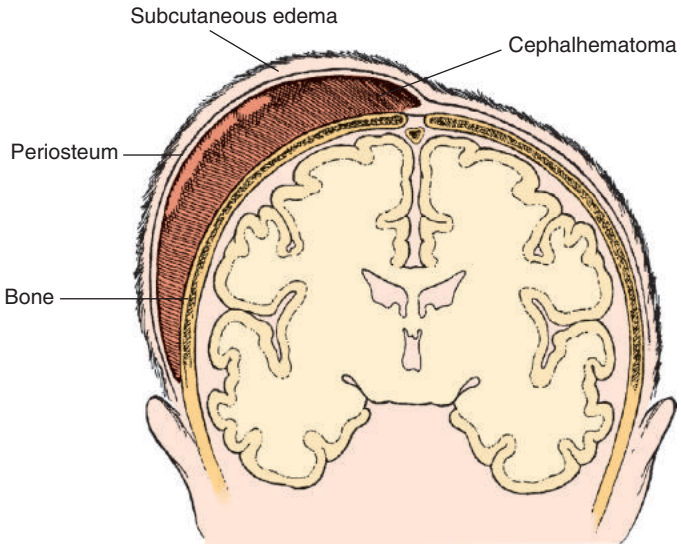
• **Fig. 13.4** A soft, bell-shaped vacuum extractor *(top)* and a rigid, mushroom-shaped vacuum extractor *(bottom).* (Courtesy Cooper Surgical, Trumbull, CT, USA.)

A 1994 metaanalysis of 1375 women in nine trials comparing soft and rigid vacuum extractor cups demonstrated that soft cups were more likely to fail to achieve a vaginal delivery, because of more frequent detachments (odds ratio [OR] 1.65, 95% confidence interval [CI] 1.19 to 2.29), but were associated with fewer scalp injuries (OR 0.45, 95% CI 0.15 to 0.60) and no increased risk of maternal perineal injury.[28] For example, the risk of scalp laceration with the rigid Kiwi OmniCup (Clinical Innovations, Murray, Utah) was reported to be 14.1%, compared with 4.5% using a standard vacuum device ($P = .006$). These and other authors concluded that handheld soft bell cups should be considered for more straightforward occiput-anterior deliveries, and that rigid M cups should be reserved for more complicated deliveries, such as those involving larger infants, significant caput succedaneum (scalp edema), occiput-posterior presentation, or asynclitism.

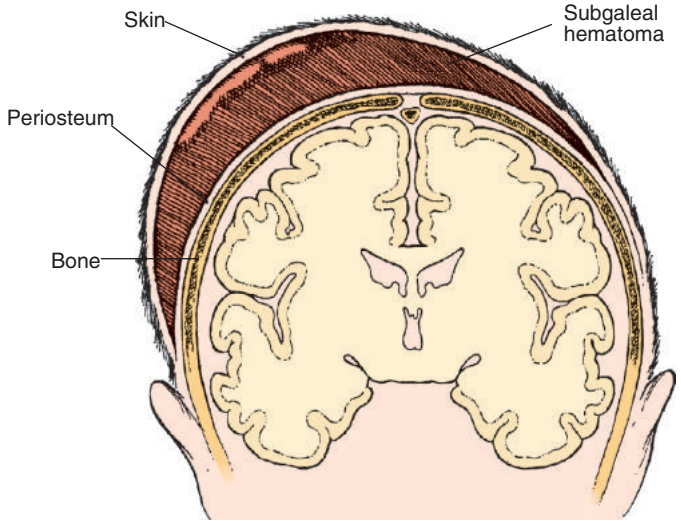
The vacuum extractor is used in the United States but is not free from neonatal injury. In addition to superficial scalp lacerations or abrasions, the use of the vacuum has been associated with cephalhematoma and subgaleal hemorrhages.

Cephalhematoma occurs when the force created by the vacuum results in the rupture of diploic or emissary vessels between the periosteum and outer table of the skull; this fills the potential space that exists between the two with blood. Although cephalhematomas are often cosmetically alarming, they are limited to traveling along one cranial bone, because the firm periosteal attachments limit further extravasation of blood across suture lines. Thus, large amounts of blood cannot usually collect in this space, and serious neonatal compromise from this bleeding is rare. In a randomized trial of continuous and intermittent vacuum application, Bofill et al. examined factors associated with increasing the risk of cephalhematoma; it was found that only asynclitism and traction time were independently related to this complication.[29] There was a clear relationship between increasing time of vacuum application (up to 6 minutes) and cephalhematoma. Interestingly, Hartley and Hitti did not find a significant independent association of neonatal injury with continuous versus intermittent vacuum, or with decreasing gestational age or increasing birth weight. Furthermore, the number of detachments was not correlated with cephalohematoma.[30] These results were further corroborated by Teng et al., who conducted a prospective observational study of 134 vacuum extractions and found that only increasing total duration of vacuum application was associated with neonatal injury.[31] Meta-analysis of randomized trials comparing vacuum to forceps extractions showed that vacuums are more likely to fail to deliver the baby and lead to increased rates of cephalhematoma and retinal hemorrhage (Fig. 13.5).[32]

Subgaleal hemorrhage poses much more risk for the neonate. It occurs when emissary veins above the skull and periosteum rupture, with blood dissecting through the loose tissue underlying the cranial aponeurosis, unimpeded by suture lines. A tremendous amount of blood, potentially the entire neonatal blood volume (approximately 250 to 350 mL), can fill this space and thus can compromise the neonate's condition.[28] Much of the literature about this rare complication of vacuum extraction was published in the 1970s and early 1980s, with few recent studies to detail associated risk factors. Plauche, in his classic paper published in 1979 on vacuum-related neonatal injury, identified only 18 cases of subgaleal hematomas among 14,276 cases of vacuum-assisted births, in contrast to a mean incidence of cephalhematoma of 6%.[33] These morbidity estimates are derived from data that are approximately 30 to 40 years old; nevertheless, Teng et al. noted an incidence of cephalhematoma of 8%, and 0.7% for subgaleal hemorrhage in



• **Fig. 13.5** A cephalhematoma is a hemorrhage that occurs under the periosteum of the skull and is thus confined to a defined space with limited capacity for expansion. (Adapted from Gilstrap LC, Cunningham FG, Hankins GDV, et al. *Operative Obstetrics*. 2nd ed. Stamford, CT: Appleton and Lange; 2002.)



• **Fig. 13.6** A subgaleal hematoma spreads along subcutaneous soft tissue planes and has no immediate barrier to expansion, creating the potential for significant neonatal hemodynamic compromise. (Adapted from Gilstrap LC, Cunningham FG, Hankins GDV, et al. *Operative Obstetrics*. 2nd ed. Stamford, CT: Appleton and Lange; 2002.)

their more recent investigation, which agrees well with Plauche's estimates.[34] The incidence in the United States in the last two decades is unknown as there was no specific Current Procedural Terminology code to distinguish subgaleal hemorrhage from cephalhematoma or other forms of minor scalp injury until after 2010.

A study from Australian investigators evaluated 37 cases of subgaleal hemorrhage at a single tertiary care center accrued over a period of 23 years, with an estimated prevalence of 1.54/10,000 total births. The finding was that this complication occurred most often in primigravidae, and that a large proportion of these infants (89.1% compared with 9.8% of the general control population) had an attempted vacuum extraction (Fig. 13.6).[35] Failed vacuum extraction has been identified as a risk factor for subgaleal hemorrhage when comparing infants born after a successful vacuum extraction (OR 7.3, 95% CI 5.5 to 9.7).[36]

The choice of which instrument to use, forceps or vacuum, is usually determined by the obstetric care provider, depending on the skill level and experience with either method. There have been several randomized trials exploring the instrument used for operative vaginal delivery.[16,37,38] In 2002, the Cochrane Library pooled the results from 10 randomized trials comparing neonatal morbidity and successful vaginal delivery between these two devices. This analysis found that the vacuum was more likely than forceps to fail (OR 1.69, 95% CI 1.31 to 2.19) and was associated with a greater likelihood of cephalhematoma (OR 2.38, 95% CI 1.68 to 3.37) and retinal hemorrhage (OR 1.99, 95% CI 1.35 to 2.98). However, the overall serious complication rate was low, and there was no difference in long-term morbidity between groups.[39]

A significant problem with the use of vacuum extraction is the difficulty some providers experience in abandoning the procedure and opting for cesarean delivery. One of the best cohort studies found that 82% of successful vacuum extractions occurred with the first three pulls.[40] When more than three pulls were necessary, signs of trauma were present in 45% of babies. Without clear progress toward vaginal delivery, as evidenced by progressive descent with each pull, attempts at further vaginal birth are not supported by the literature.

Shoulder Dystocia

Shoulder dystocia is arguably the most dreaded complication in obstetrics. The problem posed by this entity is that although it may be highly anticipated, it is also unpredictable and can appear despite the most cautious measures taken to prevent it. *Shoulder dystocia* is defined as the delivery of the fetal head with an impaction of the fetal shoulder girdle against the pubic symphysis, making subsequent delivery either difficult or impossible without performing auxiliary delivery maneuvers. In some cases, the posterior shoulder may be lodged behind the sacral promontory—a bilateral shoulder dystocia.

Once shoulder dystocia occurs, a series of maneuvers—which have never been tested in a prospective fashion, because of the sporadic and unpredictable nature of this complication—are used to resolve it. The first step to resolving shoulder dystocia is usually the McRoberts maneuver, which consists of hyperflexing the maternal thighs onto the abdomen. This maneuver flattens the pubic symphysis and sacral promontory and facilitates delivery of both the anterior and posterior shoulders. The only prospective randomized study of prophylactic McRoberts maneuver in anticipation of shoulder dystocia showed no benefit to this strategy.[41] If the McRoberts maneuver is unsuccessful, the next intervention is typically applying suprapubic pressure to remove the anterior shoulder from its impacted state behind the pubic symphysis. If these two maneuvers fail, either rotational maneuvers or extraction of the posterior fetal arm are usually tried. The Woods screw rotational maneuver or the Rubin rotational maneuver is used in an attempt to rotate the infant's shoulders to relieve the impaction of the shoulder against the pubic bone. Alternatively, delivery of the posterior arm can be accomplished by inserting the operator's hand into the vagina and grasping the fetal wrist of the posterior arm, then guiding it through the vaginal introitus. It is often necessary to perform an episiotomy to have sufficient room in the vagina to accomplish this maneuver. An alternative maneuver to fetal manipulation is the Gaskin maneuver, where the mother is

moved from the lithotomy position to a hands and knees position. Next, the posterior fetal shoulder, which is now at the 12 o'clock position, is delivered with gentle downward traction. If the dystocia remains unresolved, the Zavanelli maneuver (cephalic replacement) can be performed. After the fetal head is rotated from occiput transverse to occiput anterior, it is flexed and pushed back in the birth canal, and the child is delivered by emergent cesarean section. McRoberts maneuver, suprapubic pressure, or both will relieve shoulder dystocia in more than 50% of instances. Cephalic replacement should be rarely necessary.

Greater attention has been on enhanced practitioner training for shoulder dystocia by means of simulation.[42] Multiple studies have demonstrated multidisciplinary, simulation-based training of shoulder dystocia increases recognition of shoulder dystocia and reduced the incidence of fetal injury and brachial plexus injury.[43–45] At the current time, multidisciplinary team training with a standard management protocol and postevent debrief appears to offer the best strategy to minimize brachial plexus injury once shoulder dystocia has occurred.

The prevalence of shoulder dystocia varies depending on the population studied and the presence of various risk factors known to predispose women to this obstetric emergency. Estimates range from 0.2% to 1% in a low-risk population to 20% in higher-risk groups.[1,27,30,34] Maternal obesity, fetal macrosomia, history of prior shoulder dystocia, and maternal diabetes mellitus are the most common associated variables, but are not of sufficient prognostic power to be clinically useful in predicting shoulder dystocia.[1,34]

Because shoulder dystocia has the potential to cause significant neonatal morbidity and mortality, efforts have been made to predict its occurrence; unfortunately, no clinical guidelines have been clinically tested or proved. Ultrasound examination is commonly used in patients with suspected fetal macrosomia or diabetes to detect large birth weight infants who might be more likely to suffer shoulder dystocia. Third-trimester sonographic examination has an accuracy of ±10% to 15% in the prediction of fetal weight and is thus not highly reliable.[27,28,33] In addition, if ultrasound examination were completely reliable, the fetal weight cutoff that would prompt an elective cesarean section has not yet been determined.

If practitioners use ultrasound to evaluate estimated fetal weight (EFW), then two strategies have been suggested to avoid shoulder dystocia: labor induction or cesarean section. A recently published randomized clinical trial in France based on ultrasound evaluation of EFW has clarified the risks and benefits of labor induction to avoid shoulder dystocia and subsequent fetal injury.[45] In this trial 800 patients with EFWs greater than the 95th percentile for gestational age were randomized to either induction of labor or observation at 37 to 39 weeks gestation. At study conclusion, shoulder dystocia was reduced from 8% to 4% (OR 0.47, CI 0.26 to 0.86). Predetermined composite of fetal injury was also reduced from 6% to 2% (CI 0.15 to 0.71). There were, however, no brachial plexus injuries or deaths in either group. In addition, the occurrence of cesarean delivery was similar in both groups (28% induced versus 32% expectant management: OR 0.89, CI 0.72 to 1.09). Currently in the United States, labor induction prior to 39 weeks is an adverse quality metric and has financial consequences for both the hospital and obstetric provider. Whether this indication will gain acceptance as an exception to this quality metric remains to be determined.

Rouse et al. (1996)[46] elaborated further on the use of ultrasound to prevent shoulder dystocia. In their decision analysis, they showed that if one chose to perform an elective cesarean section for all women without diabetes but who had sonographically predicted macrosomia (EFW >4000 g), 2345 cesarean sections would need to be performed to prevent one permanent brachial plexus injury. If the 4500-g cutoff were selected, 50% more cesarean deliveries would be needed to prevent one permanent brachial plexus injury. In the mother with diabetes, if one chooses a cutoff of 4500 g or greater, 443 cesareans would need to be done to prevent one permanent injury, a trade-off that most practitioners now believe is acceptable. The conclusions from this decision analysis have been borne out by several other investigators who have established that the risk of nerve injury certainly increases with rising birth weight, but the large number of macrosomic infants who have a normal, spontaneous vaginal delivery without sequelae does not justify a policy of elective cesarean for macrosomia alone in a nondiabetic population.[37,47–49] Some believe that an EFW of greater than 5000 g provides an acceptable cutoff in nondiabetic patients to offer elective cesarean.

The ideal management of shoulder dystocia would minimize the occurrence of permanent fetal injury or death. According to one systematic review, the risk of permanent brachial plexus impairment, if recognizable at birth, was 15% to 20%.[49] In another study, a prospective investigation evaluated the natural history of recovery following a birth-related brachial plexus injury of infants referred to a tertiary care, multidisciplinary neurologic center. Enrollment required identification of injury in the newborn period, initial evaluation at the center between 1 and 2 months of age, and lack of antigravity movement in the shoulder or elbow persisting until 2 weeks of age. In this group of children subject to ascertainment bias (as those injuries resolving before 2 weeks of age would not have been included in the results), complete neurologic recovery was documented in 66%, 20% had minimal impairment, and 14% had persistent, severe weakness.[50]

At present there is no universally accepted method to prevent shoulder dystocia. A previous study demonstrated that operative vaginal delivery, especially vacuum delivery, of a fetus weighing greater than 4 kg could increase the risk of shoulder dystocia.[51] Thus it is prudent to consider avoiding difficult forceps or vacuum delivery if a patient is thought to have an infant weighing more than 4000 g, especially if she has diabetes or a past history of shoulder dystocia.

Prospective studies have shown that brachial plexus injury is related to the extent of provider-applied traction to the fetal head and neck.[52] However, there is a large variation in the provider assessment of traction used in deliveries of infants with brachial plexus injuries recognizable at birth.[52] Several studies evaluating simulated shoulder dystocia measured traction forces applied by the birth attendant.[53–55] Provider experience, gender, and body habitus were not associated with the amount of force applied during delivery. Family medicine providers applied more force than obstetrics/gynecology providers. A significant number of all providers (19/47, 40%) applied greater than 100 newtons to the fetal neck, a suggested but not proven threshold for brachial plexus injury.[55]

Currently, delivery training emphasizes that only axial traction be applied to the fetal neck, which should minimize stretch on the brachial plexus during delivery. Unfortunately, some infants are injured with minimal or no force being applied to the fetal neck, which suggests that some injuries occur as a result of a combination of labor and the standard techniques used to deliver all infants, and are therefore not provider-dependent.[55]

The documentation of the events surrounding shoulder dystocia is important, as is the discussion of the current and future

status of an infant delivered with a birth injury after shoulder dystocia. The recurrence risk of shoulder dystocia is approximately 10% to 15%, which is similar to the risk of shoulder dystocia with a known fetal weight of 4500 g. Both obstetric and pediatric providers should debrief a shoulder dystocia event immediately after the delivery. Regardless of whether fetal injury has occurred, it is optimal for both providers to discuss the delivery events and subsequent newborn treatment plans if necessary with the mother before discharge. The diagnosis of shoulder dystocia is an obstetric diagnosis made at the time of delivery, which is based upon delivery findings as defined above. There is a published update on the acute management of shoulder dystocia that can be reviewed for those readers interested in further detail on this topic.[56]

Finally, it is important to note that **shoulder dystocia is not a diagnosis that can or should be made by the pediatric team or based on observations of the neonate after delivery**.

Vaginal Breech Delivery

Three to four percent of all infants at term will present in the breech position at the time of delivery.[56,57] There are three main types of breech presentations as follows:

1. Footling breech with one (single footling) or both (double footling) lower extremities presenting.
2. Frank breech with both thighs flexed, but legs extended.
3. Complete breech with both thighs and legs flexed.

The vaginal delivery of a singleton footling breech carries attendant risks of cord prolapse and head entrapment, and the consensus among obstetricians is that this presentation should be delivered by cesarean section (unless the fetus is a second twin: see later discussion on Twin Delivery). The frank breech has a lower risk of these adverse events occurring and thus could potentially be delivered vaginally. The complete breech presentation will convert to frank or footling during labor, and the appropriate management scheme for delivery depends on which leading fetal part will descend.

The mechanics of vaginal breech delivery are as follows. The frame of reference for the presenting part is the sacrum (i.e., sacrum anterior, posterior, or transverse). In the absence of urgent fetal indications, the singleton breech is allowed to deliver passively with maternal expulsive efforts until the infant has been delivered past the umbilicus. At this point the legs are gently reduced, and the trunk and body are gently rotated to bring the sacrum anteriorly. With the appearance of the scapula below the maternal symphysis, the arms are then delivered by gently sweeping them across the chest. Every effort is then made to keep the neck from extending during the delivery of the after-coming head; this is accomplished during delivery of the body by an assistant exerting suprapubic pressure on the fetal head to keep it flexed. Once the body has delivered, the delivery of the head is accomplished by either the Mauriceau–Smellie–Veit maneuver or with Piper forceps directly applied to the fetal vertex. In the Mauriceau–Smellie–Veit maneuver, one hand extends along the posterior neck and occiput and applies pressure to prevent hyperextension, while the other hand gently applies downward traction against the maxilla to flex the head forward as the head is delivered.

The feasibility of vaginal breech delivery and its safety have been the subject of much debate throughout the past half century. With the advent of safe, expedient cesarean delivery in the United States, many obstetricians have favored cesarean section as the method of choice for management of the breech presentation at term. However, even in institutions with availability for expedient cesarean deliveries, there are situations such as precipitous delivery, out-of-hospital delivery, severe fetal anomalies, fetal death, or a mother's preference for vaginal birth that make it essential for clinicians to maintain the skills needed for a vaginal breech delivery. The literature to support this point of view has produced conflicting conclusions, and its interpretation is consequently difficult. Unfortunately, there are only two randomized trials that have explored the question of which delivery route is best for the term singleton frank breech fetus.[58,59]

Collea et al. (1980)[58] randomized 208 women with a singleton frank breech presentation at term to vaginal delivery or cesarean section; they found a low overall risk of permanent birth injury or neonatal morbidity in the vaginal delivery group, although the incidence of neonatal morbidity was higher in the vaginal delivery group. Of note, a majority of conditions listed as morbidities (hyperbilirubinemia, meconium aspiration, mild brachial plexus injury) had resolved by hospital discharge. In addition, decreased neonatal morbidity with cesarean section was offset by a striking increase in higher maternal risk in the operative group. It must be remembered that this study was published in 1980, and standards of maternal and neonatal care have changed dramatically since then.

Hannah et al. (2000)[59] published a large, multicenter, multinational trial that randomized 2088 women at 121 centers in 26 countries to planned vaginal birth or planned elective cesarean section. Criteria for enrollment were frank or complete term singleton breech with no evidence of fetal macrosomia. The investigation was halted when preliminary results showed that there were significantly increased neonatal mortality and severe morbidity in the vaginal breech arm compared with the cesarean arm (5.0% vs. 1.6%). This conclusion was not altered by the experience of the delivering obstetrician or maternal demographic factors such as parity and race. Maternal morbidity between both groups was comparable. The difference in outcome was even more striking in countries such as the United States, with a low national perinatal mortality rate (5.7% vs. 0.4%).

Criticisms of this study are that the patients enrolled did not have computed tomography (CT) pelvimetry performed, which in some institutions is standard practice before considering a vaginal breech delivery. Furthermore, subjects did not have continuous fetal monitoring, but rather intermittent fetal auscultation every 15 minutes. In addition, the capability of various centers to perform emergent cesarean sections differed markedly, and this could have potentially affected the neonatal morbidity and mortality rate. Nevertheless, it is unlikely that another large study will ever be performed to examine this issue, and the ultimate results are difficult to dispute given the excellent study design and adequate sample size.

The Term Breech Trial Collaborative Group published outcomes of children after planned cesarean birth versus planned vaginal birth for breech presentation at term. In this analysis they followed 923 of the 1159 enrolled subjects (79.6%) to 2 years of age. The risk of death or neurodevelopmental delay was no different between the planned cesarean section versus the planned vaginal groups (14 children [3.1%] vs. 13 children [2.8%]; RR 1.09, 95% CI 0.52 to 2.30).[59]

In addition, there are several large retrospective series describing neonatal outcomes with the vaginal approach, most of which suggest that vaginal delivery in carefully selected patients carries a low risk of long-term neonatal morbidity and mortality.[28,34,60–62]

A recent metaanalysis assessing the risks of planned vaginal breech delivery versus planned cesarean section for term breech birth included studies between 1993 and 2014 and included 27 articles with a total sample size of 258,953 women and reported the relative and absolute risks of perinatal mortality and morbidity in relation to mode of delivery. They reported that the absolute risks of perinatal mortality, fetal neurologic morbidity, birth trauma, a 5-minute Apgar score less than 7, and neonatal asphyxia in the planned vaginal delivery group were 0.35%, 0.7%, 0.7%, 2.4%, and 3.3%, respectively. They concluded that perinatal mortality and morbidity in planned vaginal breech delivery was significantly higher than with planned cesarean delivery, which is consistent with the aforementioned authors.[63] The truly interesting point raised by all these investigators is that, aside from the issue of cesarean versus trial of labor, singleton breech infants regardless of mode of delivery have an increased risk of morbidity compared with their vertex counterparts. Breech infants had higher incidences of neonatal intensive care unit (NICU) admissions, eventful hospital courses, hip dislocation, and traumatic morbidity (soft tissue trauma, fracture, facial nerve paralysis, and brachial plexus palsy). Thus both the obstetrician and pediatrician must be aware that the infant in breech presentation requires careful attention upon birth for the presence of these potential factors. Furthermore, the risk of developmental hip dysplasia is increased in infants born in breech presentation. The American Academy of Pediatrics has a clinical practice guideline for early detection of developmental dysplasia of the hip in these infants.[64]

Diro et al. (1999)[60] evaluated 1021 term singleton breech deliveries occurring at their institution over a 4-year period. Infants with a clinically adequate pelvis and frank breech presentation with an EFW less than 3750 g were allowed a trial of labor. They found an overall cesarean rate of 85.6%; however, for women allowed to deliver vaginally, the success rate, defined as vaginal delivery, was 50% (19 of 38 patients) for nulliparous women and 75.8% (116 of 153 patients) for multiparous women. The length of NICU stay was higher for the group delivered vaginally (17.4% vs. 12.1%; $P = .036$), but major morbidities between operative and vaginal delivery were not significantly different. Long-term outcome was not evaluated. Of note, the women in this cohort had pelvic dimensions evaluated clinically, and not by x-ray or CT pelvimetry, as has been performed in other studies.

Norwegian investigators examined the neonatal outcomes of 30,681 singleton term breech deliveries, between 1991 and 2011, identified using the Medical Birth Registry in Norway. They compared planned vaginal deliveries with planned cesarean deliveries across two time periods: January 1991 to November 2000 and November 2000 to December 2011. They identified an increase in cesarean delivery from 34.4% to 51.3% across these study periods. They also found that early neonatal mortality in the first 0 to 6 days after delivery declined from 0.10% to 0.04% ($P = .04$). During the second time period, 30.7% of term breech presentations delivered vaginally. There were eight deaths in the planned vaginal and four in the planned cesarean groups, although the difference was not statistically significant (OR 2.11, 95% CI 0.64 to 7.01). Neonatal outcomes were significantly worse in the planned vaginal delivery groups across both time periods.[62]

The delivery of the vaginal breech is an emotional issue; physicians trained in the art of the vaginal breech delivery maintain that for an appropriately selected candidate, vaginal breech delivery has acceptable neonatal risk and has the advantage of sparing the mother significant operative morbidity. Proponents of cesarean delivery further state that the level of resident training in the art of the singleton vaginal breech delivery has markedly diminished, with most graduating senior residents having performed few such births. Nonetheless, many practitioners will be required to assist in vaginal birth of a breech infant in unplanned situations. The acquisition of skills necessary to competently perform this procedure may need to be learned and practiced with simulation-based training, because the opportunities for training in most residency programs are few. ACOG has recommended that the decision regarding the mode of delivery should depend on the experience of the healthcare provider. However, cesarean delivery will be the preferred mode of delivery for most physicians because of the diminishing expertise in vaginal breech delivery.

Multifetal Delivery

Twin births account for 3% of births in the United States.[65] With the advent of assisted reproductive technologies, the incidence of multifetal pregnancies has increased, particularly higher-order multiples. The incidence of twin gestations in patients undergoing in vitro fertilization is approximately 20% in the United States,[66] and it is 1% to 3% for higher-order multiples. However, the frequency of twin gestation in the United States has most recently decreased from 2018 (32.6/1000 births) to 2019 (32.1/1000 births).[65] Of twin gestations, 80% are dizygotic and 20% are monozygotic. Twin gestations account for 17% of preterm births and approximately 25% of infants of low birth weight and very low birth weight. The perinatal mortality rate of twins is sevenfold that of singletons, of which a small fraction is due to problems associated with delivery. The mode of delivery for twins is well delineated by several studies, and the issues surrounding the choice of vaginal birth versus cesarean delivery are outlined in the following sections.[67]

Twin Delivery

Vertex–Vertex

Approximately 40% of twin gestations will be in a vertex–vertex presentation prior to delivery.[68] It is almost universally accepted that the appropriate method of delivery is vaginal if both twins are vertex. The first infant is delivered like a singleton infant. The second infant is delivered in a similar fashion, but care must be taken not to rupture membranes before the head is well engaged, because this may increase the risk of cord accident. Of note, the delivery of the second twin does not necessarily occur immediately after the first.

Vertex–Nonvertex

Vertex–nonvertex presentation occurs 38.4% of the time prior to delivery.[68] The first twin is usually delivered vaginally. The options for delivery of the second twin are as follows: cesarean section, breech extraction, or attempts at external cephalic version and vertex delivery of the second twin if successful. The optimal delivery choice for the second twin has been the subject of much controversy. Many obstetricians claim that cesarean section is the safest approach to the nonvertex twin,[69] whereas others claim that vaginal delivery affords equivalent neonatal outcome, sparing the mother from an unnecessary surgical procedure.[70] There is only one randomized trial including 60 vertex-nonvertex twin deliveries of planned vaginal delivery with plan for breech extraction of the second twin versus cesarean section in this situation. Maternal morbidity and hospital stay were increased in the surgical group, but there were no differences in neonatal outcome.[70]

Hogle et al. (2003)[71] performed a metaanalysis to determine whether a policy of planned cesarean or planned vaginal birth is preferable for twins. They found only four studies with a total of 1932 infants that met their inclusion criteria. There were no significant differences in maternal morbidity, perinatal or neonatal mortality, or neonatal morbidity between the two groups. They did find significantly fewer low 5-minute Apgar scores in the planned cesarean group, principally because of a reduction among breech first twins. They concluded that, if twin A is vertex, "there is no evidence to support planned cesarean section for twins." In contrast, Smith et al. (2005)[67] published a retrospective cohort study of 8073 twin births after 36 weeks gestation in Scotland between 1985 and 2001. There was a death of either twin in two of 1472 (0.14%) deliveries by planned cesarean and in 34/6601 (0.52%) deliveries by other means ($P = .05$; OR for planned cesarean 0.26, 95% CI 0.03 to 1.03). They concluded that planned cesarean may reduce the risk of perinatal deaths of twins at term by 75% despite the lack of statistical significance in outcomes between the two groups. The data also suffer from the fact that 30 of the 36 deaths were in second twins, and there were no data regarding fetal presentation.[72]

There are several large cohort studies examining the issue of feasibility and safety of total breech extraction of the nonvertex second twin. These studies have almost unanimously reached the similar conclusion that the neonatal outcome for nonvertex second twins delivered vaginally is similar to the vertex first twin, but is not statistically different from those second twins delivered by cesarean section, regardless of birth weight or gestational age.[1,27,28,30,33,34] Hartley and Hitti (2005)[30] conducted a retrospective analysis of birth certificates and fetal and infant death certificates for 5138 twin pairs selected from those born in Washington State from 1989 to 2001. They concluded that if prompt vaginal delivery of twin B does not occur, the benefits of vaginal delivery for twin A might not outweigh the risks of distress and low Apgar scores in twin B and vaginal plus cesarean delivery for the mother.[30]

The available body of evidence supports attempts at vaginal delivery of the nonvertex second twin. Of course, the responsible obstetrician must choose a management plan most compatible with his or her experience and training. For those not versed in the techniques of successful vaginal breech extraction, cesarean delivery might be a more prudent plan. As in the case of the singleton vaginal breech, simulation training may play a role in the acquisition and maintenance of skills for safe vaginal breech birth.[55]

In addition to cesarean delivery and total breech extraction, there is the option of external cephalic version (i.e., attempting to turn a nonvertex fetus to vertex by abdominal manipulation). Studies have shown that this option is associated with a higher failure rate at successful vaginal delivery and other complications (such as cord accident and malpresentation not amenable to vaginal delivery) when compared with primary breech extraction or cesarean section.[28,44]

If the vaginal approach is chosen, once the first twin is delivered the obstetrician inserts a hand into the uterine cavity and, under sonographic guidance if necessary, finds the feet of the second twin. Once the feet are firmly grasped, they are brought down into the vagina, and the membranes are then ruptured. Traction is applied to the fetus along the pelvic curve; once the body has been delivered through the introitus, delivery of the arms, shoulders, and after-coming head proceed in a fashion similar to that of a singleton breech.

Nonvertex–Nonvertex

The most uncommon combination for presenting position at birth is nonvertex–nonvertex, which occurs 19.1% of the time.[68] Because of the theoretical risk of interlocking twins, as well as the recent data showing the greater morbidity for the singleton vaginal breech (see the preceding discussion), cesarean section is the recommended choice for delivery of the nonvertex–nonvertex presentation.

Monochorionic, Monoamniotic Twins

Monochorionic, monoamniotic twins share a single intraamniotic space and thus have a higher risk of cord and extremity entanglement during delivery. It is commonly accepted that the optimal mode of delivery is a planned cesarean section before labor ensues.

Higher-Order Multiple Gestations

Most perinatologists would suggest cesarean delivery for triplets and higher-order multiples.[48] Although this practice is common, the data mandating cesarean delivery are far from conclusive.

A Dutch study compared the outcomes of triplets delivered vaginally and abdominally at two institutions.[50] One hospital favored cesarean section, whereas, at another, trial of labor was offered to all appropriate candidates. The success of vaginal delivery was relatively high (34 of 39 women [87%]). There was a higher incidence of neonatal mortality and postdelivery depression (as estimated by Apgar score) in the hospital favoring operative delivery compared with the vaginal delivery group. The biases inherent in this study are obvious, although the reported findings have been corroborated by several other reports from single institutions that offer trial of vaginal delivery to triplet gestations.[16,17,37] Vintzileos et al. (2005)[73] attempted to estimate the risks of stillbirth and neonatal and infant deaths in triplets, according to mode of delivery; they used the "matched multiple birth" data file that was composed of triple births that were delivered in the United States during 1995 through 1998 and found that 95% of all triplets were delivered by cesarean delivery. Vaginal delivery (all vaginal) was associated with an increased risk for stillbirth (RR 5.70, 95% CI 3.83 to 8.49) and neonatal (RR 2.83, 95% CI 1.91 to 4.19) and infant (RR 2.29, 95% CI 1.61 to 3.25) deaths. They concluded that cesarean delivery of all three triplet fetuses is associated with the lowest neonatal and infant mortality rate and that vaginal delivery among triplet gestations should be avoided.[73] Most of the data on triplet births consist of small cohort studies and not randomized trials, and the possibility of type II or beta errors exists in the interpretation of many of these studies.[27,33] Thus, delivery of triplet gestations vaginally, while not an unreasonable approach, has nearly disappeared from the practice of modern obstetrics in favor of routine cesarean delivery. Currently, quadruplets and other higher-order multiples are usually delivered by cesarean section.[74]

Vaginal Birth After Cesarean: Neonatal Concerns

The overall cesarean delivery rate in the United States increased 60% from 1996 to 2009, from 20.7% to 32.9%. Since 2009, the rate has declined slightly to 31.7% in 2019, but nearly one-third of births continue to be delivered by cesarean each year.[65] Surgery carries the maternal risks of increased blood loss, prolonged hospital stay, and longer recovery time compared with vaginal delivery.

During the 1980s to 1990s, efforts were made to encourage women to attempt a trial of labor after a prior cesarean delivery because the success rates of a VBAC are reasonable, varying from 60% to 80%, depending on the indications for the prior cesarean delivery.[75]

Whereas VBAC rates remained relatively high in the United Kingdom at 33% (range 6% to 64%), VBAC rates in the United States underwent a dramatic decrease, from a high of 28.3% in 1996 to less than 1% in 2006.[76,77] This decrease occurred primarily because VBAC can result in uterine dehiscence, in which the prior scar asymptomatically separates or, more seriously, uterine rupture occurs. In 2010, the Eunice Kennedy Shriver National Institute of Child Health and Human Development and the Office of Medical Applications of Research of NIH convened a Consensus Development Conference that sought to address the national trend away from VBAC in the United States. The conference highlighted the relative efficacy and safety VBAC and concluded that a trial of labor may be a reasonable option for appropriately selected patients.[78] As a result, VBAC rates in the United States have continued to rise in recent years, with reports as high as 13.3% in 2018, a 7% increase from 2016.[79]

A full discussion of VBAC, the studies supporting its safety, and the controversies surrounding its feasibility is beyond the scope of this chapter, and the interested reader is urged to consult *Williams Obstetrics*, 25th ed., for further details. This discussion instead focuses on neonatal risks from VBAC, particularly from its most dreaded complication, uterine rupture. There are no randomized controlled trials comparing neonatal outcomes among women undergoing a trial of labor after previous cesarean delivery versus an elective repeat cesarean delivery.

Observational studies have uniformly shown a risk of uterine rupture with VBAC on the order of 0.5% to 1%.[27,77] Feingold et al. (1988)[27] performed a large retrospective study evaluating 20,095 women with a history of prior cesarean delivery and found the following risk of uterine rupture:

- 0.16% if the woman elected for a repeat cesarean delivery
- 0.52% if VBAC occurred as a result of spontaneous labor
- 0.77% if labor was induced without prostaglandins
- 2.5% if labor was induced with prostaglandins

Thus, VBAC carries the lowest risk if labor is spontaneous. However, it is currently a well-accepted practice to induce and augment labor during a VBAC using mechanical dilation and/or oxytocin as the risk of uterine rupture remains less than 1%. The use of prostaglandins is not recommended during any portion of the labor process in patients with a prior uterine scar because of the increased risk of uterine rupture.[80]

There are few large, well-designed studies specifically evaluating neonatal rather than maternal outcomes after VBAC. Kamath et al.[81] performed a retrospective cohort study of 672 women who had one prior cesarean section and then underwent a trial of labor. They found that infants born by cesarean delivery had higher rates of admission to the NICU (9.3% compared with 4.9%) and higher rates of oxygen supplementation for delivery room resuscitation (41.5% compared with 23.2%).

Yap et al. (2001)[82] retrospectively evaluated 38,027 deliveries occurring at a single tertiary care institution and found 21 cases of uterine rupture; 17 occurred after a history of a prior cesarean delivery. The two neonatal deaths that occurred were a result of either prematurity (23-week-gestation fetus) or multiple congenital anomalies. All liveborn infants were discharged from the hospital without neurologic sequelae. Thus, the ultimate neonatal outcome despite uterine rupture was favorable. However, all deliveries occurred in a tertiary care institution with readily available obstetric anesthesiologists, neonatologists, and obstetricians. Most deliveries after diagnosis of rupture occurred within 26 minutes.

A third group of investigators retrospectively identified 99 cases of uterine rupture occurring over a period comprising 159,456 births.[28] Thirteen of these ruptures occurred before the onset of labor. There were six neonatal deaths, but four of these occurred in women with uterine rupture at admission and thus were never given a trial of labor. There were five cases of perinatal asphyxia, but once again it is not detailed whether these occurred in women allowed a trial of labor or in those who had ruptured on presentation to the hospital. Moreover, many of these women had an undocumented prior scar, which in some institutions would warrant an elective repeat cesarean section. The aforementioned study evaluating 20,095 women with a prior cesarean delivery and their subsequent risk of rupture found a neonatal mortality of 5.5%.[83] However, because this was a population-based study, it was not specified whether these deliveries occurred in tertiary care institutions with the capability of performing emergent operative rescue procedures in the event of uterine rupture.

A summary document was published by the Agency for Healthcare Research and Quality in 2010 on the infant benefits and harms after VBAC using relevant studies from 1966 to 2009.[84] The composite neonatal morbidity from elective repeat cesarean delivery compared to trial of labor after previous cesarean delivery in term infants demonstrated: antepartum stillbirth 0.21% vs 0.1%; intrapartum stillbirth 0% to 0.004% vs. 0.01% to 0.04%; hypoxic ischemic encephalopathy 0% to 0.03% vs. 0.089%; neonatal mortality 0.06% vs. 0.11%; NICU admission 1.5% to 17.6% vs. 0.8% to 26.2%; and respiratory morbidity 2.5% vs. 5.4%. In addition, in comparison to an elective repeat cesarean delivery, there is evidence that vaginal delivery after a previous cesarean delivery is associated with greater risks of long-term neurodevelopmental impairment and upper-extremity motor impairment, caused, respectively, by greater risks of perinatal hypoxic-ischemic encephalopathy and brachial plexus injury.[85] Available information does not provide a precise estimate of the relative risks for infants delivered after a trial of labor versus elective cesarean delivery.

It is appropriate to offer women VBAC, but they must be counseled carefully about the potential risk of uterine rupture. Careful documentation of the informed consent and labor management must be completed. When uterine rupture occurs, timely delivery is essential. Among 36 cases of uterine rupture, it was demonstrated that umbilical cord pH levels and 5-minute Apgar scores were normal in neonates delivered within 18 minutes after suspected uterine rupture compared to the occurrence of poor long-term outcomes when the decision-to-delivery times was greater than 30 minutes.[86] Moreover, VBAC should occur in delivery facilities capable of rapidly performing an emergent cesarean section because this improves the likelihood of minimizing adverse neonatal sequelae.[80]

Umbilical Cord Abnormalities

The term *cord accident* usually refers to adverse events affecting the fetus that occur as a result of an umbilical cord abnormality that leads to obstruction of blood flow through the umbilical cord and ultimately stillbirth. This heterogeneous term encompasses umbilical cord prolapse, in which the cord delivers through the cervix and compression by a fetal part results in a significantly increased

risk of asphyxia; it also includes such entities as compromised fetal microcirculation, cord entrapment, knots, torsions, and strictures which can lead to fetal compromise.

The incidence of in utero cord accidents is not clearly known, because the diagnosis is often one of exclusion after an in utero fetal demise. However, in one retrospective review of 496 stillbirths, 94 cases were associated with umbilical cord abnormalities: 45 (48%) had compromised fetal microcirculation; 27 (29%) had cord entrapment; 26 (27%) had knots, torsions, or stricture; and 5 (5%) had cord prolapse.[87]

Because cord prolapse is the only cord abnormality where prompt intervention can prevent fetal death, it will be the focus of the rest of this discussion. The standard of care in cases of cord prolapse is to proceed immediately with cesarean section as quickly as possible while an assistant elevates the presenting fetal part with a hand in the vagina to prevent compression of the umbilical cord. It is also of paramount importance to have appropriate pediatric support available at the time of delivery because the newborn is likely to be depressed and require resuscitation. One large, population-based study compared 709 cases of cord prolapse occurring among 313,000 deliveries to matched controls and found that low birth weight, male sex, multiple gestations, breech presentation, and congenital anomalies all increased the risk of umbilical cord prolapse.[88] Not surprisingly, cord prolapse was associated with a high neonatal mortality rate (10%), which was reduced if cesarean rather than vaginal delivery was performed.

Suggested Readings

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14

Obstetric Analgesia and Anesthesia

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KEY POINTS

- Labor results in significant pain for many women that is individualized, dynamic, and unpredictable.
- Although the effects of obstetric analgesia and anesthesia on the fetus and neonate are typically benign, there is potential for adverse neonatal effects.
- During pregnancy, labor, and delivery, women undergo fundamental changes in anatomy and physiology that affect all organ systems, significantly alter pharmacokinetic and pharmacodynamics responses to many drugs commonly used in anesthesia, and have important implication for anesthetic administration.
- Opioids are commonly used systemic medications for labor and delivery but are administered with limitations on both dose and timing because they readily cross the placenta and are associated with a risk of neonatal respiratory depression in a dose-dependent fashion.
- Neuraxial analgesia (epidural, spinal, combined spinal-epidural, and dural puncture epidural techniques) is the most widely used and most effective method for labor analgesia. Epidural labor analgesia is a catheter-based technique that provides continuous analgesia during labor using administration of medication into the epidural space. Neuraxial analgesia does not increase the risk for cesarean delivery, and with use of more current techniques is no longer associated with an increased use of instrumented vaginal delivery (forceps or vacuum).
- For the patient without an epidural catheter, spinal anesthesia is the most common regional anesthetic technique used for cesarean delivery. Use of general anesthesia for cesarean delivery is typically reserved for situations where neuraxial anesthesia is contraindicated or emergent delivery is needed.

Labor results in significant pain for many women that is individualized, dynamic, and unpredictable. Modern techniques for labor analgesia and obstetric anesthesia, essential for operative and cesarean delivery, provide an effective and safe alternative to women seeking reduced pain with childbirth. Although the effects of obstetric analgesia and anesthesia on the fetus and neonate are typically benign, there is potential for adverse neonatal effects. This chapter introduces some of the scientific background and clinical techniques used in providing obstetric analgesia and anesthesia, as well as some of the potential maternal and neonatal complications.

Anatomy of Labor Pain

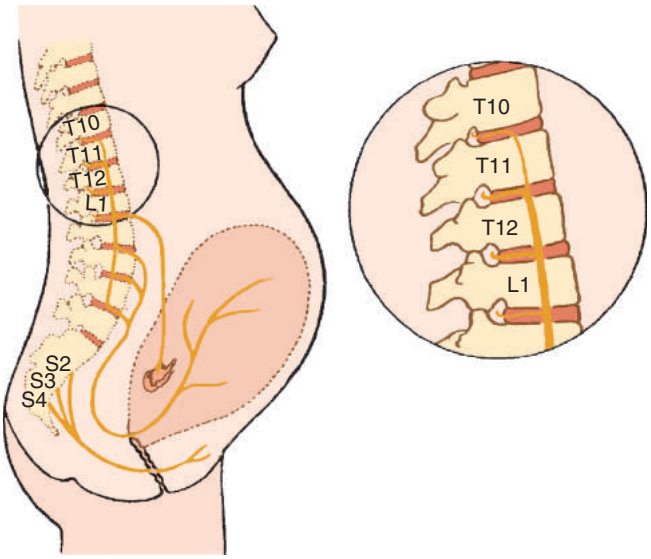
Labor is a continuous process divided into three stages. The first stage of labor begins with cervical dilation accompanied with regular uterine contractions. The second stage of labor begins when the cervix is fully dilated and concludes with the delivery of the neonate. The third stage ends with the delivery of the placenta. Contraction of the uterus, dilation of the cervix, and distention of the perineum cause pain during labor and delivery. Somatic and visceral afferent sensory fibers from the uterus and cervix travel with sympathetic nerve fibers to the spinal cord (Fig. 14.1). These fibers pass through the paracervical tissue and course with the hypogastric nerves and the sympathetic chain to enter the spinal cord at T10 to L1. During the first stage of labor (cervical dilation), the majority of painful stimuli are the result of afferent nerve impulses from the lower uterine segment and cervix, as well as contributions from the uterine body causing visceral pain (poorly localized, diffuse, and usually described as "a dull but intense aching"). These nerve cell bodies are located in the dorsal root ganglia of levels T10 to L1. During the second stage of labor (pushing and expulsion), afferents innervating the vagina and perineum cause somatic pain (well localized and typically described as "sharp"). These somatic impulses travel primarily via the pudendal nerve to dorsal root ganglia of levels S2 to S4. Pain during this stage is caused by distention and tissue ischemia of the vagina, perineum, and pelvic floor muscles, associated with descent of the fetus into the pelvis and delivery. Neuraxial analgesic techniques that block levels T10 to L1 during the first stage of labor must be extended to include S2 to S4 for effective pain relief during the second stage of labor.

Changes in Maternal Physiology and the Implications

During pregnancy, labor, and delivery, women undergo fundamental changes in anatomy and physiology. These alterations are caused by changing hormonal activity, biochemical shifts associated with increasing metabolic demands of a growing fetus, placenta, and uterus, and mechanical displacement by an enlarging uterus.[1–3]

Maternal Circulatory System

Cardiac output increases during pregnancy, reaching an output 50% greater than the prepregnant state by the third trimester. The increase in cardiac output is due in part to both increases in heart rate (15% to 25%) and stroke volume (25% to 30%).[4] During labor, maternal cardiac output increases during the first and second stages, reaching an additional 40% above prelabor values in the second stage.[5] Each uterine contraction results in the autotransfusion of 300 to 500 mL of blood back into the maternal



• **Fig. 14.1** Parturition pain pathways. Nerves that accompany sympathetic fibers and enter the neuraxis at the T10, T11, T12, and L1 spinal levels carry afferent pain impulses from the cervix and uterus. Pain pathways from the perineum travel to S2, S3, and S4 via the pudendal nerve. (From Miller RD, Pardo MC. Basics of Anesthesia. 6th ed. Philadelphia: Elsevier Saunders; 2011, Fig. 33.3.)

central circulation. The greatest increase in cardiac output occurs immediately after delivery, when values can increase as much as 75% above predelivery levels. This abrupt increase in cardiac output is secondary to the loss of aortocaval compression, autotransfusion from the contracted uterus, and decreased venous pressure in the lower extremities.[6]

Maternal systemic vascular resistance decreases 20% in normal pregnancy by term gestation. This results in a 5% to 20% decrease in systemic blood pressure starting near mid-gestation with diastolic pressures decreasing more than systolic. Reduced cardiac output and hypotension can occur when a pregnant woman is in the supine position because of aortocaval compression by the gravid uterus. Significant supine aortoiliac artery compression occurs in 15% to 20% of term pregnancies and vena caval compression is universal, often as early as 13 to 16 weeks gestation. Therefore, supine positioning is avoided during anesthetic administration in the second and third trimesters. Vena caval compression also contributes to lower extremity venous stasis and increased risk of thromboembolus. Significant lateral tilt is typically employed during cesarean delivery as 30 degrees is often required to relieve venacaval compression.[7] This improves cardiac output and preserves uterine blood flow. Because neuraxial blockade can impair compensatory increases in sympathetic tone that facilitates maintenance of blood pressure during supine positioning, maternal tilt positioning is also frequently employed with administration of neuraxial labor analgesia to help preserve uterine blood flow.

Physiologic (dilutional) anemia of pregnancy occurs as a result of a greater increase in plasma volume (45% to 55%) than in red blood cell volume (20% to 30%) by term gestation. Average blood loss at delivery—approximately 500 mL for vaginal delivery and 1000 mL for cesarean delivery—is well tolerated because of this expanded blood volume and autotransfusion (about 500 mL) from the contracted uterus after delivery.[1]

Maternal Airway and Respiratory Systems

Beginning early in pregnancy, maternal capillaries become engorged causing mucosal edema and tissue friability throughout the pharynx, larynx, and trachea. These changes can make ventilation by mask, laryngoscopy, and tracheal intubation more challenging. In addition, the presence of comorbidities such as preeclampsia, upper respiratory tract infections, and active pushing with increased venous pressure during the second stage of labor further exacerbate airway tissue edema.[8]

At term, minute ventilation is increased approximately 45% to 50%, as a result of an increase in tidal volume and a small increase in respiratory rate. In addition, oxygen consumption is increased by more than 20%, and functional residual capacity is decreased by 20%. The combination of these changes (increased oxygen consumption and decreased oxygen reserve) result in a state promoting rapid desaturation during periods of apnea. The changes in both airway and respiratory physiology during pregnancy make ventilation and intubation more difficult and increase the potential for complications.[9] A multi-institutional database of adverse obstetric anesthesia events noted rates of failed maternal intubation for cesarean delivery were approximately 1:533, although none of these 10 failed obstetric intubations resulted in maternal mortality.[10]

Maternal Gastrointestinal System

The gravid uterus increases intragastric pressure and causes the stomach and esophagus to reposition, resulting in decreased competence of the esophageal sphincter. Elevated progesterone and estrogen levels further reduce esophageal sphincter tone and placental gastrin decreases gastric pH. Consequently, most pregnant women experience symptoms of gastric reflux.[11] Furthermore, gastric emptying is delayed by active labor, pain and administration of opioids. Delayed gastric emptying and decreased competence of the esophageal sphincter cause an increased risk of pulmonary aspiration with induction of general anesthesia, which has important implications for airway management that are discussed in detail in the General Anesthesia section.

Uterine and Fetal Circulation

Uterine weight and blood flow increase throughout gestation from approximately 100 mL/min before pregnancy to approximately 700 mL/min (10% of cardiac output) at term gestation, with 80% of the uterine blood flow perfusing the intervillous space (placenta) and 20% supporting the myometrium. Uterine vasculature has limited autoregulation and remains (essentially) maximally dilated under normal conditions during pregnancy.

Maternal uterine blood flow decreases as a result of either reduced uterine arterial perfusion pressure or increased arterial resistance. Decreased perfusion pressure can result from systemic hypotension secondary to reduced cardiac preload from hypovolemia, aortocaval compression, or significant decreases in vascular resistance from the initiation of neuraxial anesthesia or induction of general anesthesia. Uterine perfusion pressure can also decrease from increased uterine venous pressure associated with vena caval compression (e.g., supine position), uterine contractions (particularly during prolonged contractions and uterine tachysystole), or significant increase in intra-abdominal pressure (pushing during second stage or seizure activity). Despite these

potential effects, phenylephrine (alpha-adrenergic) is useful for treating maternal hypotension secondary to neuraxial anesthesia, and it has been demonstrated to result in less fetal acidosis and base deficit compared to treatment with ephedrine (primarily beta-adrenergic) in many clinical trials.[12–14] If treated promptly, transient maternal hypotension does not lead to fetal depression or neonatal morbidity. Additionally, phenylephrine is the vasopressor of choice for preventing and managing hypotension during cesarean delivery.[15]

Placental and Fetal Drug Transfer

Exchange of drugs across the placenta occurs by passive diffusion, facilitated diffusion, transporter-mediated mechanisms, and vesicular transport. Most drugs less than 1000 Da molecular weight, if not ionized, cross the placenta by diffusion. The maternal drug concentration is usually the primary determinant of maternal-fetal transfer but maternal protein binding, molecular weight, lipid solubility, and drug ionization also contribute to maternal-fetal exchange. Although there is at least some placental transfer of most drugs, placental transfer of a variety of drugs is severely limited. During administration of anesthesia, drugs employed with significantly reduced placental transfer include succinylcholine, nondepolarizing muscle relaxants, heparin, and glycopyrrolate. Anesthetic drugs that readily cross the placenta include volatile anesthetic agents, opioids, and benzodiazepines. Local anesthetics and meperidine cross the placenta in a nonionized state, but once in the fetal circulation with a relatively lower pH, they become more ionized and can accumulate. (See the Neuraxial Local Anesthetics section.)

Analgesic Options for Labor and Vaginal Delivery

The pain of labor is highly variable and described by many women as severe. Factors influencing the patient's perception of labor pain include duration of labor, maternal pelvic anatomy in relation to fetal size, use of oxytocin, parity, participation in childbirth preparation classes, fear and anxiety about childbirth, attitudes about and experience of pain, and coping mechanisms. The choice of analgesic method resides primarily with the patient. The medical condition of the parturient, stage of labor, urgency of delivery, condition of the fetus, and availability of qualified personnel are also factors. Many different techniques are available to alleviate labor and delivery pain, and none appears to increase the risk of cesarean delivery.[16–19] Analgesia refers to pain relief without loss of consciousness; regional analgesia denotes partial sensory blockade in a specific area of the body, with or without partial motor blockade. Regional anesthesia is the loss of sensation, motor function, and reflex activity in a limited area of the body. General anesthesia results in the loss of consciousness, and the goals for providing general anesthesia typically include hypnosis, amnesia, analgesia, and skeletal muscle relaxation.

Techniques for labor analgesia must be safe for both mother and fetus and individualized to satisfy the analgesic requirement and desires of the parturient; they also must accommodate the changing nature of labor pain and the evolving, varied course of labor and delivery (e.g., spontaneous vaginal delivery, instrumentally assisted vaginal delivery, and cesarean delivery). The current approaches to pain relief are outlined in Box 14.1.

Nonpharmacologic Analgesia

There are a variety of nonpharmacologic techniques for labor analgesia. Although many seem to reduce labor pain perception, most lack the rigorous scientific methodology for the useful comparison of these techniques to pharmacologic methods. Although data are limited, acupuncture, acupressure, transcutaneous electrical nerve stimulation, relaxation, and massage all demonstrate a modest analgesic benefit during labor.[20,21] Other techniques such as hypnosis and intradermal water injections have not been shown to be more effective than placebo. A meta-analysis reviewing the effectiveness of a support individual (e.g., doula, friend, hospital staff, family member) noted that parturients with a support individual used fewer pharmacologic analgesia methods, had a decreased length of labor, experienced less dissatisfaction, had a lower incidence of operative or cesarean deliveries, and had improved 5-minute neonatal Apgar scores.[22]

Systemic Medications

Opioids are the only commonly used systemic medications for labor and delivery, but are administered with limitations on both dose and timing because they readily cross the placenta and are associated with a risk of neonatal respiratory depression in a dose-dependent fashion. Although pain relief from the administration of systemic opioids is frequently inadequate for the duration of labor, this option can be beneficial for short-term analgesia, particularly in early labor. Opioids are inexpensive, easy to administer, and do not require a trained anesthesia provider. However, they have a high rate of maternal side effects (sedation, respiratory depression, dysphoria, nausea, pruritus), can decrease fetal heart rate variability and fetal movements, and carry a potential risk of neonatal respiratory depression and changes in neurobehavior. Systemic administration of opioids at doses that are safe for mother and newborn provides some labor pain relief, but do not have the analgesic efficacy of regional techniques. Systemic opioids are recommended for administration in the smallest doses possible with minimization of repeated dosing to reduce the accumulation of drug and metabolites in the fetus. Larger doses would risk excessive maternal sedation, maternal respiratory depression, loss of protective airway reflexes, newborn respiratory depression, and impairment of both early breastfeeding and newborn neurobehavior.

Opioids differ in pharmacokinetics, pharmacodynamics, method of elimination, and the presence of active metabolites, but all readily cross the placental barrier through passive diffusion. Systemic opioids are most useful for patients with minimal

• BOX 14.1 Techniques for Labor Analgesia

Nonpharmacologic methods
Systemic opioids
Inhaled nitrous oxide
Regional techniques
- Epidural
- Spinal
- Combined spinal-epidural
- Dural puncture epidural
- Paracervical block
- Pudendal block

to moderate pain, precipitous labor, or contraindications to neuraxial blockade, such as a coagulopathy.

Meperidine remains the most widely used opioid worldwide for labor analgesia. Maternal half-life of meperidine is 2 to 3 hours, with the half-life in the fetus and newborn being significantly greater and more variable at values between 13 and 23 hours.[23] In addition, meperidine is metabolized to an active metabolite (normeperidine) that has a longer maternal half-life of 13 to 23 hours and significantly accumulates in the fetus after repeated doses. With increased dosing and shortened time interval between dose and delivery, there is greater neonatal risk of lower Apgar scores and newborn oxygen saturations as well as prolonged time to sustained respiration, abnormal neurobehavior, and more difficulty initiating successful breastfeeding.[24,25]

Morphine was used more frequently in the past but is rarely used today. Its onset of action is about 20 minutes with a prolonged duration of analgesia (3 to 4 hours). The half-life is longer in neonates compared with adults, and it produces significant maternal sedation. Although mostly metabolized into an inactive metabolite (morphine-3-glucuronide), about a third of administered morphine is transformed into an active metabolite (morphine-6-glucuronide) with significant analgesic properties. Obstetricians sometimes administer intramuscular morphine (10 to 15 mg) in combination with 25 mg of promethazine in latent labor to provide analgesia and facilitate rest. A recent prospective cohort study found that use of this "morphine sleep" did not impact the mode of delivery, maternal complications, or cause adverse neonatal outcomes.[26]

Fentanyl is a synthetic opioid with a short duration of action (approximately 30 minutes), no active metabolites, and a ratio of fetal to maternal plasma concentrations of approximately 1:3. In small intravenous (IV) doses of 50 to 100 μg over an hour, there were no significant differences in Apgar scores, respiratory depression, or neurobehavior scoring compared with newborns of mothers who did not receive fentanyl.[27] In addition, a comparison of equianalgesic doses of IV fentanyl compared with IV meperidine[28] demonstrated a decreased frequency of maternal nausea, vomiting, and prolonged sedation in the fentanyl group. Furthermore, neonates whose mothers received meperidine required naloxone more often compared with the fentanyl-exposed infants. There was no difference in the neuroadaptive testing scores of the two groups of infants. Fentanyl administration by a patient-controlled analgesia (PCA) device is occasionally used during labor for patients with contraindications to neuraxial analgesia. In a retrospective study, neonatal depression correlated with maternal dose even when the fentanyl PCA was terminated approximately 20 minutes prior to birth.[29]

Remifentanil, an ultra-short-acting opioid rapidly metabolized by nonspecific serum esterases, is significantly metabolized by the fetus, with umbilical artery–to–vein ratios of approximately 0.3.[30] Remifentanil administered by a PCA device is an analgesic option for women who have contraindications to neuraxial blockade. The primary benefit of choosing this rapidly metabolized drug is to minimize opioid-related side effects on the neonate. Remifentanil can be used effectively for labor with PCA dosing, but it is difficult to achieve satisfactory analgesia without potential of significant maternal respiratory depression, requires more intensive monitoring and nursing, and is therefore typically reserved for patients with contraindications to epidural anesthesia.[31] In a prospective randomized controlled trial comparing the effectiveness of epidural analgesia to a remifentanil PCA with optimized settings, epidural analgesia was significantly more effective than PCA for labor analgesia. In addition, they observed more sedation and oxygen desaturation during remifentanil analgesia, but there was no difference between groups in fetal and neonatal outcomes.[32] A meta-analysis of randomized controlled trials comparing epidural analgesia with remifentanil PCA found improved pain scores among parturients randomized to epidural analgesia.[33] A more recent equivalence trial performed between remifentanil PCA and epidural analgesia found remifentanil was inferior to epidural analgesia for satisfaction of pain relief and pain relief scores.[34] A retrospective study comparing use of remifentanil and fentanyl PCA for labor analgesia found a greater rate of transient maternal desaturation with remifentanil, but use of fentanyl resulted in greater need for assisted neonatal ventilation or administration of supplemental oxygen.[35]

Inhaled Nitrous Oxide

The use of nitrous oxide is widespread in Canada, Australia, Scandinavia, the United Kingdom, and other parts of the world, and recently its use has increased in the United States.[36] Nitrous oxide is a weak analgesic but can provide satisfactory pain relief and anxiolysis for some parturients. It is inhaled intermittently in a 50% mixture with oxygen and provides satisfactory labor analgesia for some women but is a much less effective analgesic compared to epidural analgesia. Side effects are minimal with nausea, dizziness, and drowsiness among the most common. Uterine contractility is not affected, and the degree of maternal cardiovascular and respiratory depression is very mild. At a 50% concentration (without co-administration of opioids or other sedatives), nitrous oxide is insufficient to cause unconsciousness or loss of protective airway reflexes. Nitrous oxide provides a safe analgesic option for laboring women when administered with appropriate equipment by trained personnel, essential to ensure safety (i.e., limiting the nitrous oxide concentration, avoiding administration of a hypoxic mixture, avoiding co-administration of other agents).[37] It can be used during the first, second, or third stage of labor. The effects of nitrous oxide are quickly reversed with discontinuation because of its rapid respiratory elimination, and it does not cause neonatal depression regardless of duration of administration.[38] When administered with appropriate scavenging equipment, there does not appear to be concern regarding occupational exposure.[37,39] Despite its historical use, rigorous scientific studies are lacking to adequately assess its overall efficacy, safety, and long-term effects on the fetus and newborn. Use of nitrous oxide for labor analgesia has no adverse impact on neonatal Apgar scores and there are no data to suggest adverse effects on fetal heart rate, umbilical cord gases, newborn respiration, or neonatal neurobehavioral measures.[40] Additionally, in comparing large populations with low rates of fetal nitrous oxide exposure during labor (United States) and high rates (Australia, Canada, Scandinavia, and United Kingdom), there is no evidence of cognitive differences.[41]

Neuraxial (Regional) Analgesia

Neuraxial analgesia, including epidural, spinal, dural puncture epidural (DPE), and combined spinal-epidural (CSE) techniques, is the most widely used method for labor analgesia in the United States.[42] Neuraxial techniques typically involve epidural and spinal administration of local anesthetic agents, and often the co-administration of epidural and spinal opioid analgesics. Administration of other adjuvants, such as epinephrine and clonidine, decrease the dose of local anesthetics required for

analgesia but are used much less frequently than opioids and do not appear to offer a significant advantage in most situations.[43,44] Lower blood pressures observed with use of clonidine as a neuraxial adjunctive resulted in the US Food and Drug Administration (FDA) issuing a "black box" warning for its use during labor.

Neuraxial Local Anesthetics

Local anesthetics reversibly block nerve impulse conduction via voltage-gated sodium channels. Their chemical structures are secondary or tertiary amines, which are weak bases. Local anesthetics differ in their onset, peak plasma concentration, potency, and duration based on their lipid solubility, protein binding, site of injection, and concentration. Vascular absorption of local anesthetics limits the safe dose that can be administered. Bupivacaine and ropivacaine are the most commonly used local anesthetics for neuraxial labor analgesia and are extremely safe when appropriately used for epidural or intrathecal administration. An accidental, large intravascular dose of any local anesthetic can result in local anesthetic systemic toxicity (LAST) that can lead to seizure, cardiac arrest, and/or death. If LAST occurs, administration of a lipid emulsion and initiation of advanced cardiac life support is critical to optimizing outcome.[45]

Local anesthetics have a high lipid solubility and a low ionized fraction. However, the lower pH of the fetus has the potential to increase the fraction of ionized molecules, decrease lipid solubility, and result in ion trapping. Therefore in a distressed acidotic fetus, higher concentrations of local anesthetic can accumulate (ion trapping) and result in decreased neonatal neuromuscular tone. If a direct intravascular or intrafetal injection of local anesthetic occurs, significant toxicity and depression can develop, signified by bradycardia, ventricular arrhythmia, and severe cardiac depression with acidosis.

Neuraxial Opioids

Epidural administration of lipid-soluble opioids (e.g., sufentanil, fentanyl) provides rapid analgesia equivalent to that of systemic administration but remains inferior to that of dilute concentrations of local anesthetics. When lipid-soluble opioids are co-administered with local anesthetics in the epidural space, they decrease the total local anesthetic dose required and lower the minimum local anesthetic concentration needed to achieve adequate labor analgesia.[46,47] The most common maternal side effects of conventional doses of epidural fentanyl or sufentanil are pruritus and nausea. Typical doses used for labor analgesia do not adversely affect the neonate. However, much of the opioid administered in the epidural space crosses to the maternal plasma and the potential for respiratory depression is both dose-dependent and a function of the time between administration and birth.

Subarachnoid (i.e., spinal, intrathecal) opioid injections provide effective maternal labor analgesia. Analgesic effects of spinal opioids are more potent than epidural or systemic opioid administration but are of limited duration (2 hours or less). Spinal fentanyl or sufentanil administration is often performed as part of a CSE technique (discussed in the following section). Intrathecal opioids are often combined with small doses of local anesthetic (e.g., 2.5 mg bupivacaine), decreasing the dose of opioid needed and the incidence of pruritus and nausea.[48,49] Use of high-dose intrathecal opioids (e.g., sufentanil 7.5 µg) is associated with increased risk of fetal bradycardia and severe pruritus even without the presence of hypotension.[50] The mechanism for fetal bradycardia is uncertain but may be from uterine hyperactivity following the rapid analgesia. A systematic review of studies comparing intrathecal opioids to other methods of labor analgesia noted an increase in fetal bradycardia (odds ratio [OR] 1.8; 95% CI, 1.0 to 3.1) and increased maternal pruritus (RR, 29.6; 95% CI, 13.6 to 64.6), but the risk of cesarean delivery because of FHR abnormalities was similar.[51]

Neuraxial Techniques for Labor Analgesia

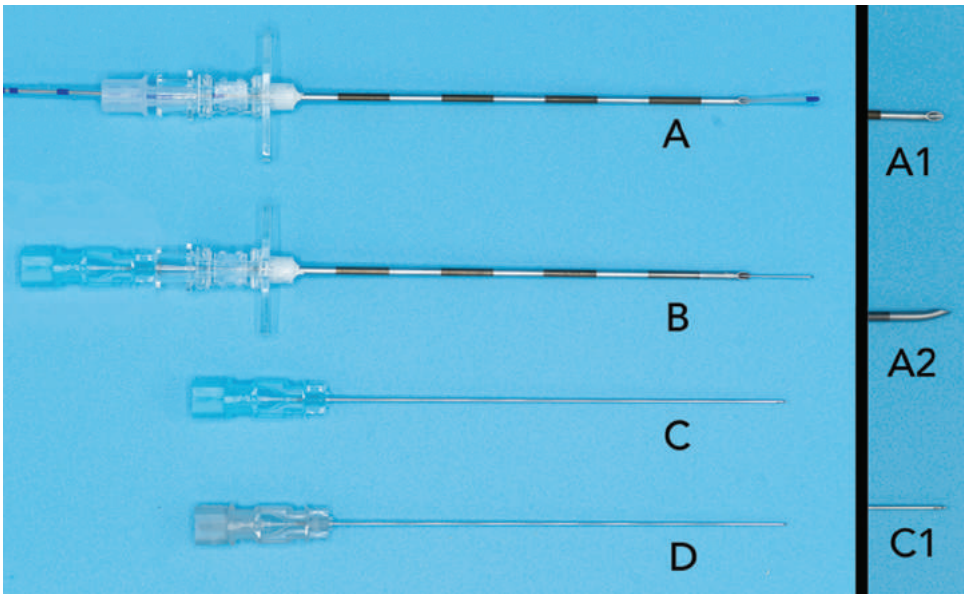
Neuraxial techniques represent the most effective form of labor analgesia, and they achieve the highest rates of maternal satisfaction.[40,52] The patient remains awake and alert without sedative side effects, maternal catecholamine concentrations are reduced, hyperventilation is avoided, cooperation and capacity to participate actively during labor are facilitated, and predictable analgesia can be achieved, superior to the analgesia provided by all other techniques.

Epidural Analgesia

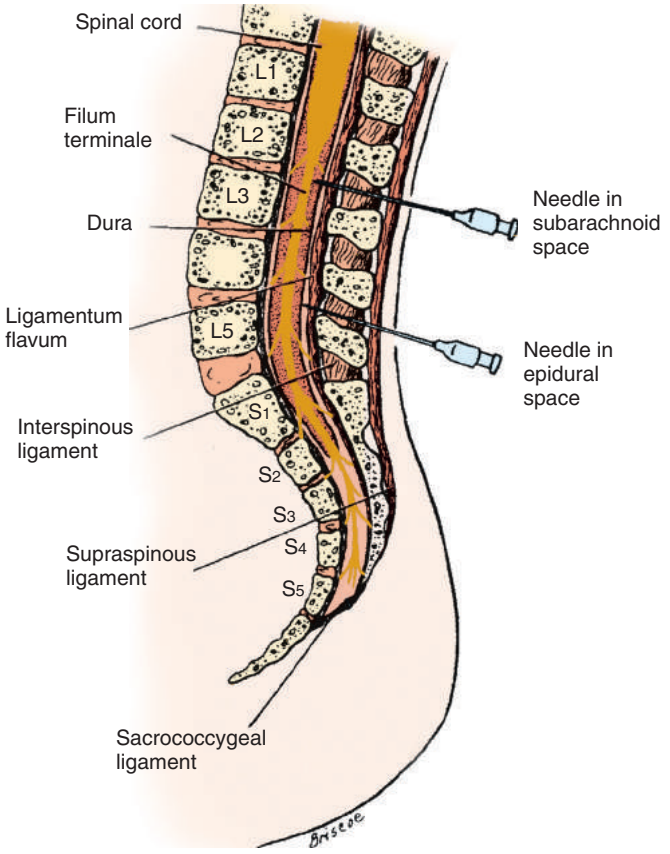
Epidural labor analgesia is a catheter-based technique that provides continuous analgesia during labor by administration of medication into the epidural space. A catheter is inserted through a specialized needle (Fig. 14.2) that is directed between vertebral spinous processes in the mid to lower lumbar region. The needle traverses the skin and subcutaneous tissues, supraspinous ligament, interspinous ligament, and the ligamentum flavum, and is advanced into the epidural space (Fig. 14.3). The tip of the needle does not penetrate the dura, which forms the boundary between the intrathecal or subarachnoid space and the epidural space. To locate the epidural space, a tactile technique called "loss of resistance" is used. The tactile resistance noted with pressure on the plunger of an air- or saline-filled syringe dramatically decreases as the tip of the needle is advanced through the ligamentum flavum (dense resistance) into the epidural space (no resistance). The epidural space has an average depth of approximately 5 cm from the skin. Once the needle is properly positioned, a catheter is inserted through the needle, and the needle is removed. The catheter is secured and analgesia is achieved by administration of local anesthetics, opioids, or both (see Neuraxial Local Anesthetics and Neuraxial Opioids sections) and maintained throughout the course of labor and delivery. The catheter can also be used for administration of anesthesia for cesarean delivery and postoperative analgesia when necessary.

After epidural placement, the catheter is aspirated to help ensure inadvertent intravascular or intrathecal placement has not occurred. Additionally, a "test dose" of lidocaine (e.g., 45 mg) and epinephrine (e.g., 15 mcg) is typically administered. If the catheter is inadvertently placed into an epidural vein, the epinephrine causes transient maternal tachycardia and the lidocaine can cause maternal tinnitus and metallic taste. If the catheter is inadvertently positioned into the subarachnoid space, the small dose of lidocaine results in the rapid onset of profound maternal analgesia and significant lower extremity weakness. By test dosing the catheter, possible disastrous complications such as total spinal anesthesia or cardiovascular collapse are significantly minimized.

Epidural medications can be administered in bolus doses or by continuous infusion. Patient-controlled epidural anesthesia (PCEA) is a delivery technique allowing the patient to self-administer small boluses of epidural analgesics with or without a background infusion. Studies comparing PCEA with continuous infusion technique have found decreased local anesthetic



• **Fig. 14.2** Photograph of typical needles and catheters used for neuraxial analgesia and anesthetic techniques. (A) Epidural needle (18-gauge Tuohy) with catheter inserted through needle and magnification of tip shown at right (A1, A2); (B) Epidural needle (Tuohy) with spinal needle (25-gauge Whitacre) inserted through needle for use in combined spinal-epidural or dural puncture epidural technique. (C) Spinal needle (25-gauge Whitacre) with magnification of tip shown at right (C1); (D) Spinal needle (27-gauge Whitacre).



• **Fig. 14.3** Schematic diagram of lumbosacral anatomy showing needle placement for subarachnoid and lumbar epidural blocks. (From Rosen MA, Hughes SC, Levinson G. Regional anesthesia for labor and delivery. In Hughes SC, Levinson G, Rosen MA, eds. *Shnider and Levinson's Anesthesia for Obstetrics*. 4th ed. Baltimore: Lippincott Williams & Wilkins; 2002:125.)

requirements, less anesthesia provider intervention, equivalent or improved patient satisfaction, equivalent or decreased motor blockade, and no significant differences in effects on the fetus or neonate.[18] Using PCEA delivery with a background infusion compared to PCEA alone further improves labor analgesia, reduces the need for clinician boluses, and does not increase maternal or neonatal adverse effects.[53]

More recently, programmed intermittent epidural bolus (PIEB) delivery provides automated fixed epidural boluses at scheduled intervals. Similar to continuous infusions, PIEB can be used alone or with a PCEA dosing technique. A recent systematic review of the literature comparing PIEB with continuous epidural infusion found PIEB reduces the risk of breakthrough pain, reduces local anesthetic usage, and improves maternal satisfaction.[54]

Dilute solutions of bupivacaine or ropivacaine are typically chosen for epidural labor analgesia, while more potent concentrations of 2% lidocaine or 3% chloroprocaine are often administered for cesarean delivery. As an example of the difference in concentrations, bupivacaine solutions of 0.1% or less are typically used for labor analgesia, while 0.5% bupivacaine would be needed to produce adequate surgical anesthesia for a cesarean delivery. Dilute solutions of local anesthetics minimize the motor blockade and preserve the perception of pelvic pressure with descent of the fetus during labor. Most practitioners routinely use low concentrations of local anesthetics with co-administration of an opioid (e.g., 2 µg/mL fentanyl) for its synergistic effect.

Effects on the Progress of Labor and Rate of Operative Delivery

The use of epidural analgesia has been associated with a prolonged second stage of labor and increased rates of assisted and cesarean delivery. A recent systematic review of the literature found neuraxial analgesia was associated with a modest increase in the mean duration

of the second stage (approximately 15 minutes) when compared to use of systemic opioids for labor analgesia.[52] This increase in the duration of the second stage is not harmful to the mother or infant and does not require intervention when the fetal status remains reassuring and labor continues to progress[55]; however, allowing a longer duration of the second stage for women who receive epidural analgesia may provide significant benefit on an individual basis.[56]

In addition, this systematic review found neuraxial labor analgesia was associated with an increased rate of instrumented vaginal delivery (RR 1.44, 95% CI 1.29 to 1.60, 30 trials, 9948 women); however, a secondary analysis of trials conducted after 2005 found this effect was no longer present (RR 1.19, 95% CI 0.97 to 1.46).[52] The use of epidural analgesia has no effect on the rate of cesarean delivery.[52] Although it was previously believed that placement of an epidural early in labor (<4 cm dilation) increased cesarean delivery rates, randomized controlled clinical trials comparing women receiving either systemic opioids or neuraxial analgesia in early labor (both spontaneous and induced), demonstrated no difference in rates of cesarean delivery.[16,17] Consequently, both American College of Obstetricians and Gynecologists (ACOG) and the American Society of Anesthesiologists guidelines recommend that maternal request is sufficient justification for epidural analgesia, and should not depend on an arbitrary amount of cervical dilation.[18,57]

Spinal Analgesia

Spinal analgesia can be administered as either a single injection or by a continuous infusion. In parturients without epidural analgesia, single injection spinal analgesia can be administered in the second stage of labor near the time of anticipated delivery. A small dose of a local anesthetic, opioid, or both is injected into the subarachnoid space. This dose of local anesthetic is far less than that used for spinal anesthesia for cesarean delivery, and it has minimal effects on motor nerve function. Compared with epidural analgesia, it has the benefits of a more rapid onset, a lower failure rate, and is technically easier and quicker to perform. It has the significant disadvantage of a finite effective duration (approximately 90 minutes). Single injection analgesia is usually reserved for multiparous women with spontaneous onset of labor and advanced cervical dilation,[58] but also can be useful in certain circumstances such as forceps-assisted delivery for a woman without epidural analgesia.

When inadvertent dural puncture occurs during epidural placement, a catheter can be inserted into the intrathecal space. Although the incidence of headache is high after dural puncture with the 17 to 19 gauge needle used for epidural catheter placement, a metaanalysis of 13 studies with 1044 obstetric patients found placement of an intrathecal catheter reduced the incidence of postdural puncture headache, when compared to relocating the epidural catheter following inadvertent dural puncture (RR = .82, $P = .018$).[59] Placement of an intrathecal catheter also decreased the need for treatment with an epidural blood patch (RR = .62, $P = .004$).[59] An intrathecal catheter provides excellent analgesia and also can be used to provide surgical anesthesia if cesarean delivery is required. However, care must be taken to ensure the spinal catheter is not mistaken for an epidural catheter since dosing errors could result in significant maternal morbidity.

Combined Spinal-Epidural Analgesia

CSE labor analgesia is a variation of neuraxial analgesia that combines the lumbar epidural technique and use of a single intrathecal dose to initiate spinal analgesia. After placement of the epidural needle, but before insertion of the epidural catheter, a spinal needle is passed through the indwelling epidural needle (see Fig. 14.2), puncturing the dura, and a small dose of local anesthetic and/or opioid is administered. Segmental analgesia results more rapidly than with epidural administration of local anesthetics. Comparing CSE to epidural labor analgesia, CSE has a faster onset of effective analgesia (especially in spread to sacral roots), decreased catheter failure for both labor analgesia and conversion to anesthesia for cesarean delivery, and increased maternal satisfaction.[40] However, CSE is associated with more pruritus and fetal heart rate changes, including fetal bradycardia with accompanying uterine hypertonus associated with higher doses of intrathecal opioids.[60–62] There is no difference seen for mode of delivery, maternal hypotension, postdural puncture headache rate, or need for blood patch.

Dural Puncture Epidural Analgesia

Similar to CSE, DPE involves use of the epidural needle to locate the epidural space and a spinal needle passed through the indwelling epidural needle to puncture the dura (see Fig. 14.2). However, no medication is administered into the intrathecal space. After the dural puncture, the spinal needle is withdrawn and a catheter is advanced into the epidural space. The dural puncture facilitates migration of epidural administered medications into the intrathecal space. The dural puncture is usually performed with a 25 g pencil-point needle since the smaller 27 g needle has not shown benefit.[63,64] Compared to an epidural without intentional dural puncture, DPE has a higher incidence of bilateral sacral analgesia, lower incidence of asymmetric block, and reduced need for top-up doses.[65] In addition, compared to the CSE technique, DPE results in less maternal pruritus, hypotension, or uterine tachysystole.

Contraindications and Complications of Neuraxial Techniques

Certain conditions contraindicate neuraxial procedures; these include patient refusal, infection at the needle insertion site, significant coagulopathy, hypovolemic shock, increased intracranial pressure from mass lesion, and inadequate provider expertise. Other conditions such as systemic infection, neurologic disease, and mild coagulopathies should be evaluated on a case-by-case basis. Human immunodeficiency virus infection is not a contraindication to regional technique in the pregnant patient.[66]

Rare but life-threatening complications can result from administration of regional anesthesia. Current rates of injury secondary to epidural catheter placement include epidural abscess/meningitis (1:63,000), epidural hematoma (1:250,000), and serious neurologic injury (1:36,000).[10] Other serious complications result from accidental IV or intrathecal injections of local anesthetics. An unintended bolus of IV local anesthetic causes dose-dependent consequences ranging from minor side effects (e.g., tinnitus, perioral tingling, mild blood pressure and heart rate changes) to major complications (e.g., LAST manifesting as seizures, loss of consciousness, severe arrhythmias, and cardiovascular collapse). The severity depends on the dose, type of local anesthetic, and preexisting condition of the parturient. Measures that can minimize the likelihood of accidental intravascular injection include careful aspiration of the catheter before injection, administration of a test dose, and incremental administration of therapeutic doses.

A "high spinal" (or total spinal) can result from an unrecognized epidural catheter placed subdural, migration of the catheter

during its use, or an overdose of local anesthetic in the epidural space (i.e., high epidural). Both high spinals and high epidurals can result in severe maternal hypotension, bradycardia, loss of consciousness, and blockade of the motor nerves to the respiratory muscles. An analysis of serious complications in over 250,000 obstetric anesthetics noted high neuraxial block (1:4300), respiratory arrest (1:10,000), and anesthesia related cardiac arrest (1:128,000).[10]

Treatment of complications resulting from both intravascular injection and high spinal is directed at restoring maternal and fetal oxygenation, ventilation, and circulation. Intubation, vasopressors, fluids, and advanced cardiac life support algorithms are often required. LAST is treated with administration of a lipid emulsion in addition to basic and advanced cardiac life support.[45] In any situation of maternal cardiac arrest with unsuccessful resuscitation, the fetus should be delivered by cesarean delivery if the mother is not resuscitated within 4 minutes of the arrest. This guideline for emergent operative delivery increases the chances of survival for both the mother and neonate.[67–69]

A variety of less severe complications such as inadequate analgesia, headache, and hypotension can occur with neuraxial blockade. The retrospective rates of inadequate epidural analgesia or inadequate CSE analgesia requiring catheter replacement were 7% and 3%, respectively, at a US academic center.[70] Interestingly, a recent meta-analysis found no difference in the need for catheter replacement between DPE and epidural techniques.[71] The rate of accidental dural puncture during epidural catheter placement is 1.5%, and approximately half of these will result in a severe headache,[72] which is typically managed with analgesics or an epidural blood patch if necessary. Hypotension (decrease in systolic blood pressure greater than 20%) secondary to sympathetic blockade is the most common complication of neuraxial blockade for labor analgesia, with rates approximately 14%.[60] Prophylactic measures include left uterine displacement and hydration. Although standards for timing, amount, and hydration fluid remain controversial, dehydration should be avoided, and there may be a reduction in hypotension following spinal anesthesia if a co-load of IV crystalloid or colloid preload is administered.[15] Hypotension treatment consists of further uterine displacement, IV fluids, and vasopressor administration. A prophylactic phenylephrine infusion and small boluses of either phenylephrine or ephedrine can be used to treat hypotension.[15] Although ephedrine (primarily β-adrenergic) was historically used, phenylephrine (primarily α-adrenergic) is associated with less fetal acidosis. If treated promptly, maternal hypotension does not lead to fetal depression or neonatal morbidity.

A rise in core maternal body temperature and fever are associated with labor epidural analgesia. Although it was originally believed that all women who had epidural analgesia gradually increased their core temperature, more current studies suggest that only about 20% of women who receive epidural labor analgesia develop a fever and the remaining 80% have no increase in core body temperature.[73] Although the etiology of the maternal temperature rise remains uncertain, an association with noninfectious inflammation mediated by proinflammatory cytokines is supported most consistently in the literature.[74] It is not associated with a change in white blood cell count or with an infectious process, and treatment is not necessary.[73] In addition, the fever associated with epidural labor analgesia does not increase the incidence of neonatal sepsis and need not affect neonatal sepsis evaluation.

Other potential side effects from neuraxial blockade during labor include pruritus, nausea, shivering, urinary retention, motor weakness, and a prolonged block.

Paracervical and Pudendal Blocks

A paracervical block is rarely used to provide pain relief during the first stage of labor and is no longer mentioned in the current ACOG guidelines on obstetric analgesia and anesthesia.[57] A paracervical block is associated with a 15% rate of fetal bradycardia,[75] which is usually limited to less than 15 minutes, and treatment is supportive. A pudendal block is infrequently used to provide pain relief during the second stage of labor at the time of delivery. In most centers this technique is used when epidural or spinal techniques are unavailable. A sheathed needle is guided to the vaginal mucosa and sacrospinous ligament just medial and posterior to the ischial spine, blocking sensation of the lower vagina and perineum. The technique can provide analgesia for vaginal delivery or uncomplicated instrumental delivery, but the rate of failure is high.[76] For parturients with cervical dilation ≥7 cm, administration of spinal analgesia with low-dose bupivacaine and fentanyl provided better labor pain relief and maternal satisfaction than administration of a pudendal block.[77] Complications include LAST, ischiorectal or vaginal hematoma, and, rarely, fetal injection of local anesthetic.

Anesthesia for Cesarean Delivery

In the United States, the vast majority of cesarean deliveries are performed with neuraxial anesthesia. It offers the advantages of less anesthetic exposure to the neonate, has the benefit of an awake mother at the delivery, allows for placement of neuraxial opioids to decrease postoperative pain, and avoids the risks of maternal aspiration and difficult airway associated with general anesthesia. However, the use of general anesthesia is sometimes required if regional anesthesia is contraindicated (e.g., coagulopathy, hemorrhage) or for emergent deliveries (e.g., fetal bradycardia, uterine rupture). Benefits of general anesthesia compared with regional anesthesia include a secure airway, controlled ventilation, rapid and reliable onset, and potential for less hemodynamic instability.

Epidural Anesthesia for Cesarean Delivery

Epidural anesthesia is an excellent choice for surgical anesthesia when an indwelling, functioning labor epidural catheter is in place. Epidural anesthesia provides the ability to titrate the desired level of anesthesia and extend the block time if needed. The volume and concentration of local anesthetic agents used for surgical anesthesia are larger than those used for labor analgesia. Typically the anesthesiologist attempts to provide a dense block from the T4 level to the sacrum. This technique does not always alleviate the visceral pain and pressure sensation associated with peritoneal manipulation, and adjuvant drugs are occasionally necessary. Epidural block failure rates for cesarean delivery following use of a labor epidural are known to be greater in the urgent setting compared to elective cases and range between 1.7% to 19.8%.[78] A systematic review of observational trials found the risk of failed labor epidural conversion to surgical anesthesia was increased with an urgent need for cesarean delivery, care by a nonobstetric anesthesiologist, and a greater number of top-up boluses.[79] In some cases,

conversion to general endotracheal anesthesia may be required. Antiemetics are frequently administered to decrease nausea and vomiting associated with the cesarean delivery and hemodynamic effects from the dense neuraxial blockade. Epidural morphine is typically administered near the end of the procedure to decrease postoperative pain for up to 24 hours.

Spinal Anesthesia for Cesarean Delivery

For the patient without an epidural catheter, spinal anesthesia is the most common regional anesthetic technique used for cesarean delivery. The block is technically easier than epidural blockade, more rapid in onset, and more reliable in providing surgical anesthesia from the midthoracic level to the sacrum.[80] The risk of profound hypotension is greater with spinal anesthesia than with epidural anesthesia because the onset of the sympathectomy is more rapid. However, this risk can be nearly eliminated by avoidance of aortocaval compression, cohydration, and appropriate use of vasopressors.[15] Data suggest that spinal anesthesia can be used safely for patients with preeclampsia.[81] A typical spinal anesthetic often consists of hyperbaric bupivacaine with fentanyl and morphine added to improve the block quality and decrease postoperative pain. A hyperbaric solution facilitates appropriate local anesthetic spread in the cerebral spinal fluid to a position near T4. The duration of a single injection spinal anesthetic is variable but normally provides adequate surgical anesthesia for greater than 90 minutes. In selected circumstances, the use of a CSE technique offers the advantage of a spinal anesthetic, with rapid onset of a dense block and the ability to administer additional local anesthetic through the epidural catheter if the procedure lasts for an extended time.

General Anesthesia

Use of general anesthesia for cesarean delivery is typically reserved for situations when emergent delivery is needed or neuraxial anesthesia is contraindicated. Based on data from 1997 to 2002, the case fatality risk ratio attributed to general anesthesia is 1.7 times that of neuraxial anesthesia, with two-thirds of the mortality associated with general anesthesia caused by intubation failure or induction problems.[82] Appropriate airway examination, preparation for unanticipated events, and familiarity with techniques and algorithms for difficult intubation are critical for providing a safe general anesthetic. A multiinstitutional database of adverse obstetric anesthesia events noted rates of failed intubation to be approximately 1:533, although none of these 10 failed obstetric intubations resulted in maternal mortality.[10]

After denitrogenation of the lungs (i.e., preoxygenation), general anesthesia is induced by rapid-sequence administration of an IV induction agent, followed by a rapidly acting muscle relaxant. The trachea is intubated with a cuffed endotracheal tube, and a surgical incision is made after confirmation of tracheal intubation and adequate ventilation. Anesthesia maintenance can include a combination of inhaled halogenated agents (e.g., sevoflurane), propofol, opioid analgesics, nitrous oxide, benzodiazepines, and additional muscle relaxants if needed. These additional IV agents are normally administered after the baby is delivered to avoid placental transfer to the neonate. Before delivery of the baby, the primary anesthetic for the incision and delivery is the IV induction agent because there is little time for uptake and distribution of the inhaled agents into the mother or fetus.[83] If intubation attempts fail, the cesarean delivery may proceed if the anesthesiologist communicates that it is possible to reliably ventilate the mother's lungs with either facemask or laryngeal mask airway.[18] Halogenated agents are often partially replaced with other anesthetic agents following delivery to decrease the adverse effects on uterine muscular tone (atony).

Induction Agents

Anesthesiologists use a variety of agents to rapidly induce unconsciousness. Among the most common are propofol, etomidate, and ketamine. Each agent represents a different biochemical class, and each has specific advantages and cardiovascular effects.

Propofol is rapid in onset with an umbilical artery to umbilical vein (UV) ratio of 0.7.[84] Propofol administration has no significant effect on neonatal behavior scores with typical induction doses of 2.5 mg/kg, but larger doses (9 mg/kg) are associated with newborn depression.[85] The lack of neonatal effects is unclear but may be caused by redistribution into maternal vascular-rich tissue beds, first-pass metabolism by the neonatal liver, additional dilution by the fetal circulation, and higher fetal brain water content. Among patients with hemodynamic compromise (e.g., maternal hemorrhage), typical doses of propofol can result in significant hypotension and decreased cardiac output.

Etomidate also has a quick onset of action because of its high lipid solubility. It rapidly crosses the placenta and rapid hydrolysis and redistribution results in a relatively short duration of action. Unlike propofol, etomidate has minimal effects on the cardiovascular system and is often used for parturients with hemodynamic instability. In addition, etomidate can cause involuntary muscle tremors, has higher rates of nausea and vomiting, and can increase the risk of seizures in patients with decreased seizure thresholds. At typical induction doses (0.3 mg/kg), etomidate administration can cause decreased neonatal cortisol production (<6 hours), but the clinical significance remains uncertain.[86]

Ketamine, a structural analogue to phencyclidine, is an analgesic, hypnotic, and amnestic with minimal respiratory depressive effects. In contrast to propofol, sympathomimetic characteristics of ketamine increase arterial pressure, heart rate, and cardiac output through central stimulation of the sympathetic nervous system. Similar to etomidate, it is an appropriate choice for a patient in hemodynamic compromise. With typical induction doses (e.g., 1.5 mg/kg) no neonatal depression is noted.[87] Larger doses can increase uterine tone and reduce uterine arterial perfusion. Even in low doses, ketamine has profound analgesic effects.

Nitrous Oxide

Inhaled nitrous oxide is often used as part of maintenance for general anesthesia because of its minimal effects on maternal hemodynamics and uterine tone. Alone, it is insufficient to provide adequate anesthesia for cesarean delivery. It rapidly crosses the placenta with increasing UV-to-maternal artery ratios of 0.37 in the first 2 to 9 minutes increasing to 0.61 at 9 to 14 minutes.[88] The effects of nitrous oxide on the neonate for such brief exposures are not significant.[37] (Additional information about nitrous oxide is found in the section Inhaled Nitrous Oxide section.)

Inhaled Halogenated Anesthetics

Isoflurane, sevoflurane, and desflurane are all halogenated hydrocarbons that differ in chemical composition, physical properties, biotransformation, potencies, and rates of uptake and elimination.

In clinical use, specialized vaporizers deliver these volatile liquid agents so that the inhaled concentrations can be carefully titrated by anesthesiologists because of the relatively profound cardiovascular effects and potential for uterine muscle relaxation. These agents are important components of general anesthesia for cesarean delivery but readily cross the placenta. Placental transfer of inhalation agents is rapid because these are nonionized, highly lipid-soluble substances of low molecular weight. The concentrations of these agents in the fetus depend directly on the concentration and duration of anesthetic in the mother. Although general anesthesia is often utilized when emergent cesarean delivery is required, neonatal depression is more likely due to the previously compromised fetus than the impact of anesthesia. A depressed fetus will likely become a depressed neonate. A Cochrane review of 16 studies comparing neuraxial blockade versus general anesthesia in otherwise uncomplicated cesarean deliveries found that "no significant difference was seen in terms of neonatal Apgar scores of six or less and of four or less at five minutes and need for neonatal resuscitation."[89] The authors concluded that there was no evidence to show that neuraxial anesthesia was superior to general anesthesia for neonatal outcome.

Experimental animal studies have demonstrated neuronal apoptosis in the developing brain when a variety of agents are administered to induce and maintain general anesthesia.[90,91] Implications for the fetus and neonate from the extremely brief anesthetic exposures associated with cesarean delivery are currently unknown because of a lack of human studies and difficulties extrapolating animal study methodology to humans. The FDA published a warning in 2016 that "repeated or lengthy use of general anesthetic and sedation drugs during surgeries or procedures in children younger than three years or in pregnant women during the third trimester may affect the development of children's brains."[92] This warning is based on concern that anesthetic or sedative drugs that bind to GABA or NMDA receptors could result in long-term effects on children's learning and behavior, although nitrous oxide was not included in the list of agents. No specific anesthetic agent (e.g., halogenated anesthetics, propofol, ketamine) is known to be safer than another, and there is no association known between use of general anesthesia for urgent cesarean delivery and neurocognitive disability later in life.[93]

For neonatal outcome, the "induction-to-delivery" interval is not as important as the uterine "incision-to-delivery" interval, during which uterine blood flow can be compromised and fetal asphyxia can occur. A long induction-to-delivery time can result in a neonate who is lightly anesthetized but should not be associated with fetal asphyxia. If inhaled anesthetics are administered to the mother for a prolonged period, neonatal anesthesia evidenced by flaccidity, cardiorespiratory depression, and decreased tone can be anticipated.[94] In such cases, the infant would be lightly anesthetized and should easily respond to basic, supportive treatment measures focused on effective ventilation; cardiopulmonary resuscitation should not be necessary. Ventilation will allow elimination of the inhalation anesthetic by the infant's lungs. Rapid improvement of the infant should be expected. Otherwise, a search for other causes of depression is imperative. For these reasons, it is critical that clinicians experienced with neonatal ventilation are present at cesarean deliveries during which the mother receives general anesthesia and the time from skin incision to delivery is expected to be prolonged (e.g., known percreta, large fibroids), or maternal condition necessitates an atypical induction and maintenance of anesthesia. A discussion of the operative and anesthetic plan by the neonatologist, obstetrician, and anesthesiologist is crucial for optimizing the outcome of neonates in these situations.

Neuromuscular Blocking Agents

Succinylcholine remains the skeletal muscle relaxant of choice for obstetric anesthesia because of its rapid onset and short duration of action. In appropriate doses (1 to 1.5 mg/kg), intubating conditions are achieved within 45 seconds. Because it is highly ionized and poorly lipid-soluble, only small amounts cross the placenta. Side effects include increased maternal potassium levels and myalgias. Succinylcholine is also a known trigger agent for malignant hyperthermia in susceptible individuals. This depolarizing neuromuscular blocking agent is normally hydrolyzed in maternal plasma by pseudocholinesterase and usually does not interfere with fetal neuromuscular activity. Although pseudocholinesterase activity is decreased in pregnancy, neuromuscular blockade by succinylcholine is not significantly prolonged. If large doses are administered (2 to 3 mg/kg), it results in detectable levels in umbilical cord blood, and extreme doses (10 mg/kg) are needed for the transfer to result in neonatal neuromuscular blockade.

Rocuronium is a rapid-acting, nondepolarizing neuromuscular blocker that is an acceptable alternative to succinylcholine. It has the benefit of not being a triggering agent of malignant hyperthermia or elevating serum potassium levels. It provides adequate intubating conditions in approximately 60 to 90 seconds in appropriate doses. Unlike succinylcholine, it has a much longer duration of action, decreasing maternal safety in the event the anesthesiologist is unable to intubate or ventilate the patient. However, with a large IV dose of sugammadex (16 mg/kg), neuromuscular block with rocuronium can be rapidly reversed.[95] Sugammadex is a α-cyclodextrin that inactivates aminosteroid nondepolarizing neuromuscular blocking drugs. Studies have found sugammadex to be effective and safe to use at the conclusion of a cesarean delivery.[96] The Society for Obstetric Anesthesia and Perinatology currently recommends avoidance in pregnant woman undergoing nonobstetric surgery.[97] Sugammadex reduces unbound progesterone in pharmacologic studies and could theoretically impact the length of pregnancy, however there is insufficient evidence regarding the safety of its use during pregnancy.

During cesarean delivery under general anesthesia, nondepolarizing neuromuscular blocking agents are typically not required but can be titrated to improve operating conditions. Under normal circumstances, the poorly lipid-soluble, highly ionized, nondepolarizing neuromuscular blockers (i.e., rocuronium, vecuronium, cisatracurium) do not cross the placenta in amounts significant enough to cause neonatal muscle weakness.[98] This placental impermeability is only relative, however, and neonatal neuromuscular blockade can be present when large or repeated doses are administered.[99]

The diagnosis of neonatal depression secondary to neuromuscular blockade can be made on the basis of the maternal history (e.g., prolonged administration of neuromuscular blockers, history of atypical pseudocholinesterase), the response of the mother to neuromuscular blocking drugs, and the physical examination of the newborn. The paralyzed neonate has normal cardiovascular function and good color, but lacks spontaneous ventilatory movements, has muscle flaccidity, and shows no reflex responses. The anesthesiologist can place a nerve stimulator on the neonate and demonstrate the classic signs of neuromuscular

TABLE 14.1 Perioperative Drugs and Breastfeeding Compatibility

Drug Class	Compatible	Limited Data[a]	Avoid
Anesthetics	Bupivacaine, etomidate, lidocaine, midazolam, nitrous oxide, propofol, ropivacaine, volatile agents	Dexmedetomidine, ketamine	Diazepam
Analgesics	Acetaminophen, fentanyl, hydromorphone,[b] morphine,[b] neuraxial opioids, NSAIDs (oral), oxycodone, remifentanil	Ketorolac	Codeine, meperidine, tramadol
Antiemetics	Dexamethasone, metoclopramide, ondansetron	Droperidol, haloperidol	
Other	Atropine, gadolinium, glycopyrrolate, iodinated contrast, NDMRs, neostigmine, succinylcholine, sugammadex[c]		

[a]Limited studies available; recommend monitoring infant for adverse effects.
[b]Close monitoring is recommended.
[c]The effects of sugammadex on breast feeding in the early postpartum period are currently unknown, and may affect the patient's ability to establish breastfeeding successfully.
Based on table in Kraus MB, Dodd SE, Sharpe EE. "Sleep and keep": dispelling myths of "pump and dump" from an anesthesiologist's perspective. *J Womens Health (Larchmt)*. 2020 Oct;29(10):1243–1245. Additional info obtained from Drugs and Lactation Database (LactMed) website, part of the National Library of Medicine's Toxicology Data Network. https://www.ncbi.nlm.nih.gov/books/NBK501922/?report=classic. Accessed June 23, 2021.
NSAIDs, Nonsteroidal antiinflammatory drugs; *NDMRs*, nondepolarizing muscle relaxants.

blockade. Treatment consists of ventilatory support until the neonate excretes the drug, up to 48 hours. Reversal of nondepolarizing relaxants with cholinesterase inhibitors may be attempted (e.g., neostigmine, 0.06 mg/kg), but adequate ventilatory support is the mainstay of treatment. Concomitant administration of an anticholinergic (e.g., atropine, glycopyrrolate) with neostigmine is normally necessary to prevent severe bradycardia from muscarinic side effects of the increased acetylcholine.

Breastfeeding and Perioperative Medications

It is considered safe for a mother to breastfeed after general and/or neuraxial anesthesia.[100] Recommendations for the safety of anesthetics are guided by pharmacologic data. Breast milk drug levels follow passive diffusion and maternal plasma concentrations. After surgery, once the patient is awake and able, she can breastfeed or pump her breasts. Resumption of normal mentation occurs once medications have redistributed from the plasma compartment (and milk). Additionally, the dose the infant receives is affected by bioavailability, and peripartum drugs that are administered intravenously to the mother have a different bioavailability when consumed orally by the infant. Medications commonly used for anesthesia and their compatibility with breastfeeding are summarized in Table 14.1. It is not recommended that women pump and discard milk after exposure to anesthetic medications.

Mothers can also safely breastfeed when standard prescribed doses of opioids are administered for postoperative analgesia after cesarean delivery. However, caution is recommended if larger opioid doses are required, the infant is premature or susceptible to apnea, or for mothers also taking other sedating medications. Meperidine and codeine carry increased risk. Meperidine use among breastfeeding mothers can result in neonatal respiratory depression; cases of neonatal cyanosis, bradycardia, and apnea have been reported.[101] (Cobb 2015). Codeine is also avoided since there is significant transfer to breast milk and infants with genetic polymorphisms are at risk for respiratory depression.[102] A resource for clinicians to obtain current information is the Drugs and Lactation Database (LactMed) website, part of the National Library of Medicine's Toxicology Data Network.[103]

Summary

This chapter serves as a general overview of the changes in maternal physiology during pregnancy and briefly discusses options and techniques for both labor analgesia and anesthesia for cesarean delivery. Its purpose is to allow the pediatrician and neonatologist a better understanding of the decisions and concerns of the anesthesiologist and the implications of his or her interventions. To provide the best care for mother and child, excellent communication is required between the obstetrician, pediatrician, anesthesiologist, and nursing staff. Only by facilitating these lines of communication and obtaining input from each discipline can patient care and safety be optimized.

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15

Perinatal Transition and Newborn Resuscitation

NOORJAHAN ALI AND TAYLOR SAWYER

KEY POINTS

- Preparation prior to delivery helps ensure timely and effective newborn resuscitation.
- Delayed umbilical cord clamping following delivery may have a significant impact on newborn outcomes.
- Establishing adequate ventilation is key to the perinatal transition.
- The laryngeal mask airway is an alternative to intubation in newborns at ≥34 weeks' gestation and > 2000 g.
- Devices such as carbon dioxide detectors, pulse oximetry, electrocardiography, and respiratory function monitors can be helpful during resuscitation.
- Postresuscitation care is critical to ensure optimal outcomes after newborn resuscitation.

The transition from fetal to neonatal life is a dramatic and complex process. Individuals who care for newborns must monitor the progress of this transition and be prepared to intervene when necessary. In most births, the perinatal transition occurs without the need for assistance. However, when assistance is needed, the presence of healthcare providers skilled in neonatal resuscitation can be lifesaving. Globally, 2.9 million neonates die every year.[1] Approximately 1 million of those babies die on the day they are born.[2] While newborn resuscitation cannot eliminate all early neonatal deaths, properly performed newborn resuscitation can save lives and reduce subsequent morbidities.[3]

Attempts to revive nonbreathing newborns have been described throughout recorded time.[4,5] In the past 50 years, significant attention has been focused on improving the scientific evidence behind the process of newborn resuscitation. Although the sophistication of resuscitation efforts has changed over time, the basic goal of inflating the newborn's lungs and initiating spontaneous breathing has remained constant.[4,5] With collaboration between the American Heart Association (AHA) and the American Academy of Pediatrics, the Neonatal Resuscitation Program (NRP) was started in 1987 to address the specific needs of the newborn.[6] The goal of the NRP is to teach healthcare providers the cognitive, technical, and behavioral skills needed for newborn resuscitation.[7]

To ensure that resuscitation methods taught in resuscitation training programs are based on the best available scientific evidence, the International Liaison Committee on Resuscitation (ILCOR) was formed. ILCOR meets on a regular basis to review the world's literature on resuscitation.[8] The findings form ILCOR reviews are translated into AHA Guidelines for Cardiopulmonary Resuscitation and Emergency Cardiovascular Care. Major changes to resuscitation guidelines generally occur every 5 years. The 2020 AHA guidelines on neonatal resuscitation[9] and the content of the 2021, 8th edition, *Textbook of Neonatal Resuscitation* are referenced throughout this chapter.[10]

Transition from Fetal to Extrauterine Life

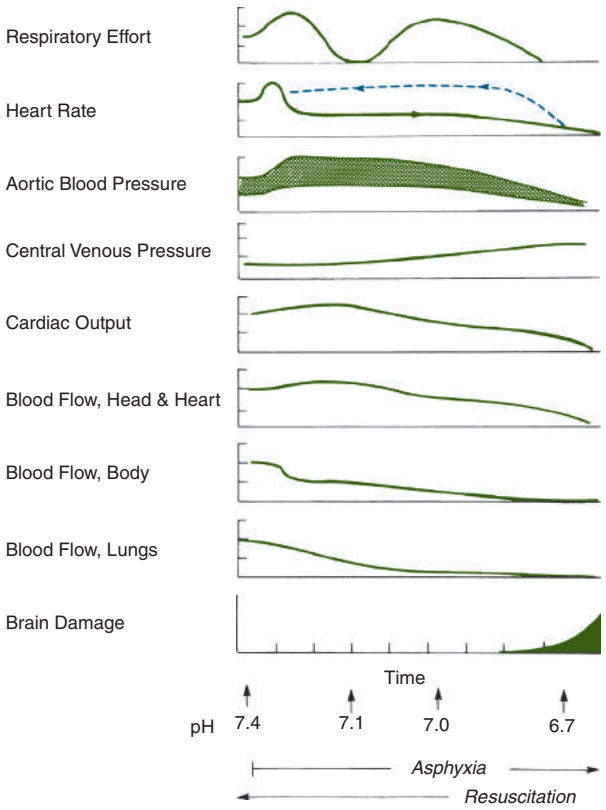
The fetus exists in the protected environment of the uterus, where temperature is closely controlled, the lungs are filled with fluid, continuous fetal breathing is not essential, and the gas exchange organ is the placenta. The transition that occurs at birth requires the newborn to increase heat production, initiate continuous breathing, replace lung fluid with air/oxygen, and significantly increase pulmonary blood flow so that gas exchange can occur in the lungs. Understanding this transitional process and knowing how to effectively assist the process helps guide the practice of newborn resuscitation. The key elements necessary for a successful transition to extrauterine life involve changes in thermoregulation, respiration, and circulation.

In utero, the fetal core temperature is approximately 0.5°C greater than the mother's temperature.[11] Heat is produced by metabolic processes and is lost through the placenta and skin.[12] After birth, the temperature gradient between the newborn and the environment becomes much greater, and heat is rapidly lost through radiation, convection, conduction, and evaporation. To adapt to the increased heat loss, the newborn must produce heat through other mechanisms, such as lipolysis of brown adipose tissue.[13] If heat is lost faster than it is produced, the newborn will become hypothermic. Preterm newborns are at particular risk of increased heat loss because of immature skin, a greater surface area to body weight ratio, and decreased brown adipose tissue stores.

The fetus lives in a fluid-filled environment, and the developing alveolar spaces are filled with lung fluid. Lung fluid production decreases in the days before delivery,[14] and the remainder of lung fluid is reabsorbed into the pulmonary interstitial spaces after delivery.[15] As the newborn takes its first breaths after birth, a negative intrathoracic pressure of approximately 50 cm H_2O is generated.[16] The alveoli become filled with air, and with the help of pulmonary surfactant, the lungs retain a small amount of air at the end of exhalation that is known as the functional residual capacity (FRC).

Although the fetus makes breathing movements in utero, these intermittent efforts mobilize fluid in physiologic dead space and are not required for fetal gas exchange. Continuous spontaneous breathing is maintained after birth by several mechanisms, including the activation of chemoreceptors, decrease in hormones that inhibit respirations, and the presence of natural environmental stimulation.

Spontaneous breathing can be suppressed at birth for several reasons, most critical being the presence of acidosis due to fetal hypoxia. Dawes described the fetal breathing response to acidosis in different animal species.[17] The physiologic effects that occur with worsening acidosis are shown in Fig. 15.1. When the pH is decreased, animals have a relatively short period of apnea followed by gasping. The gasping rate then increases until breathing ceases again for a second period of apnea. The first apnea period (e.g., "primary apnea") can be reversed with tactile stimulation. The second apnea period (e.g., "secondary apnea") requires assisted ventilation to establish spontaneous breathing. At birth, the timing of the onset of acidosis is unknown, and therefore apnea in the newborn could be either primary or secondary. Therefore, tactile stimulation should be attempted in the presence of apnea at birth to resolve primary apnea, but if it is not quickly successful, assisted ventilation should be started to resolve secondary apnea. In the absence of acidosis, a newborn can develop apnea because of exposure to respiratory-suppressing medications such as maternal anesthetics, narcotics, and magnesium.



• **Fig. 15.1** The sequence of cardiopulmonary changes with asphyxia and resuscitation. Time is on the horizontal axis. Asphyxia progresses from left to right; resuscitation proceeds from right to left. Units of time are not given. If there is complete interruption of respiratory gas exchange, the entire process of asphyxia from extreme left to right could occur in approximately 10 minutes. It could take much longer with an asphyxiating process that only partly interrupts gas exchange or does so completely but only for repeated brief periods. With resuscitation, the process reverses, beginning at the point to which asphyxia has proceeded. The blue dotted line is the reversal of asphyxia with resuscitation. (Modified from Dawes G. *Foetal and Neonatal Physiology*. Chicago: Year Book; 1968; and Avery GN. *Neonatology*. Philadelphia: JB Lippincott; 1987.)

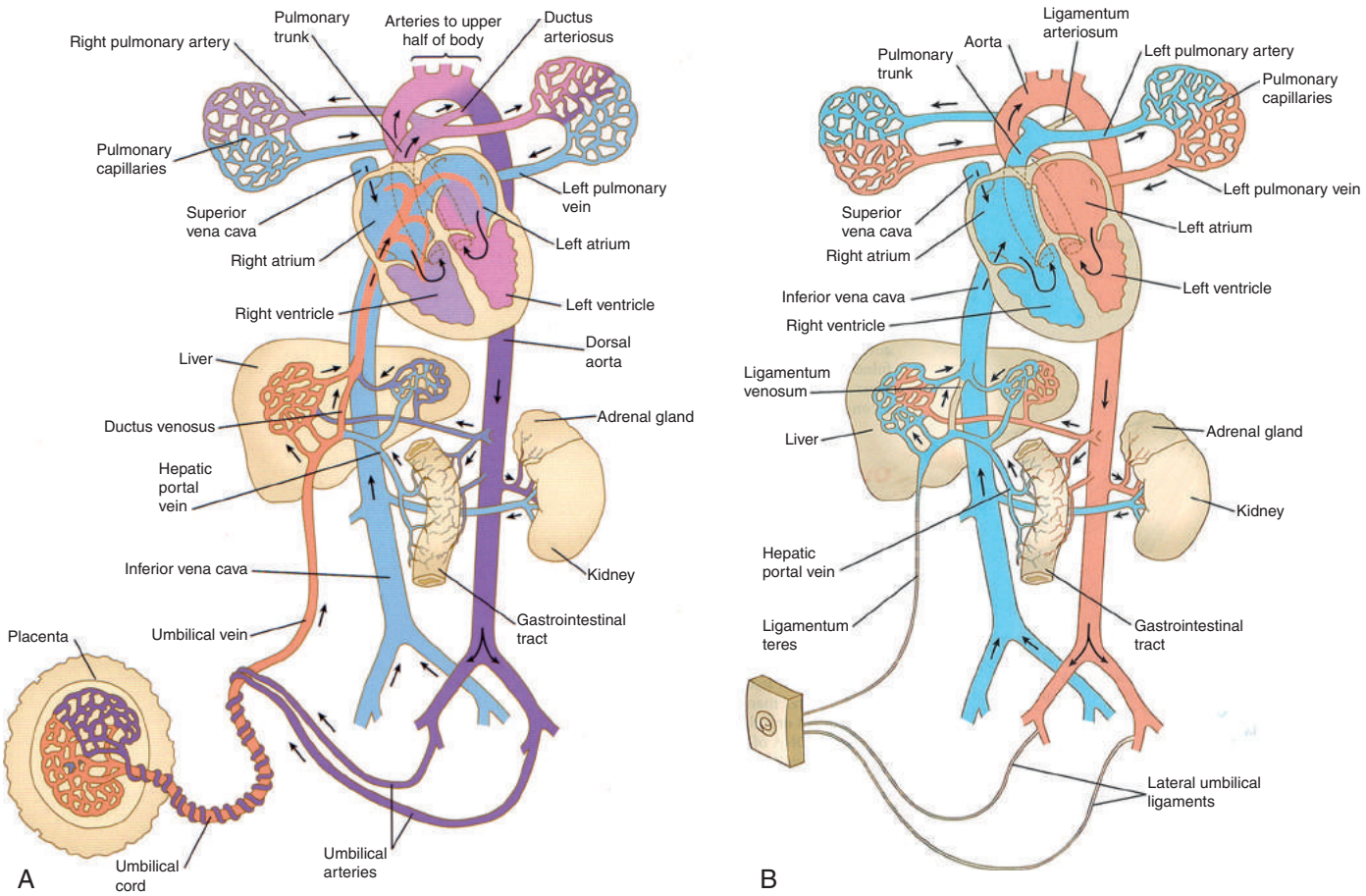
The fetal circulation is unique because gas exchange occurs in the placenta. Fig. 15.2 outlines the changes from fetal to neonatal circulation around the time of birth. Fetal channels, including the ductus arteriosus and foramen ovale, allow most of the blood flow to bypass the lungs. In the fetus, pulmonary blood flow is approximately 8% of the total cardiac output. With the closure of the ductus arteriosus and foramen ovale after birth, the neonatal lungs receive 100% of the cardiac output. When the low-resistance placental circulation is removed after birth, the newborn's systemic vascular resistance rises, while the pulmonary vascular resistance falls because of lung expansion, increased arterial and alveolar oxygen tension, and the release of local vasodilators.

The average fetal oxyhemoglobin saturation averages around 50% with a range between 20% to 80% at different points in the fetal circulation.[18,19] After birth, the oxyhemoglobin saturation rises gradually over the first 5 to 15 minutes of life to 90% or greater as the air spaces are cleared of fluid.[10] In cases of disordered transition secondary to asphyxia, meconium aspiration, pneumonia, or extreme prematurity, the lungs may not be able to develop efficient gas exchange, and thus the oxygen saturation may not increase as expected. Also, in some situations the normal drop in pulmonary vascular resistance may not fully occur, resulting in decreased pulmonary blood flow, and continued right to left shunting through the aforementioned fetal channels, a phenomenon known as persistent pulmonary hypertension of the newborn.

Birth Environment and Preparation for the Delivery

The birth environment should facilitate the transition to neonatal life and accommodate the needs of a newborn resuscitation. Hospitals and birthing centers differ in their physical layouts of the birth environment and the procedures used to prepare for delivery. Wherever the resuscitation is performed, a few key elements must be considered. The room should be warm enough to prevent excessive newborn heat loss, bright enough for assessment of the newborn's clinical status, and large enough to accommodate the necessary personnel and equipment to care for the baby. When a preterm birth is expected, the temperature in the birth environment should be set to approximately 23°C to 25°C (74°F to 77°F).[10]

In most cases, it is possible to use perinatal risk factors to predict which newborns will need resuscitation. According to the *Textbook of Neonatal Resuscitation*, every birth should be attended by at least one qualified provider skilled in the initial steps of newborn care and positive pressure ventilation (PPV), whose only responsibility is managing the newborn.[10] If risk factors are present, at least two qualified people should be present to manage the newborn.[10] If the need for extensive resuscitation is anticipated, then a qualified team capable of performing endotracheal intubation, chest compressions, emergency vascular access, and medication administration should be present at the time of birth.[10] Table 15.1 lists several risk factors associated with the need for mask ventilation or intubation.[20] Using these risk factors can



• **Fig. 15.2** The fetal circulation at term: (A) before birth and (B) after birth. (A) The path of oxygenated blood returning from the placenta is shown in *orange*. It mixes with deoxygenated blood returning from the fetal systemic veins shown in *light blue*. There is intracardiac mixing of blood as shown. The upper body receives a higher oxygen content than the lower body, as deoxygenated blood enters the descending aorta via right to left flow at the ductus arteriosus. After birth, the pulmonary and systemic circulations are completely separated as shown in (B). (From Carlson BM. *Human Embryology and Developmental Biology*. 5th ed. Philadelphia, PA: Mosby; 2014:436, 469.)

TABLE 15.1 Risk Factors Associated With the Need for Mask Ventilation or Intubation[a]

	High-Risk Deliveries[b]	Very High-Risk Deliveries[c]
Preterm delivery	34–37 weeks' gestational age	<34 weeks' gestational age
Birth weight	2.0–2.5 kg	<2 kg
Antepartum factors	Intrauterine growth restriction Fetal anemia or isoimmunization Polyhydramnios Gestational diabetes	Fetal hydrops Other major fetal anomalies Conditions that compromise respiratory transition
Intrapartum factors	Chorioamnionitis/maternal fever Maternal general anesthesia Emergency cesarean section Intrapartum hemorrhage Placental abruption Meconium-stained amniotic fluid Fetal heart rate trace concerns Forceps or vacuum delivery Breech presentation Shoulder dystocia	Fetal bradycardia Cord prolapse Uterine rupture Acute or severe complication of labor

[a]There is insufficient data to determine risk factors for chest compressions or medication administration; however, the same risk factors likely apply.
[b]Odds ratio less than 5 as compared to term controls.
[c]Odds ratio ≥5 as compared to term controls.
Adapted from Sawyer T, Lee HC, Aziz K. Anticipation and preparation for every delivery room resuscitation. *Semin Fetal Neonatal Med.* 2018 Oct;23(5):312–320.

guide delivery attendance decisions and may improve resource utilization.[20]

Institutions can improve newborn resuscitation team readiness in several ways. These include simulation-based training, encouraging task-oriented role assignment, reviewing resuscitation videos, using delivery room checklists, and debriefing after resuscitations. A systematic review of simulation-based team training in neonatal resuscitation found improved team performance and technical performance in simulation-based evaluations 3 to 6 months later.[21] Task-oriented role assignments include specific role(s), list of tasks, and the location where each newborn resuscitation team member should stand. Task-oriented role assignment training has been associated with improved behavioral skills during simulated neonatal resuscitation.[22] Many institutions regularly review videos of newborn resuscitations as a quality improvement process to identify areas of resuscitation performance that need improvement.[23,24] Current neonatal resuscitation guidelines recommend the use of a standardized checklist before every birth.[9] An example of a checklist is shown in Fig. 15.3. Checklist use during neonatal resuscitation has been found to be helpful in improving overall

DR Resuscitation Check list Patient Label Here

Pre-Resuscitation Briefing

Leader _____

MD(s) _____

RN(s) _____

RT(s) _____

☐ Introductions/Roles

☐ Discuss Plan, communication expectations

- Special considerations?
- Additional personnel/equipment?
- **"If any team member sees any developing problem or concern, I want to have it brought to my attention as soon as possible."**
- **Please call back all orders from Leader (e.g. "PIP is now 40")**

Pre-Resuscitation Checklist

Lead Resuscitator

- ❖ **Need urgent assistance, call x10770**
- ☐ Ensure briefing completed and introductions done
- ☐ Ensure RT checklist done
- ☐ Ensure RN checklist done
- ☐ Check status with resident receiving infant

Respiratory Therapy

- Brings RT bag (bring surfactant for < 28 weeks)
- Sets up Neopuff (30/5 and FiO2 .40, flow 8-10), appropriate masks
- Pedicap
- Sets up hand bag, checked (black bag if expecting difficult resus.)
- Intubation equipment checked, appropriate sized tubes
- Suction set at 80-100 mmHg, catheters, meconium aspirator if needed
- Pulse ox on and probe out
- NICO ET CO2 sensor
- Turn on video recorder

Nursing

- If crash C/section (call 2nd RN/MD) ensure line is set up, Epi drawn up.
- Barney bag
- Radiant warmer on MANUAL at 100%, probe and cover available, hat
- Stethescope
- Plastic wrap for < 28 weeks, Chemical mattress for <25 weeks
- ECG Leads

Debrief

Did we have all the information we need to admit this patient? Y/N

What did we do well? (Resident, Nurse, RT, Fellow, Attending in that order)

_____

What can we improve upon? _____

Do we need follow-up on any items? _____

• **Fig. 15.3** Delivery room resuscitation checklist. *DR*, Delivery room; *ET*, endotracheal; *FiO_2*, oxygen concentration; *MD*, medical doctor; *NICO ET*, NICO Monitor (Philips-Respironics, Inc.; Wallingford, CT); *PIP*, peak inspiratory pressure; *RN*, registered nurse; *RT*, respiratory therapist.

communication and rapidly identifying issues to be addressed by institutional leaders.[25] A performance-based postresuscitation debriefing should be completed after every resuscitation.[26] The debriefing is a critical opportunity for teams to identify what went well, what could be improved, and what issues require follow-up.

Umbilical Cord Management

After birth, blood flow in the umbilical arteries and vein usually continues for a few minutes. The additional blood volume transferred to the baby during this time is known as a placental transfusion. During the first 30 seconds after birth, the newborn's blood volume can increase by at least 12 mL/kg because of placental transfusion.[27–31] The timing of umbilical cord clamping influences the amount of placental transfusion and subsequent plasma and red blood cell volume of the newborn.[32,33]

The benefits and risks of delayed cord clamping (longer than 30 seconds) at birth have been closely examined. Cord clamping within 30 seconds may interfere with normal transition because it leaves fetal blood in the placenta that could have gone to the newborn. Delayed cord clamping is associated with higher hematocrit after birth and better iron levels in infancy.[34–46] Although developmental outcomes after delayed cord clamping have not been comprehensively assessed, iron deficiency is associated with impaired motor and cognitive development.[47–49] Based on the perceived benefits, the American College of Obstetricians and Gynecologists recommends delayed cord clamping for at least 30 to 60 seconds after birth in vigorous term and preterm infants.[50] For uncomplicated term or late preterm births, neonatal resuscitation guidelines include deferring cord clamping until after the newborn is placed on the mother and assessed for breathing and activity.[9] Delaying cord clamping may also be reasonable in preterm newborns because it reduces the need for blood pressure support, blood transfusions, and may improve survival.[51–58] Early cord clamping should be considered in cases when placental transfusion is unlikely to occur, such as maternal hemodynamic instability/hemorrhage, placental abruption, or placenta previa.[50] There is insufficient data at this time to make a recommendation on delayed cord clamping for newborns requiring PPV after birth, but this is an area of active research.[9]

Cord milking is an alternative to delayed cord clamping and can be used when the cord must be cut immediately after birth for medical reasons.[59] Cord milking is performed by gently squeezing a short segment of the cord with the thumb and forefingers and slowly pushing the blood within the cord towards the newborn's abdomen three to four times. Cord milking may be helpful for full-term newborns born by cesarean delivery, where there is a concern that delayed cord clamping may not provide an adequate placental transfusion.[27] Compared with delayed cord clamping, cord milking results in greater blood flow to and from the heart, higher hemoglobin levels, and higher blood pressure in neonates born by cesarean delivery.[60] Studies are ongoing to investigate the risks and benefits of umbilical cord milking in preterm newborns and in newborns requiring resuscitation. At the time of this writing, cord milking should be avoided in babies less than 28 weeks' gestational age, because it has been associated with brain injury.[61]

Newborn Resuscitation

Fig. 15.4 shows the neonatal resuscitation algorithm from the 8th edition (2021) *Textbook of Neonatal Resuscitation*.[10] Here, we examine several concepts and interventions in the practice of neonatal resuscitation and review the evidence that supports the current neonatal resuscitation guidelines.

Initial Steps

Preparing for the birth includes antenatal counseling, a team briefing, and an equipment check using a checklist. Before delivery, the following four "prebirth questions" should be asked of the obstetric team.[10]

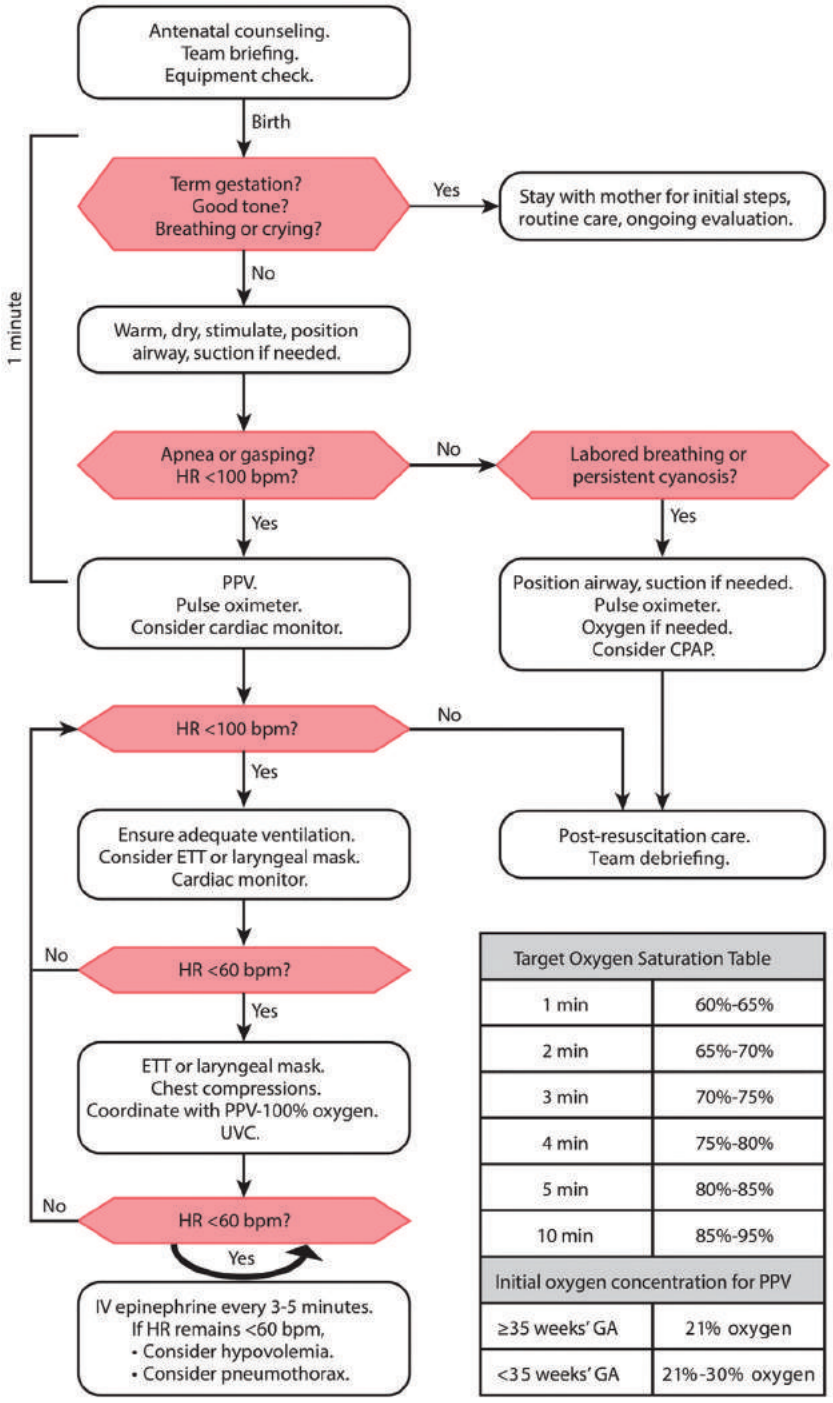
What is the gestational age?
Is the amniotic fluid clear?
Are there any additional risk factors?
What is the plan for umbilical cord management?

After the birth, an initial assessment includes a visual inspection of the newborn to determine if it is term gestation, has good muscle tone, and if it is breathing or crying. If the newborn is preterm, has poor muscle tone, or is not breathing or crying, it should be moved to the radiant warmer and the initial steps of newborn resuscitation should begin.

The initial steps of newborn resuscitation include warming, drying, tactile stimulation, and positioning the airway. Radiant warmers are used to maintain the newborn's body temperature between 36.5°C and 37.5°C.[10] For preterm and low-birth-weight newborns, warming adjuncts including plastic wraps or bags, hats, exothermic mattresses, and warmed humidified inspired gases may reduce the risk of hypothermia.[9] Drying the newborn is done by placing the baby on a warm towel or blanket and gently drying fluid on the skin. Drying is not necessary for newborns less than 32 weeks' gestation. Preterm infants less than 32 weeks' gestation should be immediately covered in plastic or their torso and extremities placed inside a plastic bag to reduce evaporative heat loss.[10] Tactile stimulation should be limited to drying and rubbing the back and soles of the feet. Appropriate positioning of the airway includes placing the newborn supine with the head in a neutral or "sniffing" position to facilitate maintenance of an open airway.

Routine suction of the airway is not indicated in newborns that are vigorous or crying.[10] Avoiding unnecessary suctioning helps prevent reflex bradycardia resulting from laryngeal stimulation.[9] The mouth and nose may be suctioned with a standard bulb syringe if there is visible fluid obstructing the airway, if the baby is having difficulty clearing their secretions, or if there is concern for obstructed breathing. Suctioning of secretions from the airway should be performed if the baby is not breathing, is gasping, has poor tone, and before PPV is given.[10]

Neonates born through meconium-stained amniotic fluid are at risk of aspirating meconium and developing meconium aspiration syndrome. For many years, routine management of nonvigorous newborns with meconium-stained amniotic fluid included endotracheal intubation and tracheal suctioning to remove meconium from the trachea in hopes of preventing meconium aspiration syndrome. In 2015, after careful deliberation, the neonatal resuscitation guidelines were changed to eliminate routine tracheal intubation and suctioning due to insufficient evidence of clinical benefit and concern for possible harm. A specific concern with routine tracheal suctioning was that it delayed the start of PPV in nonvigorous newborns. Neonatal resuscitation guidelines continue to recommend against routine direct laryngoscopy and tracheal suctioning for nonvigorous newborns born through meconium-stained fluids.[9] However, tracheal suction with a meconium aspirator can be beneficial in newborns who have evidence of airway obstruction while receiving PPV.[62]



• **Fig. 15.4** 2020 Neonatal Resuscitation Algorithm. (Used with permission from Weiner GM, Zaichkin J. *Textbook of Neonatal Resuscitation (NRP)*. 8th ed. American Academy of Pediatrics and American Heart Association; 2021.)

Positive Pressure Ventilation

Assisted ventilation with PPV is the most important step in newborn resuscitation. Approximately 3% to 5% of newborns require PPV to initiate spontaneous respirations at birth. The need for PPV is more common in preterm newborns than term newborns. The indications for PPV include apnea, gasping, and bradycardia (heart rate less than 100 bpm).[10] A PPV rate of 40 to 60/min is recommended in newborns.[9,10] PPV can be delivered noninvasively with a pressure delivery device and face mask or with a laryngeal mask airway (LMA). PPV can be delivered invasively using an endotracheal tube (ET). Pressure delivery devices used for newborn resuscitation include self-inflating bags, flow-inflating (e.g., anesthesia) bags, and T-piece resuscitators. Each device has its advantages and disadvantages. Currently, there is insufficient evidence to recommend one type of PPV device over another.[9]

Proficiency is needed to perform effective PPV with a face mask. This is especially true when administering PPV to extremely low birth weight newborns. It is important to maintain an open airway for pressure to be transmitted to the lungs. This is done by

keeping the head in a neutral position and lifting the jaw towards the mask, as opposed to pushing the mask down onto the face. The mask must make a good seal on the face for air to pass to the lungs. Leakage around the mask and airway obstruction from suboptimal airway positioning are common issues with face mask PPV. Signs that the airway is open and the face mask is well-sealed include visible chest rise with each breath and, most importantly, improvement in the newborn's heart rate.[10]

It is important to provide adequate pressure for ventilation; however, excessive pressure can contribute to lung injury. Initial inflation pressures of 20 to 25 cm H_2O are recommended.[9,10] If there is no visible chest rise or improvement in the newborn's heart rate at the initial inflation pressures, the pressures can be increased in 5 to 10 cm H_2O increments. In term newborns, a peak inflation pressures of 30 cm H_2O is usually sufficient to inflate the lungs.[63–70] In preterm newborns, peak inflation pressures of 20 to 25 cm H_2O are usually sufficient.[63–70] In some cases, however, higher inflation pressures are required. The *Textbook of Neonatal Resuscitation* recommends a maximum peak inspiratory pressure (PIP) of 40 cm H_2O.[10]

Immaturity and surfactant deficiency result in lung collapse in preterm newborns. In animal studies, positive end expiratory pressure (PEEP) has been shown to maintain FRC during PPV and thus improve lung function and oxygenation.[71] PEEP may be beneficial during neonatal resuscitation; however, the evidence from human studies is limited.[9] In clinical practice, PEEP is commonly used when providing PPV to both term and preterm newborns with either a flow-inflating bag or T-piece resuscitator. The optimal PEEP has not been determined.[9] All current human studies have used a PEEP of 5 cm H_2O.[72–76]

Continuous Positive Airway Pressure (CPAP)

Both term and preterm newborns need to establish a FRC as part of the transition from fetal to extrauterine life. The use of continuous pressure throughout the breathing cycle is helpful in establishing FRC and improves surfactant function.[77–79] Continuous positive airway pressure (CPAP) is a form of continuous pressure that helps newborns keep their lungs open and lessens breathing difficulty in preterm infants.[80] CPAP should only be used in spontaneously breathing newborns. CPAP is not appropriate in newborns that are apneic, gasping, or have a heart rate less than 100 bpm.[10] CPAP is administered during resuscitation using a face mask attached to either a T-piece resuscitator or a flow-inflating bag. CPAP cannot be administered with a self-inflating bag. The desired CPAP level is achieved by adjusting the PEEP dial on the cap of the T-piece resuscitator or the flow-control valve on the flow-inflating bag. If CPAP is administered for a prolonged period, the interface can change to nasal prongs or a nasal mask attached to a CPAP device or a mechanical ventilator.[10]

Studies comparing initial respiratory management with CPAP to endotracheal ventilation report a reduction in death and bronchopulmonary dysplasia in preterm infants < 30 weeks' gestation.[81–84] Thus, it is reasonable to use CPAP rather than intubation in spontaneously breathing preterm newborns who require respiratory support at birth.[9] The recommended level of CPAP is 5 to 6 cm H_2O.[10]

Sustained Inflation

CPAP is one method to establish FRC. Another method is to use sustained inflation. Sustained inflation is performed using either a T-piece resuscitator or a flow-inflating bag to deliver a prolonged PIP at a level of 20 to 25 cm H_2O for 15 to 20 seconds.[85] Animal studies suggest that a sustained inflation may be beneficial for short term respiratory outcomes.[86] However, a 2020 ILCOR systematic review and metaanalysis on sustained lung inflations during neonatal resuscitation at birth did not find benefit in using sustained inflations.[87] In that analysis, sustained inflations were associated with an increased risk of death before discharge in newborns < 28 weeks' gestation. A 2020 Cochrane systematic review examining sustained versus standard inflations during neonatal resuscitation also found no evidence to support the use of sustained inflation.[88] The current newborn resuscitation guidelines recommend against the routine use of sustained inflations in the delivery room.[9]

Laryngeal Mask Airways

A laryngeal mask airway (LMA)—also called a supraglottic airway device—is a small elliptical mask that is attached to an airway tube. Several types and brands are available. Some include an inflatable mask while others use a soft-gel mask that does not need inflation; some have a separate port through which to insert a gastric drainage tube.

An LMA is inserted into the baby's mouth using the operator's index finger and advanced into the throat until it sits over the laryngeal opening. The soft LMA mask conforms to the shape of the hypopharynx creating a seal that occludes the esophagus and provides a channel for air to enter the lungs via the glottis. The LMA makes a better seal than a face mask and thus may improve the effectiveness of ventilation. The LMA allows both the support of spontaneous breathing and the delivery of PPV. Unlike endotracheal intubation, the LMA does not require other instruments for insertion and the newborn's vocal cords do not need to be visualized during insertion.

Using an LMA for neonatal resuscitation was first described in 1994.[89] The LMA was introduced in the neonatal resuscitation guidelines in 2000. Over the past two decades, the utility of LMAs for neonatal resuscitation has been increasingly appreciated. A 2018 Cochrane systematic review noted that LMAs were more effective than face-mask ventilation.[90] Current guidelines recommend LMA use in newborns >34 weeks' gestational age with a birth weight >2000 g when attempts at face-mask ventilation or endotracheal intubation are not feasible or are unsuccessful.[9] Because the smallest commercially available LMAs are too large for very preterm newborns, there is lack of evidence on the use of LMAs in premature infants.

Endotracheal Intubation

Endotracheal intubation is the insertion of an ET through either the mouth or nose, and into the trachea. Endotracheal intubation is performed during newborn resuscitation to assist with breathing and inflating the newborn's lungs. Endotracheal intubation (or LMA placement) should be considered in newborns with a heart rate less than 100 beats/min after initial PPV and should be performed in newborns with a persistent heart rate less than 60 beats/min after adequate ventilation with face mask PPV. Endotracheal intubation can also be performed at delivery in newborns who are difficult to ventilate with a face mask and for those with respiratory failure requiring mechanical ventilation or intratracheal surfactant administration.

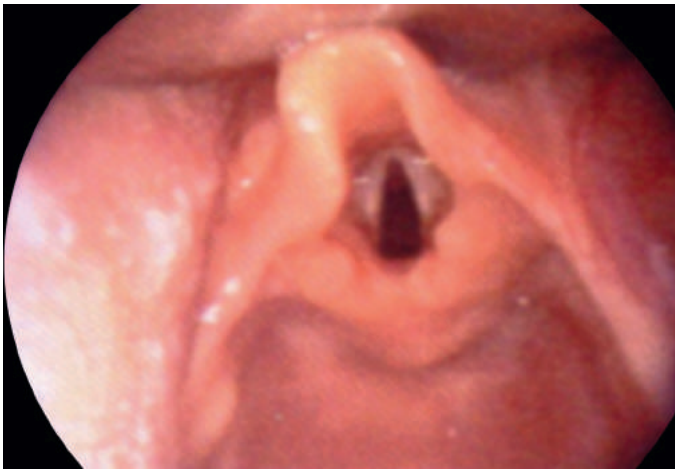
Intubation of the newborn trachea has been a part of newborn resuscitation for over 200 years.[91–94] The procedure was originally

performed using a long flexible tube directed into the newborn's airway by the operator's fingers (digital intubation).[94] The first laryngoscope for neonatal intubation was developed by the New York anesthesiologist Paluel Flagg in 1928.[95] The laryngoscope blade lifts the newborn's tongue out of the way to allow visualization of the glottis, so the ET can be visualized passing through the vocal cords. In recent years, the laryngoscope has been modified to include a video camera which allows better visualization of the glottis and provides the opportunity for other team members to see into the airway and confirm ET placement.[94] A photograph of a newborn's airway, obtained from a video laryngoscope, is presented in Fig. 15.5.

Neonatal intubation is technically challenging and often associated with adverse events. Recent studies from the multicenter National Emergency Airway Registry for Neonates report an average first intubation attempt success rate of 46% in the delivery room.[96,97] Tracheal intubation-associated events—including esophageal intubation, mainstem bronchial intubation, airway trauma, hypotension, pneumothorax, and cardiac arrest—ranged from 9% to 50% across centers.[96,97] Severe oxygen desaturation, defined as a drop in oxygen saturation during intubation by ≥20% from preintubation baseline occur in 29% to 69% of intubations.[96,97] In a recent study, the median time to a saturation decrease to <90% during neonatal intubation was only 22 seconds.[98] The low success rates, high adverse event rates, and frequent oxygen desaturations during intubation are concerning. Failed intubation in premature infants is associated with an increased incidence of severe intraventricular hemorrhage and neurodevelopmental impairment.[99,100] Concerns around intubation safety have caused the LMA to be increasingly considered as the primary advanced airway device during newborn resuscitation in near term and term infants.[10] If endotracheal intubation is performed, it should be done by an experienced provider skilled in the procedure to optimize the chance of success and to minimize adverse events.

Supplemental Oxygen

After birth, healthy newborns make the transition from the low oxygen environment of the womb to the oxygen-rich environment of room air (21% oxygen) over the course of several minutes. During that time, blood oxygen saturation levels slowly rise from approximately 60% at birth to around 90% by 10 minutes of life. When blood oxygen levels do not rise as expected, or fall after birth, supplemental oxygen is used to prevent harm from inadequate oxygen delivery to tissues (e.g., hypoxia).



• **Fig. 15.5** View of the glottis and vocal cords as the laryngoscope is gently lifted. (From Kattwinkel J, ed. *Neonatal Resuscitation Textbook*. 5th ed. Elk Grove Village: American Heart Association and American Academy of Pediatrics; 2006.)

Before 2000, neonatal resuscitation guidelines recommended a supplemental oxygen concentration of 100% for all newborns, regardless of gestation. Studies over the past two decades have raised concerns about exposure to high oxygen concentration during neonatal resuscitation.[101,102] A 2005 Cochrane systematic review found a reduction in short-term mortality for newborns resuscitated with room air (21% oxygen) as compared with 100% oxygen.[103] A 2019 ILCOR systematic review with meta-analysis reported a 27% relative reduction in short-term mortality for newborns ≥35 weeks' gestation receiving respiratory support at birth with room air as compared with 100% oxygen.[104] No difference was found in neurodevelopmental outcome of survivors.[104] Based on the short-term benefits, current neonatal resuscitation guidelines recommend room air for the initial resuscitation of newborns ≥35 weeks' gestation.

The data on supplemental oxygen use in preterm newborns <35 weeks' gestation is less clear. A 2019 ILCOR meta-analysis of randomized trials enrolling preterm newborns showed no difference in short-term mortality with low oxygen (21% to 30%) as compared with high oxygen (60% to 100%) concentrations.[105] There was also no difference in neurodevelopmental outcomes, retinopathy of prematurity, bronchopulmonary dysplasia, necrotizing enterocolitis, or major cerebral hemorrhage between the low oxygen and high oxygen groups.[105] Of note, all preterm babies who started off on 21% oxygen required supplemental oxygen during resuscitation to reach target oxygen saturations.[105] Based on a lack of evidence to support a specific initial oxygen level, current newborn resuscitation guidelines recommend anywhere between 21% and 30% for the resuscitation of newborns <35 weeks' gestation.[9,10] To ensure the safe use of oxygen, pulse oximetry with oxygen saturation targeting is recommended.

During cardiopulmonary resuscitation with chest compressions, higher oxygen concentrations may be needed to deliver adequate oxygen to the body. Also, during cardiopulmonary resuscitation with chest compressions, peripheral circulation may be so poor that the pulse oximeter will not give a reliable signal. For these reasons, neonatal resuscitation guidelines recommend that supplemental oxygen be increased to 100% when chest compressions are started.[9,10] Once the heart rate is greater than 60 beats/min and a reliable pulse oximeter signal is achieved, supplemental oxygen levels should be adjusted to achieve the target oxygen saturation based on minutes life.[10]

Chest Compressions

The need for chest compressions during newborn resuscitation is rare. It is estimated that 0.1% (1/1000) of term infants and around 15% (15/100) of preterm infants receive chest compressions—with or without epinephrine—in the delivery room.[106] Chest compressions are indicated if the newborn's heart rate remains less than 60 beats/min after at least 30 seconds of PPV that inflates the lungs.[9,10] Chest compressions mechanically pump blood through the body by either direct compression of the heart between the sternum and vertebral column ("cardiac pump theory") or via phasic increases in intrathoracic pressure ("thoracic pump theory").[106] Optimized chest compressions generate around 30% of normal organ perfusion, with preferential perfusion of the heart and brain.[107]

Performing chest compressions on a newborn is best done using the two thumb–encircling hands technique.[9] With this technique, the compressor's thumbs are placed on the lower third of the sternum, just below an imaginary line connecting the newborn's nipples. The thumbs can be placed either on top of the other or side-by-side.[10] Using two thumbs, the sternum is pressed downward to a depth of approximately one-third of the anterior–posterior diameter of the chest. The compression distance depends on the size of the newborn. One chest compression consists of the downward stroke plus the release. The release phase is critical because it allows the myocardium and coronary blood vessels to refill with blood.

Chest compressions are typically started by a team member standing at the side of the warmer near the neonate's feet. Once an advance airway is established, the team member performing chest compressions can move to the head of the bed. Having the compressor at the head of the bed frees up space for another team member to place an umbilical catheter.

The optimal approach to chest compressions and ventilation in an asphyxiated newborn is unknown.[106] The recommended compression rate in newborns is 90 compressions per minute. The recommended chest compression to PPV ratio is 3 compressions and 1 ventilation, with 90 compressions and 30 ventilations provided per minute. It is important to avoid unnecessary interruptions in chest compressions to optimize coronary and cerebral perfusion. Once started, coordinated compressions and ventilation should be continued for at least 60 seconds before pausing compressions to reassess the heart rate.[9,10] Chest compressions can be stopped when the newborn's heart rate is 60 beats/min or greater.[9,10]

Epinephrine

Epinephrine (adrenaline) is recommended for newborns with a sustained heart rate <60 beats/min after adequate assisted lung ventilation with 100% oxygen (preferably through an ET) and chest compressions.[9,10] Epinephrine produces beneficial effects during cardiac arrest primarily through its α-adrenergic receptor-stimulating properties, which increase both myocardial and cerebral blood.[108,109] The beneficial effects of epinephrine's β-adrenergic-stimulating properties during cardiac arrest are controversial because they may increase myocardial work and reduce subendocardial perfusion.[110]

The acceptable intravenous (IV) dose of epinephrine is 0.02 mg/kg.[9,10] A normal saline flush of 3 mL is recommended after the IV dose.[9,10] The acceptable endotracheal dose of epinephrine is 0.1 mg/kg. No flush is needed with endotracheal epinephrine. The dosage interval for epinephrine, either IV or endotracheal, is every 3 to 5 minutes if the heart rate remains less than 60 beats/min.[9,10] However, an intravenous dose may be given as soon as IV access is obtained if an initial dose of endotracheal epinephrine was already given.

The preferred route of epinephrine is IV. This is because IV is believed to be more effective than endotracheal administration.[111] A low-lying umbilical venous catheter provides the most rapid and reliable medication delivery method at birth.[9] If the need for advanced resuscitation is suspected prior to birth, the necessary equipment for umbilical venous catheter placement should be prepared before delivery. One method to ensure rapid IV epinephrine administration is to assign one resuscitation team member to place the emergency umbilical catheter as soon as chest compressions are initiated. In cases where IV access via a low-lying umbilical venous catheter fails or is not feasible, an intraosseous line is an acceptable alternative.[10]

Volume Expanders

Hypovolemic shock at delivery can result from extensive vaginal bleeding, acute fetal-maternal hemorrhage, bleeding vasa previa, a placental laceration, umbilical cord prolapse, a tight nuchal cord, and blood loss from a ruptured umbilical cord.[10] Newborns with hypovolemic shock may appear pale, and have delayed capillary refill and weak pulses. A persistently low heart rate that does not respond to effective ventilation, chest compressions, and epinephrine may be seen in newborns with hypovolemic shock.[9]

Volume expanders are indicated for newborns with signs of shock or a history of acute blood loss that does not respond to resuscitation. The preferred route for volume expanders is IV. However, the intraosseous route is an acceptable alternative. Volume expanders should be avoided in the absence of shock or a history of acute blood loss because large volumes of IV fluid can worsen cardiac output and further compromise the newborn.[10] Normal saline (0.9% sodium chloride) is the crystalloid volume expander of choice.[9] In cases of substantial blood loss, uncrossmatched type O, Rh-negative blood is preferred.[9] The recommended dose of volume expanders is 10 mL/kg given over 5 to 10 minutes.[9] This dose may be repeated if there is inadequate response.

Apgar Score

The assessment of a newborn was first quantified by the anesthesiologist Virginia Apgar in the 1950s and henceforth known as the Apgar score.[112] The score is based on a 10-point scale with a maximum of 2 points assigned for each of the following categories: respirations, heart rate, color, tone, and reflex irritability (Table 15.2). The Apgar score is assigned at 1 minute, 5 minutes, and then every 5 minutes thereafter if the total score is less than 7. The score was initially intended to provide a uniform, objective assessment of the newborn's condition and to compare different practices, especially obstetric anesthesia. Despite the intent of objectivity, there is often disagreement in score assignment among practitioners.[113] Low scores are associated with increased risk of neonatal death,[114] but have not been predictive of neurodevelopmental outcome.[115] Interpreting the score when interventions are being provided may be difficult. Current recommendations suggest that clinicians document the interventions used at the time the score is assigned.[116]

Delivery Room Monitoring

The modern delivery room is becoming more like the NICU with advanced patient monitoring devices used to track important physiological measures. Exhaled carbon dioxide (CO_2) detectors, pulse oximetry, electrocardiogram (ECG), and respiratory function monitors (RFM) are some of the most used monitoring devices in the delivery room. Here, we briefly examine the use of each.

Exhaled CO_2 Detector

Detection of exhaled CO_2 confirms ventilation and perfusion of the lungs. Exhaled CO_2 is evaluated by either color change

TABLE 15.2 The Apgar Score

Feature Evaluated	0 Points	1 Point	2 Points
Heart rate (beats/min)	0	<100	>100
Respiratory effort	Apnea	Irregular, shallow, or gasping respirations	Vigorous and crying
Color	Pale, blue	Pale or blue extremities	Pink
Muscle tone	Absent	Weak, passive tone	Active movement
Reflex irritability	Absent	Grimace	Active avoidance

of a colorimetric device or as an expiratory waveform using capnography. A lack or color or waveform alerts the operator to an obstructed airway, blocked ET, displaced ET, or lack of perfusion to the lungs. Airway obstruction is common when providing face mask PPV to newborns.[117,118] The site of obstruction may be laryngeal or supralaryngeal and can be alleviated with ventilation corrective steps. The use of a CO_2 detector during face mask ventilation provides visual confirmation that gas exchange is occurring in the lungs.[119] The *Textbook of Neonatal Resuscitation* supports the use of colorimetric CO_2 detectors during face mask ventilation and during ventilation corrective steps.[10]

It is important to remember that CO_2 detectors will not change color or display a waveform in the absence of pulmonary blood flow, as occurs during cardiac arrest or inadequate cardiac output. If the newborn has cardiac arrest or severe bradycardia, the CO_2 detector may not detect exhaled CO_2 even though the lungs are being ventilated. In cases of newborn cardiac arrest or severe bradycardia where chest compressions are given, the presence of exhaled CO_2 provides some evidence of effective chest compressions.

Pulse Oximetry

Pulse oximeters measure the absorption of red light passing through capillaries in the skin to estimate the percentage of hemoglobin that is saturated with oxygen. The pulse oximeter displays the oxygen saturation ranging from 0% to 100%. Oxygen saturation is different from the partial pressure of oxygen (PaO_2) measured on a blood gas, but both provide evidence of blood oxygenation. The pulse oximeter also displays the newborn's heart rate by sensing pulsatile blood flow in the capillaries. Pulse oximetry at delivery is indicated when resuscitation is anticipated, to confirm the presence of central cyanosis, when supplemental oxygen is administered, and when positive-pressure ventilation is required.[10]

The recommended location for a pulse oximeter is on the newborn's right hand or wrist. This "pre-ductal" position is preferred because it measures the blood flow from the arteries supplying the baby's right arm, which normally attach to the aorta before the patent ductus arteriosus. Oxygen saturations from "post-ductal" locations (left arm or either leg) may be lower than those from the pre-ductal location because the former receives blood from the aorta after it mixes with venous blood that bypasses the lungs through the patent ductus arteriosus. When properly placed on a newborn with adequate pulsatile capillary flow, the pulse oximeter should accurately display the heart rate and oxygen saturation within approximately 1 to 2 minutes after birth.[10] If the baby has a very low heart rate or poor perfusion, the oximeter may not be able to detect oxygen saturations or heart rate. In such cases, electrocardiography may be helpful to determine the heart rate.

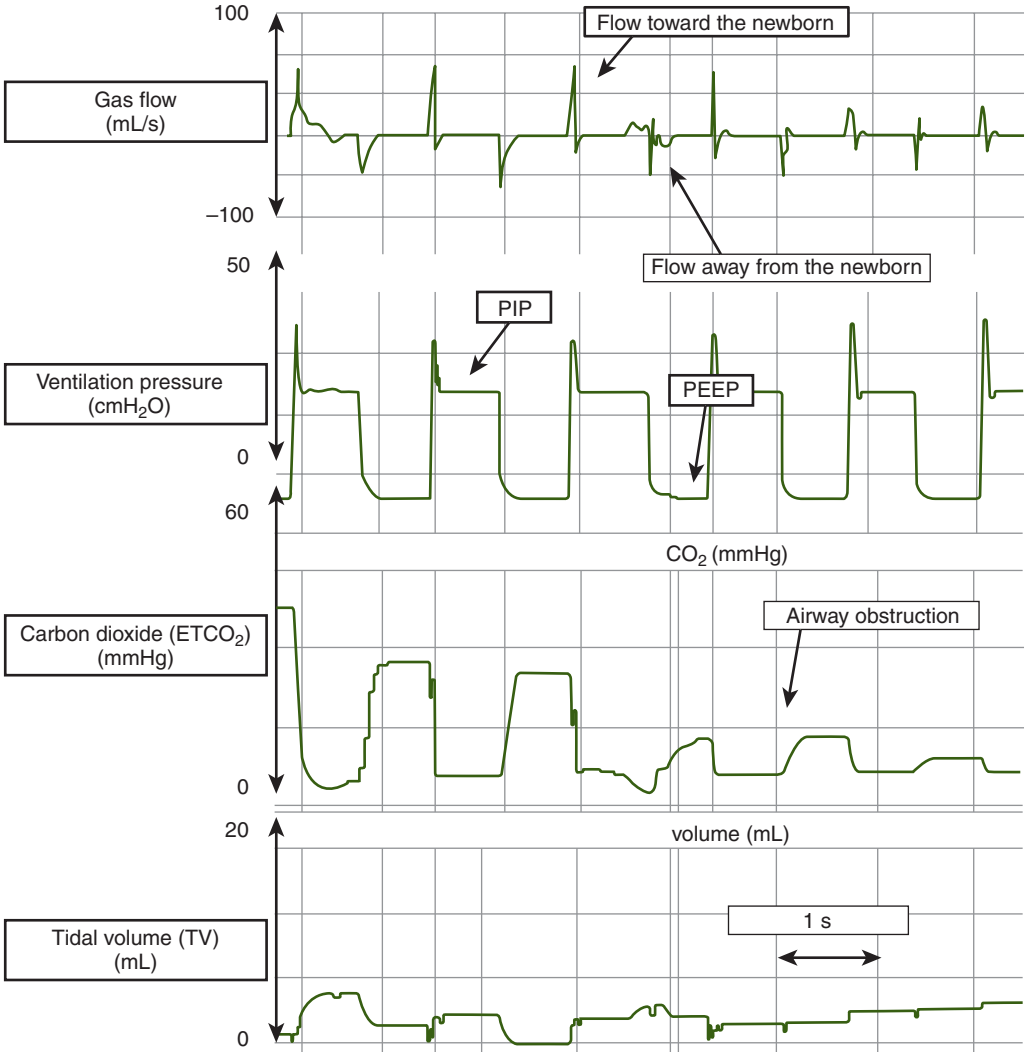
Electrocardiography

Electrocardiography (ECG) detects the electrical activity of the heart, and so is not dependent on the circulation. Placement of the 3-lead ECG electrodes includes just below the right clavicle, just below the left clavicle, and just above and left of the umbilicus. ECG provides a rapid and accurate estimation of heart rate in low perfusion states. In randomized clinical trials comparing oximetry with ECG during newborn resuscitation, ECG was faster and more accurate for newborn heart assessment compared with pulse oximetry.[120,121] Use of ECG for heart rate detection does not replace the need for pulse oximetry to evaluate oxygen saturation.[9] Neonatal resuscitation guidelines suggest that ECG be consider with the initiation of PPV and should be used after placement of an alternative airway and during chest compressions to confirm heart rate.[9,10]

Respiratory Function Monitors

A respiratory function monitor (RFM) measures delivered pressures and gas flow in and out of the lungs.[122] From pressures and gas flow data, inflation and expired volumes are calculated. Most RFMs shows continuous graphical displays of airway pressure, gas flow, tidal volumes (VT), and expired CO_2, and gas leak (Fig. 15.6).[123] Studies have shown that using an RFM during neonatal resuscitation is associated with reduced mask leak, less variation in VT, and improved ability to determine whether an ET is properly inserted in the trachea.[123]

Three types of RFMs are currently available to measure lung function at birth.[122] The devices use different techniques to measure gas flow and unique software to calculate tidal volume. At this time, none of these devices are approved for use in the United States by the Food and Drug Administration (FDA). Therefore, AHA neonatal resuscitation guidelines do not include recommendations regarding RFM use. RFMs are mentioned in the 2021 European Resuscitation Council guidelines on newborn resuscitation[124] and the clinical impact of RFM use is being examined as part of ILCOR's continuous evidence review process. RFMs may be included in future AHA guidelines if devices become FDA-approved.



• **Fig. 15.6** Display from a respiratory function monitor (rfm) showing complete airway occlusion during mask positive pressure ventilation. Adequate positive pressure ventilation is provided, and suddenly the inflation and expiratory flow curves both display fewer flow movements and no tidal volume, and reducing expiratory CO_2 is displayed. The peak inspiratory pressure and positive end-expiratory pressure are maintained during positive pressure ventilation. *$ETCO_2$*, End-tidal carbon dioxide; *PEEP*, positive end-expiratory pressure; *PPV*, positive pressure ventilation. (Courtesy of G. Schmolzer.)

Specific Problems Encountered During Resuscitation

Neonatal Response to Maternal Anesthesia/Analgesia

Medications administered to the mother during labor can affect the fetus by transfer across the placenta. The most discussed complication of intrapartum medication exposure is newborn respiratory depression after maternal opiate administration. The fetus can develop respiratory depression from the direct effect of opiates. Naloxone has been used during neonatal resuscitation as an opiate receptor antagonist to reverse the effects of acute fetal opiate exposure. However, newborns chronically exposed to opiates in utero (which may not be known at delivery) may have acute withdrawal, including seizures, if they receive a narcotic antagonist. Therefore, neonatal resuscitation guidelines do not recommend naloxone as part of the initial resuscitation of newborns with respiratory depression, regardless of whether they have been acutely exposed to opiates.[10] Such newborns should be treated with assisted ventilation.

Conditions Complicating Resuscitation

There are many perinatal and congenital conditions that can complicate newborn resuscitation. Any time a resuscitation has proceeded through the neonatal resuscitation algorithm without improvement in the newborn's clinical condition, other problems should be considered. Some of these problems may be modifiable with interventions that could improve the course of the resuscitation. For example, a pneumothorax could prevent adequate pulmonary inflation and if under tension impair cardiac function. If the pneumothorax is recognized and drained, both gas exchange and circulation can be improved.

Many other congenital anomalies can also lead to a difficult resuscitation. A complete review of the management

TABLE 15.3 Special Considerations and Modifications of Neonatal Resuscitation Guidelines for Newborns With Select Congenital Anomalies

Congenital Anomalies	Specialized Equipment and Personnel to Consider	Neonatal Resuscitation Guideline Modifications
Airway Anomalies		
Micrognathia, TEF, CHAOS, Airway mass, lymphatic malformations	Video laryngoscope, flexible fiberoptic bronchoscopy, tracheostomy, otolaryngology and/or anesthesia assistance	Rapid progression to intubation if face mask PPV is difficult; consider ex-utero intrapartum treatment (EXIT) in high-risk cases
Lung Anomalies		
CDH	Replogle to decompress stomach	Immediate intubation, "gentle ventilation" with lower pressures and tidal volumes
Congenital Heart Disease		
TGA, HLHS, uncontrolled arrhythmias	Cardiologist for possible early balloon septostomy in case of restricted atrial level shunt, PGE, defibrillator, equipment for thoracentesis, and paracentesis in cases of fetal hydrops	For mixing lesions, targeted SpO2, 75%–80%
Gastrointestinal Anomalies		
Gastroschisis, omphalocele, duodenal/intestinal atresia	Gastroschisis: Replogle, sterile bowel bag Omphalocele: Replogle, sterile bowel bag if concern for rupture Duodenal/Intestinal atresia: Replogle	Caution with positive pressure, intubate early in cases of respiratory distress
Neurological Anomalies		
Neural tube defects	Sterile nonstick gauze, foam donut or a gauze rolled into a circle for placement around the defect, latex-free dressing and gloves	Positioning on the side or in a prone position to avoid putting pressure on the lesion.
Multiples		
Conjoined twins	Duplicate equipment to accommodate both twins	Individualized assessment for placement of ECG leads, pulse oximetry, and position for optimal delivery of PPV and chest compressions

CHAOS, Congenital high airway obstruction syndrome; *CDH*, congenital diaphragmatic hernia; *HLHS*, hypoplastic left heart syndrome; *TEF*, tracheoesophageal fistula; *TGA*, transposition of great arteries.
Adapted from Ali N, Sawyer T. Special consideration in neonatal resuscitation. *Semin Perinatol*. 2022 May 25 [Online ahead of print].

of all the varied congenital anomalies that can complicate newborn resuscitation is outside the scope of this chapter. However, Table 15.3 provides a summary overview of special considerations in newborn resuscitation adapted from a recent review.[125]

Limits of Viability

The lower limit of viability has been an ongoing debate since the specialty of neonatology began. The limit has changed over time with the advent of newer equipment and increasing comfort with the care of extremely premature newborns. In the 1972 (3rd edition) of the Avery's textbook, Drs. Avery and Schaffer wrote "the lower limit of viability is probably about 28 weeks, at which time most infants weigh 2 pounds 4 ounces (1000 g)."[126] Today, the lower limit is probably a gestational age less than 22 weeks and/or birth weight less than 350 g.[127] Because of ethical and legal implications of publishing guidelines for the noninitiation of resuscitation based on gestational age or birth weight, the AHA neonatal resuscitation guidelines and the *Textbook of Neonatal Resuscitation* do not mention a lower limit of viability.[9,10] Instead, they acknowledge that decision-making should be individualized with consideration to the likelihood of survival and acceptable health outcomes.

Noninitiation and Discontinuing of Resuscitation

Even with optimal neonatal resuscitation, some babies are too sick or immature at birth to survive. Also, some conditions are so severe that the burdens of the illness and treatment outweigh the likelihood of survival. In cases where such conditions are identified at or before birth, it is reasonable not to initiate resuscitative efforts.[9,10] What is best for the newborn is the primary consideration for decisions around newborn resuscitation. Factors that should be considered include the following[10]:

The chance that the therapy will succeed.
The risks involved with treatment and nontreatment.
The degree to which the therapy, if successful, will extend life.
The pain and discomfort associated with the therapy.
The anticipated quality of life for the newborn with and without treatment.

Making the decision to discontinue resuscitation efforts after resuscitation is started is challenging. Variables to consider include the circumstances before birth, whether the resuscitation process was optimal, the availability of advanced neonatal care such as therapeutic hypothermia, and the wishes of the family.[10] Neonatal resuscitation guidelines suggest that if the heart rate remains undetectable (e.g., persistent asystole) at about 20 minutes after birth, and all steps of the resuscitation algorithm have been completed, it may be reasonable to stop resuscitation.[9,10] The decision to continue or discontinue resuscitative efforts should, however, always be individualized. The 20-minute time frame is based on case series showing small numbers of intact survivors after 20 minutes of resuscitation with no detectable heart rate.[9]

Post-resuscitation Care

While effective resuscitation is critical, optimal care after the resuscitation is also paramount. Post-resuscitation monitoring in a neonatal intensive care unit or a triage area is needed for any newborn who received prolonged PPV or advanced resuscitation (e.g., intubation, chest compressions, and/or epinephrine) at birth.[9] Frequent complications following resuscitation include hypoglycemia, hypothermia, hypotension, and persistent metabolic acidosis.

Hypoglycemia is a common problem in newborns who have received advanced resuscitation and hypoglycemia in the post-resuscitation period is associated with poorer outcomes.[128] Thus, all newborns that received prolonged PPV or advanced resuscitation should be monitored for hypoglycemia and treated appropriately.[9]

During resuscitation, many newborns experience unintentional hypothermia (temperature less than 36°C). Therefore, temperature should be monitored closely after stabilization. Hypothermic newborns should be rewarmed to avoid complications associated with low body temperature, which including increased mortality, brain injury, hypoglycemia, and respiratory distress.[9] Neonatal resuscitation guidelines suggest that warming can be done either rapidly (0.5°C/h) or slowly (less than 0.5°C/h) with no significant difference in outcomes.[9] Caution should be taken to avoid overheating during post-resuscitation care.

Newborns 36 weeks or greater who receive advanced resuscitation should be examined for clinical evidence of hypoxic-ischemic encephalopathy (HIE). The pathophysiology of HIE and its treatment with therapeutic hypothermia are discussed in detail elsewhere in this textbook. Therapeutic hypothermia decreases the secondary injury that occurs after a hypoxic-ischemic insult. The therapy is most beneficial when started as soon as possible after birth, ideally within 6 hours. Thus, a thorough assessment for HIE during the immediate post-resuscitation period is critical to determine if a newborn meets treatment criteria. Therapeutic hypothermia is not recommended in newborns less than 36 weeks' gestational age since the impact is unclear. It is the subject of ongoing research.[9]

Acknowledgment

The authors would like to acknowledge Dr. Anup Katheria and Dr. Neil Finer for their work on this chapter in the previous edition.

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16 Care of the Newborn

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KEY POINTS

- Prenatal ultrasonography can diagnose multiple newborn conditions early. However, the natural history of many common ultrasound findings is variable, and the findings may or may not represent markers of serious disease.
- The short-term and long-term benefits of breastfeeding are clear. The effects on breastfeeding rates from interventions such as formula supplementation, frenotomy, and restriction of pacifiers remain controversial, as does breastfeeding among HIV-positive mothers.
- Comprehensive guidelines for the management of common newborn conditions such as jaundice, suspected sepsis, and hypoglycemia allow standardization of care. Ongoing research is needed to optimize guidelines to improve long-term outcomes.
- With the implementation of universal maternal screening for group B streptococcus and the use of intrapartum antibiotic prophylaxis, rates of sepsis in term newborns have fallen significantly. Online aids are available to assist clinicians in assessing the risk of sepsis and developing institutional guidelines for the identification and management of infants at risk for sepsis.
- Pulse oximetry screening for critical congenital heart disease is recommended for healthy newborns.
- Late preterm infants are at risk of complications associated with prematurity and require close monitoring for respiratory problems, feeding issues, hypoglycemia, hyperbilirubinemia, infection, and thermoregulation in the postnatal period.

Introduction

Optimal newborn care facilitates a successful transition from intrauterine to extrauterine life without overmedicalization of care, provides low-risk preventive care, and identifies "high-risk" infants who would benefit from increased monitoring, testing, or intervention, thereby decreasing early preventable morbidity and mortality. Systems of care should be designed to align with the concept that the majority of newborns are healthy and require little intervention beyond the promotion of breastfeeding, and interventions for which there is clear evidence that benefits outweigh risks should be provided as unobtrusively as possible. Simultaneously, the system must maintain space and opportunity for healthcare providers to quickly and efficiently identify those neonates at risk of developing serious or life-threatening conditions.

The goal of this chapter is to provide a framework for developing an approach to the initial assessment of the apparently well newborn. This includes the identification of common neonatal problems, indications for additional monitoring, and risk-benefit analysis of testing and treatments commonly employed in the newborn nursery. Rather than providing a comprehensive prescription on how to care for healthy newborns, we hope that the reader will integrate the information provided in this chapter with expert opinion and his or her own clinical experience to provide thoughtful, evidence-based management for this unique patient population.

Initial Newborn Evaluation

In the mother-baby unit or well-baby nursery, the primary goal is to identify the small minority of babies with significant problems that may cause serious morbidity if not detected promptly, including psychosocial problems that may adversely impact both "normal" and "at risk" newborns. This goal must be accomplished with the understanding that the vast majority of babies encountered in the newborn nursery are completely healthy, despite wide variations in presentation and findings.

The Initial Assessment

The timing of the initial assessment of a healthy-appearing newborn is dependent on the condition of the newborn and parental preference. In most instances, a healthcare professional who is present at the birth will make a general appraisal of the newborn and alert the child's provider if there is an acute problem necessitating an immediate evaluation. The newborn's weight, length, and head circumference should be measured and plotted on a standardized chart; the WHO growth chart is used for term (≥37 weeks) infants, and the Fenton growth chart for preterm infants (<37 weeks).[1,2] This assessment can usually be timed so as not to interfere with breastfeeding, bonding with the family, and routine care.

The Newborn History

Before a well newborn is examined, the maternal medical history should be reviewed to identify issues that could affect the care or prognosis of the newborn. For example, a history of diabetes (gestational or prepregnancy) would lead to glucose monitoring in the newborn; and maternal medication use may prompt an assessment for possible teratogenic effects or signs of neonatal drug withdrawal or compatibility with breastfeeding. It is equally

important to review the pregnancy history, including the estimated gestational age (GA), the results of prenatal screening for genetic conditions, and the results of prenatal ultrasound examinations. Positive findings on the prenatal ultrasound deserve particular attention, as they may have implications for postnatal management. Perinatal events such as the type of delivery, length of time that membranes were ruptured, and Apgar scores should also be reviewed. Finally, it is critical to review the mother's social history to identify psychosocial risk factors that may preclude safe discharge home, or for which interventions are indicated before, or shortly after, discharge from the newborn nursery. An outline of relevant prenatal and newborn history is presented in Table 16.1.

TABLE 16.1 Key Components of the Maternal Medical, Pregnancy, and Perinatal History

Category	Components
Maternal identification	Age, gravida, parity, weeks in gestation
Maternal medical history	Significant co-morbidities, illness, medications
Current pregnancy	Singleton or multiple fetuses
	Pertinent results of laboratory tests and imaging studies
	Fetal growth (IUGR; LGA; hydrops)
Labor	Rupture of membrane (duration)
	Amniotic fluid (oligohydramnios/polyhydramnios; bloody; purulent; meconium; foul smelling)
	Signs of infection (maternal fever, elevated WBC count, tachycardia, uterine tenderness)
	Fetal tracing (tachycardia, decelerations, etc.)
Delivery	Indication if emergent delivery (abruption; preeclampsia, fetal distress/intolerance to labor)
	Route of delivery
	Method of anesthesia (general versus local)
	Medications administered
Newborn	Apgar scores and resuscitation at delivery
	Growth parameters
	Desired feeding method

IUGR, Intrauterine growth restriction; *LGA*, large for gestational age; *WBC*, white blood cell.

Prenatal Ultrasound Findings

Ultrasound screening for fetal anomalies has become increasingly routine. Major fetal organ system abnormalities can be identified, for the most part, and the mother can be referred for counseling and appropriate fetal and neonatal management. There are, however, a number of ultrasound findings that have a variable natural history and may or may not be markers for serious conditions. Moreover, these ultrasound findings may or may not result in a definitive prenatal diagnosis, highlighting the importance of postnatal assessment and management. Here we present common prenatal ultrasound findings, organized by organ system, including recommendations for prenatal and postnatal evaluation.

Central Nervous System Findings

Choroid plexus cysts are found in 2% to 4% of second-trimester fetal ultrasound examinations. They are transient, functionally benign in nature, and generally resolve spontaneously before term. Isolated choroid plexus cysts on prenatal ultrasound examination are not associated with adverse effects on fetal growth or development. Thus, without other risk factors, no further evaluation is needed in an infant with this isolated finding who has had a benign prenatal and a normal postnatal course.[3] Choroid plexus cysts may be a soft marker for aneuploidy (particularly trisomy 18) when associated with other fetal anomalies or with maternal risk factors, such as advanced maternal age. In such situations, current recommendations are to begin an appropriate prenatal evaluation, such as karyotyping.[4–6]

Agenesis of the corpus callosum is reported to occur in 0.3% to 0.7% of unselected postnatal populations. Aneuploidies have been reported in 10% to 20% of children with this prenatal ultrasound finding, and major organ system abnormalities are reported in up to 60% of affected fetuses. Therefore, fetal magnetic resonance imaging can be considered to further assess for associated anomalies and offer enhanced risk stratification. Encouragingly, the absence of the corpus callosum in an otherwise anatomically normal fetus is associated with a normal developmental outcome in 50% to 75% of cases. Postnatal management for infants with a history of agenesis of the corpus callosum on prenatal ultrasound should include a thorough physical examination, close clinical assessment, and consideration of additional imaging if not already completed prenatally.[7–10]

Mild, isolated ventriculomegaly is a relatively uncommon fetal ultrasound finding and may be a soft marker for aneuploidy, fetal infection, or other central nervous system abnormalities. As such, serial prenatal imaging studies and, in some cases, more extensive work-up are recommended. In the presence of a benign fetal assessment, most newborns appear to do well following delivery. It is important to consider close developmental follow-up and serial imaging studies, often beginning with an early postnatal cranial ultrasound, if there are additional concerns.[11–13]

Cardiac Findings

Echogenic cardiac focus is an incidental ultrasound finding in 3% to 4% of normal fetuses. Notably, there is an increased incidence (10% to 30%) in Asian populations. It may be a soft marker for chromosomal abnormalities (trisomy 21 and trisomy 13) when associated with other screening abnormalities. Further work-up may be indicated in high-risk populations. If the physical examination findings for a newborn are unremarkable and there are no other ultrasound findings, no further evaluation is suggested.[14–18]

Gastrointestinal Findings

Grade 0 or 1 echogenic bowel on a second-trimester ultrasound examination (i.e., less echogenic than bone) is considered a normal variant with a good prognosis. No special prenatal or postnatal work-up is recommended. Anything of density equal to or greater than that of bone (grade 2–3) is abnormal; differential diagnoses include cystic fibrosis (CF), trisomy 21, gastrointestinal anomalies, in utero infection, bowel ischemia or bleeding, intrauterine growth restriction, and/or impending in utero demise. Oftentimes, further prenatal work-up will have been performed, including parental CF testing, maternal serologic testing for

cytomegalovirus (CMV) and toxoplasmosis, and amniocentesis, which allows for narrowing of the differential. If unrevealing, postnatal assessment should include consideration of aneuploidy, congenital infection, and identification of associated structural anomalies. Management often entails close monitoring for feeding tolerance, with or without abdominal imaging and additional targeted testing.[3,19,20]

Cholelithiasis is an uncommon third-trimester fetal ultrasound finding that needs to be differentiated from hepatic calcification. Cholelithiasis is considered a benign condition requiring no special evaluation or treatment prenatally or postnatally, but careful clinical follow-up is recommended. An imaging examination at 1 year of age for a child with this prenatal finding may be helpful in documenting expected resolution.[21,22]

Hepatic calcifications are uncommon fetal ultrasound findings. They are often isolated, single, and, in a low-risk mother, of no significance. However, when numerous, hepatic calcifications may be markers for fetal aneuploidy, infection, meconium peritonitis, hepatic tumor, or vascular insult. A significant percentage are associated with some form of fetal disease. Neonatal management depends on the prenatal work-up and the clinical presentation in the newborn period.[23,24]

Urinary Tract Findings

Mild fetal pelviectasis is one of the more common abnormalities detected by second-trimester ultrasound, with a reported incidence of 0.5% to 5%. Diagnostic criteria differ but generally include a second-trimester renal pelvis diameter of 4 to 10 mm or a third-trimester renal pelvis diameter of 7 to 10 mm; renal pelvis diameters of ≥10 mm are always considered abnormal. Some experts consider mild fetal pelviectasis to be a soft marker for aneuploidy, especially trisomy 21. When mild fetal pelviectasis is an isolated finding, the prognosis is good, and the condition often resolves either in utero or during early childhood. In fact, the majority of children with a prenatal finding of hydronephrosis demonstrate no postnatal disease.[25–28] Experts thus recommend a postnatal follow-up renal ultrasound examination approximately 1 week after birth and, if necessary, at 1 month of life to document resolution.[3,29,30]

Evidence of hydronephrosis on prenatal ultrasound will need a postnatal evaluation with a renal ultrasound in order to identify obstructive lesions and determine the need for further evaluation for urinary reflux. The ultrasound is recommended to occur at greater than 48 hours after birth, as earlier evaluation can lead to false-negative results due to the relative oliguria that occurs on the first day of age in newborns. An ultrasound should be considered immediately after birth if evidence of obstructive uropathy is seen on the prenatal ultrasound.[29]

The Physical Examination

For the healthy newborn, the admission examination is done after the newborn completes transition and within 24 hours of birth. A complete physical examination at this time—in an orderly sequence—is designed to efficiently detect problems that were initially unapparent or are likely to soon develop (Box 16.1). The pediatrician should review the infant's growth chart to determine whether there are discrepancies in the weight, height, and head circumference percentiles and stated GA. Although the most common reason for a discrepancy is an inaccurate measurement, a valid discrepancy warrants close clinical observation or testing. If the estimated GA of the newborn is inconsistent with the growth parameters, a formal evaluation by a Dubowitz–Ballard

• BOX 16.1 Example Examination Sequence

1. Observation (is the baby sick or well?)
2. Auscultation of the anterior chest and abdomen (if the newborn is quiet and cooperative).
3. Inspect and palpate the head (the back of the head and neck will be inspected later).
4. Gently turn the head to each side (noting any restricted range of motion).
5. Inspect each ear when the head is turned.
6. Palpate the neck and clavicular areas (for masses and/or crepitus).
7. Determine overall features of facial shape and symmetry.
8. Confirm the presence or absence of abnormal findings involving skin, eyes, nose, mouth, and oral cavity.
9. Assess respiratory pattern.
10. Inspect and palpate the anterior chest and abdomen (including the umbilicus).
11. Open the diaper and palpate the femoral pulses.
12. Examine the genitalia and perineum.
13. Inspect the lower extremities for abnormal positioning to check alignment, plantar grasp, and the Babinski reflex (start by placing the thumbs on the soles of the feet with the fingers around the back of the ankles).
14. Perform the Barlow and Ortolani maneuvers.
15. Lift and abduct the legs into a frog-leg position to provide a full view of the perineum and anus. Evaluate the newborn for appropriate positioning of the anus and its patency.
16. Inspect the genitalia by gently retracting the labia majora in females or depressing the skin at the base of the penis in males. Inspect and palpate the scrotum and testes. Refasten the diaper.
17. Take an unobstructed observation of the overall shape, symmetry, and movements of the arms and hands (shirt/clothing removed).
18. Palpate each whole arm gently, starting with one of the examiner's hands on each of the baby's shoulders, and then slide down to the baby's hands, noting any swelling or discontinuities.
19. With the newborn supine, turn the head to elicit the asymmetric tonic neck reflex on each side.
20. Inspect the hands, fingers, nails, and palms. If the newborn's hand is tightly fisted, do not attempt to pry the fingers open. Instead, gently flex the wrist to 90 degrees, which will cause the fingers to relax naturally. Inspect the palms, and then elicit the palmar grasp reflex.
21. Without releasing the baby's hands, one can then perform the pull-to-sit maneuver. The pediatrician places his/her hand behind the newborn's head and neck to provide support as the newborn is gently lowered back toward the bed. When the newborn's head and shoulders are a few inches from the bed, the examiner drops his/her hand rapidly to elicit the Moro reflex. (Parents may be alerted to this portion of the examination to avoid unnecessary distress/anxiety.)
22. Next, place the hands on either side of the chest, under the arms at the shoulders, and raise the baby to an upright position. Note the strength and tone of the shoulder muscles.
23. Lower the newborn, still in an upright position, and try to elicit the supporting and stepping reflexes.
24. Turn the newborn to a prone position, suspended on the examiner's hand. Observe the newborn's posture and tone, and elicit the incurvation response (again, explanation in advance of these actions to parents who may be observing is helpful).
25. Inspect the newborn's back, from the vertex of the head down to the sacrum (pulling the diaper down, if needed).
26. Gently place the newborn back in the crib, and fully redress the newborn. Attempt to successfully soothe newborn by swaddling if the newborn has cried during the more active portions of the examination.
27. Red reflex examination can be performed at this time or at any suitable time when the eyes are open spontaneously.

assessment should be performed.[31] This scoring tool is probably the most widely used GA assessment tool in contemporary practice.[32] Detailed descriptions and a video demonstration of this examination are available online.[33,34] For greater detail on organ-specific aspects of the newborn physical exam, readers are referred to relevant sections and chapters in this textbook.

Routine Management of the Newborn

Prevention of Ophthalmia Neonatorum and Conjunctivitis

Approximately 1% to 12% of babies will develop conjunctivitis in the first 4 weeks of life.[35] Conjunctivitis can be caused by a sexually transmitted bacterium, normal skin or nasopharyngeal flora, or chemical irritation. In addition, eye discharge can be caused by obstruction of the nasolacrimal duct rather than from conjunctivitis.[36] The most worrisome infection is that of *Neisseria gonorrhea*, which can invade the cornea in a matter of hours and lead to blindness. Despite effective preventive measures known since the 1880s, thousands of children are still blinded by this infection worldwide each year.[37]

Most states in the United States have laws or regulations requiring the administration of topical antibiotic ointment to the conjunctivae of babies within a few hours of birth. This practice has been effective in reducing the cases of blindness caused by gonococcal conjunctivitis. It is moderately effective in preventing conjunctivitis caused by chlamydia. The main risk of antibiotic ointment application is chemical conjunctivitis. Silver nitrate solution instilled into both eyes immediately after birth was the standard of care for many years, but it caused a high rate of chemical conjunctivitis.[37]

A search for alternative, non-ointment-based prophylaxis that causes less irritation is ongoing. A variety of prophylactic treatments have been recommended, including 1% nitrate solution, 1% tetracycline solution, 1% erythromycin solution, 2.5% povidone-iodine solution, fusidic acid, and freshly expressed breast milk. Among those, tetracycline has been reported as the most effective.[38] There is lay literature recommending the instillation of colostrum or breast milk into the eyes of babies to prevent or treat conjunctivitis,[39,40] and limited scientific literature addressing the feasibility and efficacy of this approach.[41–43] A randomized controlled trial from Iran comparing the efficacy of colostrum versus erythromycin ointment versus placebo in preventing neonatal conjunctivitis demonstrated some degree of protection conferred by colostrum, but results should be interpreted cautiously and are insufficient to recommend practice change in the United States.[44]

Studies demonstrate that povidone-iodine solution is more effective and causes less irritation than erythromycin ointment. It is also less expensive but is not yet approved for this use by the US Food and Drug Administration.[45] A major concern is that medical errors can occur if povidone-iodine soap is mistakenly substituted for the solution; it can cause eye damage. Fusidic acid has been used for preoperative prophylaxis for a number of surgical procedures in adults; however, data on its use in newborns are limited.[38]

Vitamin K Prophylaxis

Vitamin K is necessary for biologic activation of several human proteins, most notably coagulation factors II (prothrombin), VII, IX, and X. Since placental transfer is limited, umbilical cord blood levels of vitamin K_1 (phylloquinone) are 30-fold lower than maternal levels. Intestinal bacteria synthesize menaquinone (vitamin K_2), which has 60% of the activity of phylloquinone. However, neonates have a decreased number of bacteria in their gut that manufacture vitamin K_2; thus, newborns are deficient in vitamin K at birth and are at risk of significant bleeding within the first days to months of life. There are three presentations of vitamin K–deficient bleeding (VKDB) in the neonate, with the risk dramatically reduced when intramuscular (IM) vitamin K is administered shortly after birth.

"Early" VKDB presents in the first 24 hours after birth, is not prevented by postnatal administration of vitamin K, and usually occurs in newborns born to mothers who are taking medications that cross the placenta and interfere with vitamin K metabolism. The most common of these medications include many anticonvulsants (such as phenytoin), isoniazid, rifampin, warfarin, and some antibiotics (especially cephalosporins). Early VKDB is frequently serious because of intracranial or intraabdominal hemorrhage. It is estimated that in neonates at risk of early VKDB, the incidence is as high as 12%.[46]

"Classic" VKDB occurs in newborns during the first week of life. Although the presentation is often mild, blood loss can be significant, and intracranial hemorrhages have been reported. Although estimates differ, the incidence of classic VKDB, in the absence of vitamin K supplementation, is 0.25% to 1.7%.[47]

"Late" VKDB occurs between the ages of 2 and 12 weeks and is usually severe. The mortality rate from late VKDB is approximately 20%, and half of infants with this disorder develop intracranial hemorrhages. Late VKDB is associated with exclusive breastfeeding. Human milk contains only 1 to 4 μg of vitamin K per liter, while commercially available formula contains 50 μg/L or more. In exclusively breastfed neonates who do not receive supplemental vitamin K, the incidence of late VKDB is estimated at 4.4 to 7.2 per 100,000 (or 1 per 15,000 to 1 per 20,000).[46]

IM vitamin K administered shortly after birth is effective in preventing classic and late VKDB by rapidly activating clotting factors. Since 1961, the United States has recommended 1 mg IM vitamin K for term newborns. In the early 1990s, controversy about this recommendation began after a study was published suggesting an association between IM vitamin K given at birth and childhood cancer.[48,49] The results of subsequent studies strongly suggested that there is no increased risk of solid tumors in children given IM vitamin K.[50] However, enough concern was raised over this possible association that in some countries vitamin K prophylaxis was transitioned to an oral preparation. While helping to allay fears about childhood malignancies, it was unclear whether an oral dosing regimen provided the same degree of protection against all forms of VKDB as the IM injection. Specifically, studies demonstrate that the efficacy of a single oral dose of vitamin K is similar to that of an IM dose in preventing classic VKDB, but offers less protection against late VKDB.

In a multination review, the rate of late VKDB in infants receiving various regimens of orally administered vitamin K ranged from 1.2 to 1.8 per 100,000, compared with no cases in 325,000 children receiving an IM dose.[51] The rates of VKDB in newborns receiving 2 mg orally at birth plus repeated doses over the ensuing weeks are relatively low, but still higher than in neonates administered a single dose of IM vitamin K.[52,53] Early data from the Netherlands suggested that infants receiving an oral vitamin K regimen of 1 mg at birth and 25 μg daily for up to 12 weeks was as effective as a single IM dose at birth in preventing both classic and late VKDB.[51] However, a subsequent study

from the Netherlands documented a higher rate of late VKDB (3.2 per 100,000) with the 1 mg/25 μg dosing regimen among a group of infants later diagnosed with biliary atresia,[54] a significant risk factor for VKDB. This study raised questions about the ability of an oral vitamin K regimen to offer adequate protection against VKDB for higher-risk populations when the identification of risk factors may be delayed. Finally, no cases of late VKDB were found among 396,000 Danish infants who received an oral dose of 2 mg of vitamin K at birth and 1 mg weekly until the age of 3 months.[55]

Highlighted by the reports of intracranial hemorrhages in four newborns from Tennessee who did not receive vitamin K at birth, there are concerns that the rate of parental refusal of vitamin K in the United States has been increasing.[56,57] Although there has been little widespread surveillance of the rates of vitamin K refusals, recent studies suggest that rates in North America may range from 0.5% to 3%,[58] and that rates are higher for newborns born in birthing centers than in hospitals.[59,60] Rather than being concerned about the reports linking vitamin K with childhood cancers, parents who refuse vitamin K treatment for the newborn are also more likely to refuse vaccines for their children at later ages and share many of the beliefs of other parents refusing vaccines for their children.[60] In one study the most commonly cited reason for parents refusing vitamin K treatment for their newborns was "synthetic or toxic ingredients," followed by concerns about an "excessive dose" and side effects; only 7% of those surveyed were concerned about the risks of cancer.[60]

The risks of IM vitamin K include pain at the injection site and the possibility of a serious medication error. The risks of a significant complication from the injection are probably negligible; in one study, no significant complications were reported after 420,000 injections.[61] In the United States, oral administration is complicated by the lack of an oral vitamin K preparation licensed for newborns. In some settings, infants have received the IM preparation orally. However, tolerability may be a problem, and the efficacy of this preparation when given orally may not be comparable with the oral formulations used in Europe. In addition, adherence to repeated doses of orally administered vitamin K in infants may be suboptimal. Finally, it is unknown whether the use of repeated administration of an oral vitamin K preparation in the dose range of 1 to 2 mg each week is associated with an increased risk of childhood cancers.

For parents who have questions regarding the best method to prevent classic and late VKDB, we suggest the clinician discuss the pros and cons of IM versus oral administration of vitamin K. If the parents decline IM vitamin K administration but agree to oral administration, a dose of 2 mg should be given shortly after birth, with subsequent doses until the newborn is at least 4 weeks old if breastfed. These recommendations are based on a policy report developed by the American Academy of Pediatrics (AAP) addressing prophylactic vitamin K administration, which states that *if an oral vitamin K formulation becomes licensed for use in the United States*, providers may administer 2 mg by mouth at birth, at 1 to 2 weeks of age, and at 4 weeks of age.[62]

Universal Hepatitis B Immunization

The implementation of routine hepatitis B virus (HBV) immunization during infancy has been associated with a dramatic decrease in the incidence of this infection. Between 1990 (before routine vaccination of infants) and 2004, the overall incidence of acute hepatitis B infection in the United States declined by 75% and by 94% among children and adolescents, respectively.[63] Both the Centers for Disease Control and Prevention and the AAP recommend that the initial dose of the three-dose HBV immunization series be given within the first 24 hours of life for medically stable infants with birth weight ≥ 2 kg and at day of life 30 or discharge (whichever is sooner) for infants with birth weight less than 2 kg.[64]

There are at least two advantages of providing the first dose of HBV vaccine during the initial hospital stay. First, newborns who receive a birth dose are more likely to complete their HBV immunization series on time than those who receive the first dose later.[65] Second, since a dose of HBV vaccine given within 12 hours of birth can prevent vertical transmission of HBV infections in 75% to 90% of cases, early provision of immunization serves as a "safety net" in cases where there has been an error in identifying a mother who is HBV surface antigen positive.[63] There is no evidence that administration of a birth dose of HBV vaccine leads to more evaluations for sepsis because of adverse events related to the immunization.

Newborn Feeding

Breastfeeding

There is voluminous evidence that the optimal nutrition for healthy neonates is human milk provided via the mother's breast. Growing evidence supports the role of human milk in the prevention of the early onset of allergies, prevention of adult obesity, reduction in severity and frequency of infections (including those leading to hospitalization in developed countries and those leading to death in developing countries), and increased intellectual functioning.[66] It is a public health imperative and incumbent on our society to provide systems that support breastfeeding.[67]

Support of Breastfeeding

Breastfeeding is not always the "easy and natural" undertaking it is touted to be. Primiparous mothers report more difficulties than multiparous mothers. Breastfeeding support begins with encouragement and education at prenatal visits. After birth, in-person lactation support is helpful in promoting both initiation and continuation of nursing.[68] Places of employment should provide support by having adequate maternity care leave policies, improving facilities for nursing women, and having policies allowing time and space for nursing and pumping for lactating women at the workplace.[69] Fathers and grandparents should provide a supportive social network by performing home care tasks to facilitate rest for lactating mothers.[70] Problems with nursing should trigger additional intervention with lactation specialist evaluation and advice.

In 1991, the WHO and the United Nations Children's Fund developed a program to promote breastfeeding called the Baby-Friendly Hospital Initiative (BFHI). As a comprehensive program, implementation of the 10 steps of the BFHI (Box 16.2) has been shown to significantly increase the rates of breastfeeding.[71] In addition, there is evidence of a "dose-response" relationship between the number of BFHI steps that women are exposed to and improved breastfeeding outcomes.[72]

For some of the individual steps of the BFHI, such as excluding the use of pacifiers, the evidence is contradictory.[73,74] There are a number of epidemiologic studies showing cessation of breastfeeding is associated with pacifier use, but the few randomized prospective trials done give different results. The authors of a review

• BOX 16.2 Baby-Friendly Hospital Initiative: Ten Steps to Successful Breastfeeding

1. Maintain a written breastfeeding policy that is routinely communicated to all healthcare staff.
2. Train all healthcare staff in the skills necessary to implement this policy.
3. Inform all pregnant women about the benefits and management of breastfeeding.
4. Help mothers initiate breastfeeding within 1 h of birth.
5. Show mothers how to breastfeed and how to maintain lactation, even if they are separated from their newborns.
6. Give newborns no food or drink other than breast milk unless medically indicated.
7. Practice "rooming-in"—allow mothers and newborns to remain together 24 h/day.
8. Encourage unrestricted breastfeeding (breastfeeding on demand).
9. Give no pacifiers or artificial nipples to breastfeeding newborns.
10. Foster the establishment of breastfeeding support groups and refer mothers to them on their discharge from the hospital or clinic.

concluded that among mothers who were motivated to breastfeed, pacifier use did not significantly affect the prevalence or duration of breastfeeding.[75] Sucking is a primitive brain self-soothing process. Babies with certain temperaments may benefit more than others by using sucking to self-soothe. Pacifier use has been shown in some studies since the 1970s to decrease the risk of sudden infant death syndrome (SIDS), and in premature infants, nonnutritive sucking actually enhances weight gain. The AAP developed a policy statement supporting pacifier use but recommended waiting until approximately 1 month of age.[66] The important issue is whether or not the use of a pacifier is replacing feedings, so if a mother is motivated to breastfeed and maintains a frequency of 8 to 12 feedings per day, the use of a pacifier between meals is reasonable.

Another contentious issue is the use of supplemental formula during the initial newborn period. The results of a randomized prospective trial indicated that use of limited supplemental formula was associated with increased breastfeeding rates at 3 months of age.[76] That seems contradictory to earlier studies showing a decline in nursing when formula samples or discharge packages were given to families. One key difference is that in the trial, formula use was limited to 10 mL after feedings, administered via a syringe, and supplement was discontinued once mature milk was produced. More research is needed to determine which mother–infant dyads will benefit from supplement while avoiding sabotage of breastfeeding.

From a practical standpoint, there are several evidence-based interventions during the newborn nursery stay that increase the rate and/or duration of breastfeeding. These include the use of frequent demand feedings as opposed to a rigid feeding schedule, early skin-to-skin contact between the mother and the newborn, professional advice on breastfeeding techniques, and exclusion of commercial formula from discharge packs.[77–80]

Challenges With Breastfeeding

Breast milk development is divided into three phases. The first, lactogenesis I, begins during pregnancy with breast enlargement due to the proliferation of ducts and lobules and concludes with colostrum production. Lactogenesis II occurs usually about 56 to 72 hours after delivery; gonadotropin and progesterone levels decline and prolactin level increases.[81] This phase is characterized by a rapid increase in milk volume—sometimes this is exuberant to the point of engorgement. Lactogenesis III occurs after approximately one month of nursing when the milk composition and volume are responsive to the reciprocal relationship between the mother and her baby—a demand and supply feedback loop. Some experts combine lactogenesis II and lactogenesis III into one phase.

Delays in lactogenesis II may occur after cesarean birth, in poorly controlled diabetic mothers, when there is stress during delivery, when there are retained placental fragments, and when there is pituitary failure. There are some situations when no milk production occurs, leading to frustration and feelings of failure in mothers. The timing of lactogenesis II is biologically fixed and cannot be accelerated by pumping or frequent nursing.[82,83]

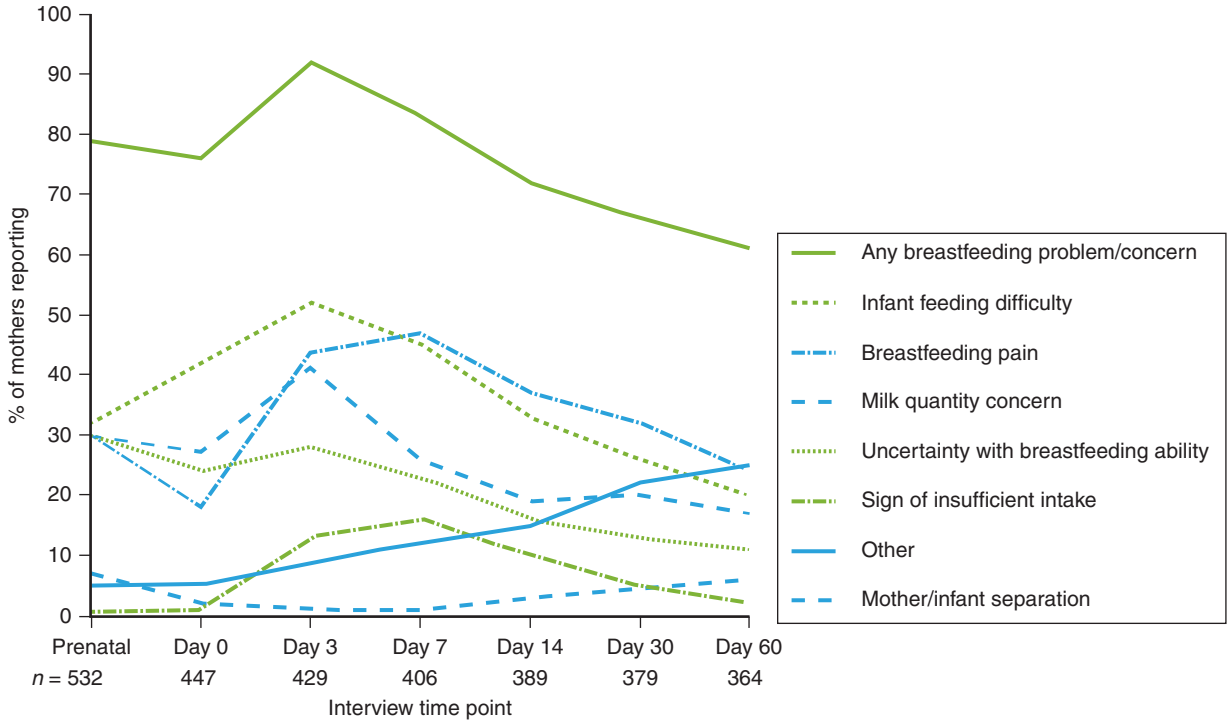
More than 90% of mothers report concern and difficulty with nursing during the first 10 days after delivery (Fig. 16.1).[84] Combined with hormonal changes and sleep deprivation, this can compound the risk of postpartum depression and early cessation of nursing. Postpartum depression screening should be conducted at pediatric health supervision visits until the infant is 6 to 12 months of age.[85]

Common issues that may lead to early cessation of breastfeeding include nipple pain, newborn jaundice, excessive weight loss or poor weight gain, concern about maternal medications, and lack of social support. There are also conditions associated with low milk volume production, including maternal factors (lack of social support, prenatal confidence and expectations about breastfeeding, timing of return to work, inadequate frequency of nursing, inadequate breast tissue, flat or inverted nipples, large breasts) and infant factors (hypotonia, drug withdrawal, asymmetric jaw, high arched palate, poor tongue motor abilities, temperamental issues).

For nipple pain, there is no treatment that is clearly advantageous (e.g., hydrogel, lanolin, breast milk, shields), but education on latch position is helpful. For most, the pain decreases within the first 7 days regardless of the treatment selected.[86] Nipple shields can be used to help decrease nipple pain, but there is some concern that their use could interfere with milk transfer.[87]

There has been a recent increase in the use of frenotomy to alleviate pain with nursing, presumably due to a tight lingual frenulum (tongue-tie or ankyloglossia). With the use of objective rating scales, the rate of tongue-tie in newborns is about 1% to 4%, but more infants are undergoing frenotomy, and there are concerns that this may be more due to anecdotal reports rather than more rigorous study.[88] Sometimes frenotomy is done to alleviate the frustrations of mothers (and lactation specialists) who are dealing with breastfeeding problems of unknown origin. There are few prospective studies evaluating frenotomy, and two recent systematic reviews did not find a consistent positive effect of lingual frenotomy on infant breastfeeding.[89,90] It may be prudent to wait until the physiologic process of lactogenesis II and early nipple pain have passed before frenotomy is considered. Although the procedure is simple and relatively free of side effects, unnecessary interventions should be avoided in newborn care whenever possible. Frenotomy for posterior tongue-tie has received extra scrutiny because evidence is lacking to support improved outcomes after treatment, and the procedure is more invasive.[91]

Jaundice became more prevalent with the resurgence of breastfeeding and is discussed in more detail elsewhere in this textbook. Consistent with efforts to promote breastfeeding, a rising bilirubin level should serve as a call for action to emphasize lactation



• **Fig. 16.1** Prevalence of reported breastfeeding concerns by mothers by newborn age. (Modified from Wagner EA, Chantry CJ, Dewey KG, et al. Breastfeeding concerns at 3 and 7 days postpartum and feeding status at 2 months. *Pediatrics*. 2013;132:e865–e875.)

support rather than lead to a separation of the newborn from the mother and artificial feeding to lower the bilirubin level.

The normal newborn is born with a surplus of extracellular free water, and in cesarean delivery births, mothers are often given additional boluses of fluids that may further hydrate the newborn.[92] It is normal, expected, and perhaps preferable that babies will lose this free water in the first 72 hours of life. This free water is protective of the newborn's fluid balance while the mother's milk comes in.[93] In cases of extra hydration, extra weight loss may be expected. The average term newborn loses about 7% of birthweight, with 12% of newborns born vaginally losing more than 10% of birthweight (Fig. 16.2).[94] The loss during the first 24 hours of life can predict those who will lose more. This is not a state of dehydration but is a normal physiologic adaption to extrauterine life, so healthcare providers should not alarm parents or suggest that there is something wrong with their baby.

With the onset of copious production of mature milk, neonates begin to gain weight and their serum sodium levels fall.[95] Newborns fed human milk regain their birthweight, on average, by the age of 8.3 days; 97.5% have regained their birthweight by 21 days.[96] In newborns who lose substantially more than 10% of their birthweight because of breastfeeding difficulties, there is the potential for significant hypernatremia, and these infants should be monitored closely.[97]

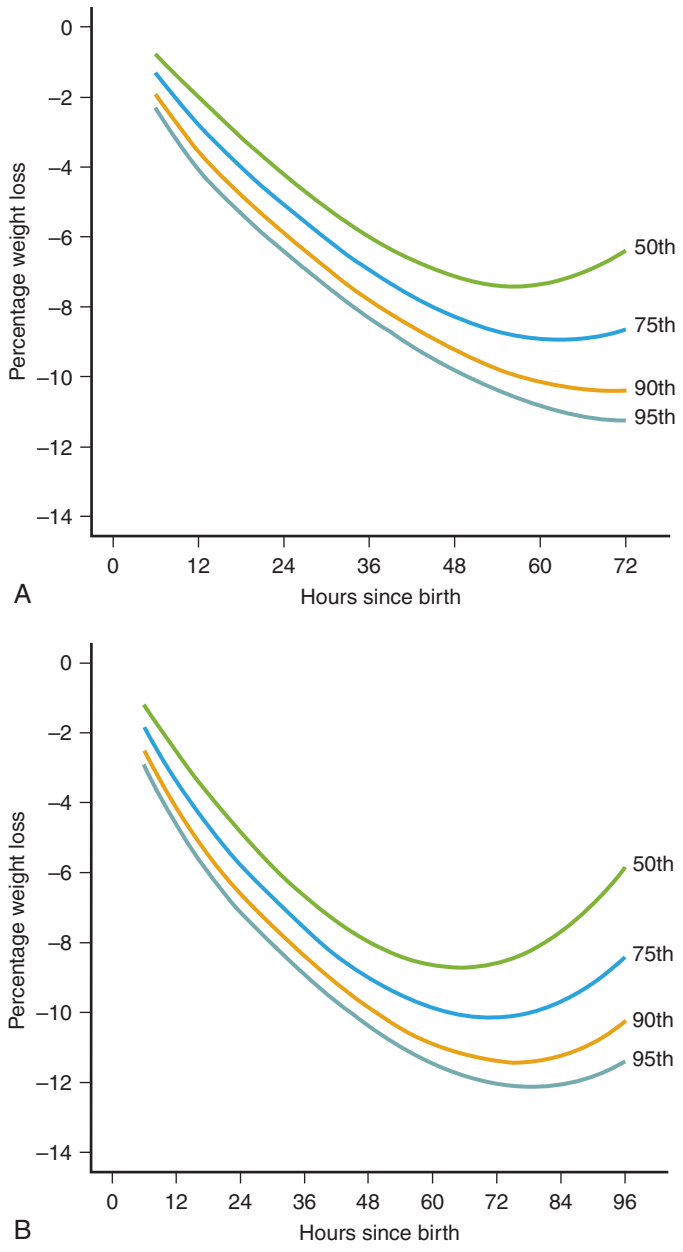
Supplementation of Breastfeeding

It is usually unnecessary to provide any nutrition or fluid to breastfed term newborns beyond human milk. Oral dextrose or commercial formula may be needed in neonates with hypoglycemia whose blood glucose levels are not responsive to breastfeeding alone. Supplementation may also be indicated in newborns who have lost more than 10% of their birthweight and/or have decreased urine and stool output or in the presence of significant hyperbilirubinemia. Supplementation should be considered a temporary intervention, and its provision should not interfere with the initiation of successful breastfeeding.

Temporary supplemental formula or expressed breast milk when available can be provided via a supplemental nursing system, finger feeding, or a bottle. Of greatest importance is close monitoring of the change in weight of the baby and continued lactation support. The use of banked or donor milk is increasing, and human milk banks have now been established in over 60 countries globally. However, there remains little authoritative guidance on the implementation, operation, and regulation of human milk banks and appropriate the use of donor milk. The importance of safe operational guidelines and a coordinating body to collate and communicate data have been recently highlighted by the milk-banking community,[98] and efforts to create the evidence base necessary for guideline development are ongoing. However, in the absence of existing evidence and guidelines, there remains concern about the routine use of donated human milk outside of the hospital setting, especially for otherwise healthy term newborns for whom there is a paucity of evidence to support its value in promoting health and mitigating illness.

Contraindications to Breastfeeding

The few absolute contraindications to breastfeeding include untreated maternal tuberculosis, evidence of current maternal cocaine use or antimetabolite drugs, and neonatal galactosemia.[99,100] Hepatitis C virus RNA has been found in the milk of mothers infected with this virus; however, the transmission of infection via breastfeeding has not been documented. Therefore, maternal hepatitis C is not considered a contraindication to breastfeeding.[99]



• **Fig. 16.2** Nomograms of weight loss in exclusively breastfed newborns born vaginally (A) or by cesarean delivery (B). (Modified from Flaherman VJ, Schaefer EW, Kuzniewicz MW, et al. Early weight loss nomograms for exclusively breastfed newborns. *Pediatrics*. 2015;135:e16–e23.)

Maternal HIV infection has historically been considered an absolute contraindication to breastfeeding. However, in more recent years, there has been an increase in the number of HIV-positive mothers expressing a desire to breastfeed. Oftentimes, mothers are from low- and middle-income countries where the risk-benefit analysis of breastfeeding is different, due to high rates of infant mortality and limited access to safe water and affordable infant formulas. In this context, although the risk of HIV transmission with breastfeeding is present, it is lower than the risk of infant morbidity and mortality from other causes, especially with strict maternal adherence to an antiretroviral medication regimen and persistently undetectable viral loads. In many instances, these mothers have breastfed previous children in their home country, and are confused or resistant when breastfeeding is not recommended upon relocation to the United States. Lastly, in some cultural communities, especially enclaves of families originally from Africa, the maternal HIV diagnosis is not disclosed to the extended family, and the mother choosing to formula feed and avoid breastfeeding raises suspicion of maternal HIV infection and stigmatizes the mother.

Although breastfeeding among HIV-positive mothers in the United States remains strongly discouraged, there are some mothers who will decide to breastfeed, despite extensive counseling. In these instances, risk-reduction strategies should be implemented to reduce the possibility of HIV transmission. For more detail, readers are referred to Chapter 34, Viral Infections of the Fetus and Newborn. In addition, comprehensive recommendations and further details regarding risk-reduction strategies can be found on the Center for Disease Control and Prevention's website.[101] Due to the complexity and clinical equipoise surrounding this issue, decision-making should include outpatient pediatric providers who will be responsible for follow-up care.

There are a number of drugs that raise concern for adversely impacting infant long-term neurodevelopment. Selective serotonin reuptake inhibitors are commonly used to treat depression and anxiety in young women. Among the drugs in this category, sertraline and paroxetine are thought to be the safest for use in breastfeeding mothers, while fluoxetine and citalopram are felt to have the most potential for toxicity in the neonate.[100] Overall, few adverse effects have been noted with the use of any of these drugs, and generally the potential risks associated with these medications are thought to be outweighed by the benefits of breastfeeding.[100] Similarly, although methadone is detectable in the breast milk of women receiving this medication, serum levels in neonates are quite low and unlikely to have a significant effect.[102] Online references are available detailing the current scientific knowledge of the effects of toxins and medications in breast milk.[103]

Formula Feeding

Commercial formula that provides adequate nutrition, vitamins, and minerals is available for infants of mothers who do not choose (or are unable) to breastfeed their infants or in those rare instances when breastfeeding is contraindicated. There are three major categories of formula used in neonates: cow's milk-based, soy, and hydrolyzed formula. Of these, cow's milk-based formula is the most commonly used. The main carbohydrate in cow's milk-based formula is lactose. Soy formulas were developed for infants with suspected cow's milk allergy. Because the main carbohydrate in soy formulas is sucrose or corn syrup, soy formula can be used in neonates with suspected galactosemia. Protein hydrolysate formulas were initially developed for use in infants who are highly intolerant to cow's milk protein.[104] They are purported to lead to fewer allergies in babies and children than cow's milk-based formula, but the evidence for this is limited.[105] All extensively hydrolyzed formulas are lactose-free.[104] Extensively hydrolyzed formulas are indicated in infants with definitive evidence of cow's milk protein allergy because 10% to 14% of such children also have a soy allergy.[106]

Traditionally, standard preparations of formulas available for use in healthy term neonates provided 0.67 kcal/mL. This caloric density was based on the calories in human milk. However, the results of some studies indicate that the average caloric density of human milk may be closer to 0.64 to 0.65 kcal/mL. Based on

these additional data, some infant formulas have been modified to provide 0.643 kcal/mL (19 kcal/oz).[107] Most commercially available formulas are fortified with iron at a concentration of 10 to 12 mg/L and vitamin D at a concentration of 400 IU/L.[108]

Mothers who elect to give their babies formula report feeling unsupported in their decision by healthcare professionals, and up to 50% feel pressured to breastfeed.[109] Although the benefits of breastfeeding should be provided to mothers who have not decided how to feed their babies, the role of healthcare providers is also to support the decision of those who have elected to provide formula feedings to their babies. It is also important to provide practical education about formula feeding to these parents; this is frequently not done in many newborn nurseries.[109]

Newborns who are fed formula can feed ad lib beginning shortly after birth. The average formula intake in term newborns during the first day of life is 15 to 20 mL/kg and is 40 to 45 mL/kg during the second day.[110] Term newborns who are formula fed during their birth hospitalization typically lose less weight than breastfed infants.[94,111] The median weight loss in formula-fed term newborns at 48 hours of life has been reported to be 2.9% of birthweight for those born vaginally and 3.7% among those born by cesarean delivery; weight loss of 7% or more during a typical newborn nursery stay in formula-fed infants is uncommon.[111]

Umbilical Cord Care

Bacterial colonization of the umbilical cord can lead to omphalitis, and in some cases may be associated with thrombophlebitis, cellulitis, or necrotizing fasciitis. To decrease the risk of bacterial infection and serious illness, many methods of umbilical cord care have been developed worldwide. Although various topical substances continue to be used, the more recent practice in high-resource settings has been towards dry umbilical cord care. Based on the results of multiple meta-analyses, dry cord care without the application of topical substances is currently recommended for hospital births and those in high resource settings; for infants born outside of the hospital in communities with high neonatal mortality rates, application of topical chlorhexidine is recommended.[112] Regardless of the method of cord care used, parents and healthcare providers should monitor for redness around the umbilical cord stump, and seek medical attention and further intervention to avoid serious bacterial illness.

Tetanus neonatorum, with the infection occurring via the umbilical cord, continues to be reported in more than 20 developing countries, resulting in 58,000 neonatal deaths per year. The condition is related to low vaccination rates in women of childbearing age, home deliveries, and certain cultural care practices. Public health efforts focusing on effective vaccination programs and the use of "clean bed" deliveries are needed to eliminate the disease.[113]

Circumcision

Neonatal circumcision is a polarizing issue for both healthcare professionals and parents. Those who favor routine circumcision highlight health benefits such as the decreased risk of urinary tract infections (UTIs), reduced risk of penile cancer, and possibly lower rates of sexually transmitted infections, including HIV.[114] Those who oppose the procedure point out that the number of circumcisions needed to be performed to prevent one of these outcomes (number needed to treat) is large, that the risks of the procedure balance out the benefits, that circumcision may lead to loss of sexual sensation, and that subjecting a neonate to a painful procedure without clear benefits may be unethical.[115] In 2012, the AAP published a policy statement concluding that the benefits of circumcision outweighed the risks of the procedure. However, these health benefits were not great enough to recommend routine circumcision in all male neonates.[116]

It is clear that circumcision reduces the risk of UTI by 3- to 10-fold.[117] However, given the low incidence of UTI in male newborns, 100 boys need to be circumcised to prevent one UTI. Similarly, although circumcision has been shown to prevent penile cancer, this is an extremely rare condition, and the number needed to treat is about 900.[118] The results of studies in three African countries indicate that circumcision reduces the risk of HIV infection by 56%.[119] In the United States, where HIV infection rates are lower, it has been estimated that circumcision might decrease the acquisition of HIV through heterosexual transmission by 16%; 298 boys would need to be circumcised to prevent one case of HIV infection.[120] There is also limited evidence suggesting that circumcision might reduce the risk of other sexually transmitted infections, including syphilis and genital herpes. However, there is no compelling evidence that circumcision reduces the risk of chlamydia or gonorrhea.[117]

Circumcision is generally a safe procedure. Although some increased bleeding is reported after 1% of circumcisions, the rate of significant complications is about 0.2%.[118,121,122] Bleeding, sometimes requiring suturing of a vessel, is the most common significant complication, followed by penile injury and infection. Infection is more common following a circumcision using a Plastibell rather than a Gomco clamp; the incidence of hemorrhage is reportedly similar after either technique.[121]

Circumcision is an uncomfortable experience for the neonate. Small amounts of sucrose solutions can be offered to the baby for soothing. Pain from the actual surgery can be significantly decreased with the use of a dorsal penile nerve block or ring block.[117] In one study, 65% of newborns who received a dorsal nerve block had no or minimal response to the initial clamping of the foreskin.[123] However, the results of a randomized controlled trial suggest that ring block provides superior analgesia compared with a dorsal penile nerve block.[124] Although topical anesthesia may be better than no anesthesia, it provides inferior pain relief compared with a dorsal penile nerve block.[125]

A poor cosmetic outcome can be caused by the removal of too little foreskin. It has been estimated that 1% to 9.5% of circumcisions are redone because of parental concern regarding the appearance. In a prospective study among boys younger than 3 years who had been circumcised with the use of either a Plastibell clamp or a Mogen clamp, the glans were fully exposed in only 35.6%. However, in older circumcised boys, the glans was fully exposed in more than 90%.[126] This suggests that parents of a circumcised infant should be counseled that the vast majority of properly done circumcisions will lead to an acceptable cosmetic appearance over time.

In the United States, the Gomco clamp is the most commonly used apparatus for performing circumcisions, followed by the Plastibell clamp and the Mogen clamp.[127] The use of the Mogen clamp, which was designed by a Jewish mohel, leads to shorter procedures and, reportedly, less pain and bleeding than the other techniques.[123,128,129] However, less foreskin is removed with the use of the Mogen clamp than with the other two techniques.[130]

Newborn Metabolic Screening

Newborn screening (NBS) for metabolic disorders began in 1962 when 29 states participated in a trial of testing for phenylketonuria (PKU). The advent of tandem mass spectrometry in the 1990s revolutionized newborn metabolic screening by increasing the number of conditions that could be tested for using a small sample of blood. In 1995, the average number of conditions included in state-mandated screening programs was 8; by 2005 this had increased to 19, with some states testing up to 46 conditions. However, this increase in conditions included in the NBS has been controversial. Not all of the conditions included meet the established criteria for screening in that there are no known effective treatments, and, in some cases, it is not known whether the targeted condition always leads to disease. In addition, increased testing results in more false-positive results, which leads to increased parental anxiety and potential overuse of medical services for confirmational testing.[131,132]

In an attempt to define a rational list of disorders for which NBS is appropriate, the American College of Medical Genetics used an iterative process to identify "core conditions" that should be included in mandatory screening programs.[133] Subsequently, federal legislation was passed to facilitate standardization of NBS across the United States. This legislation led to the development of the Recommended Universal Screening Panel, which initially included 29 core disorders. All US states currently provide testing for all of these disorders.[132] The recommended screening panel has now been expanded to include 32 core disorders and 26 secondary disorders. In addition to newborn hearing screening and screening for critical congenital heart disease (CCHD), there are nine organic acids, five fatty acid oxidation, six amino acids, two endocrine, three hemoglobin, and four other conditions included in the list of core disorders. Information on state-by-state screening programs can be found online.[134]

The most common disorders included on newborn metabolic screens in the United States are congenital hypothyroidism (1 case per 3000 to 4000 newborns) and sickle cell disease.[135,136] The incidence of PKU is approximately 1:15,000.[137] For many of the core conditions for which screening is now recommended, the incidence rates are in the range of 1:100,000 to 1:200,000.[135] For additional information, see Chapter 18, Newborn Screening.

Hearing Screening

Newborn hearing screening has become universal in the United States, with more than 98% of newborns screened in 2018, the most recent year for which national statistics are available. Every state and territory in the United States has now established an early hearing detection and intervention program and is required to provide tracking data. Newborns who do not "pass" the hearing screen in the newborn nursery should be referred for definitive outpatient testing in a timely manner. The prevalence of hearing loss among screened infants is 1.7 per 1000. Of those infants who did not pass their final screening (1.6%), 53.4% had no documented hearing loss, 10.7% had documented hearing loss, and 35.9% had no documented diagnosis.[138,139] Many other countries have adopted or are in the process of adopting universal hearing screening. Experts from the WHO endorsed universal newborn hearing screening in 2009. It is reported that 80% of early childhood hearing loss is congenital and that most cases have genetic origins or are a result of CMV infection.[140,141]

There is growing evidence that early intervention with amplification or cochlear implants can improve childhood reading, language, and communication skills.[142–145] These treatments are effective when implemented by 6 months of age, preferably younger. For infants who do not pass the hearing screen, medical providers and families should act swiftly to complete definitive diagnostic testing and refer the infant to specialists with expertise in the treatment of early hearing loss. This process is particularly challenging in rural areas and in countries with limited access to these services.[146]

It is recommended that the term "refer" be used instead of "fail" when the screen results are being discussed with families. Babies have an increased "refer" rate when born by cesarean delivery or screened during the first day of life, so performing the screen on the third or fourth day of life, or at least after 4 hours of age, will aid in decreasing false positives.[147–149] Many nurseries have adopted a two-step process using an automated otoacoustic emissions (OAE) test for the first step followed by a brainstem auditory evoked potential test in those who do not pass the automated OAE test. This process has been shown to decrease the false-positive rate.[150,151] False-negative rates are low, occurring in an estimated 0% to 2% of newborns, and are further minimized with the use of auditory brainstem response (ABR) screening.[152,153]

All newborns who are at high risk of early hearing loss should be sent directly for ABR screening. High-risk factors include premature birth, family history of early childhood or infant hearing loss, craniofacial anomalies or abnormal ear examination findings (includes microtia but not tags), and prolonged exposure to aminoglycoside antibiotics.

Screening for Critical Congenital Heart Disease

It has been estimated that approximately 25% of newborns with complex congenital heart disease (CCHD) have "critical" lesions, defined as a lesion requiring surgery and/or cardiac catheterization in the first year of life.[154] Overall, CCHD is diagnosed in fewer than half of newborns prenatally, and in only 70% to 75% of affected newborns during the birth hospitalization.[155] Further, some neonates with lesions that are amenable to surgical intervention who are not identified as having CCHD before discharge from their birth hospitalization may die from their CCHD before a clinical diagnosis is made.[155] Because of this, it is recommended that all newborns be screened with pulse oximetry for CCHD prior to discharge from their birth hospital.[156]

Pulse oximetry, a relatively inexpensive and noninvasive method of determining peripheral oxygen saturation, was chosen as the screening modality for CCHD based on the idea that most (but not all) CCHD lesions cause hypoxemia. To minimize false-positive results, pulse oximetry screening should be delayed until newborns are 24 hours or older, if possible. Conversely, since some newborns with CCHD have different oxygen saturation levels when measured preductally or postductally, false negative results are reduced by screening newborns both in the right hand (preductal) and in a lower extremity (postductal). Newborns with a measured oxygen saturation level of less than 90% at either site are classified as having a positive screen and should be evaluated by a pediatric provider on an urgent basis. Repeated screening is recommended for newborns with oxygen saturation levels between 90% and 95% or with a difference of 3 or more percentage points between the right hand and the lower extremity; if either of these findings persists after three screenings, the screen is considered positive.[157] Alternative algorithms, using only the lower extremity

as the initial screen, or modifications for high altitudes, have been adapted in some states.[158]

There were initially concerns that widescale implementation of universal pulse oximetry screening would result in a high proportion of false-positive results, leading to unnecessary and expensive evaluations of normal newborns. These concerns have been largely unfounded; a recent Cochrane systematic review including 19 studies conducted from 2002 to 2017 found routine CCHD screening to have a false-positive rate of 0.14%, with sensitivity of 77% and specificity of 99.9%. Of note, the false-positive rate for detection of CCHD was lower when newborn pulse oximetry was performed after 24 hours of age compared to when it was performed within 24 hours of birth.[159] Although an excellent screening test, because the sensitivity of pulse oximetry screening is not 100%,[160] a diagnosis of CCHD should remain high on the differential for a young infant with signs or symptoms consistent with cardiac disease, even if a negative CCHD screen was documented during the birth hospitalization.

Common Problems in Newborn Care

Hypoglycemia

Fetal blood glucose levels are approximately 70% of maternal levels. Following birth and separation from its major energy supply, the newborn's glucose level falls by half. Over the next several hours, it gradually increases to a level approaching that of older newborns. The critical factors involved in this normal adaptive process include transient inhibition of the newborn's insulin secretion and an increase in levels of the counter-regulatory hormones growth hormone, cortisol, catecholamines, and glucagon.[161] This results in the promotion of liver glycogen breakdown, gluconeogenesis, and tissue lipolysis.

Risk Factors

Infants with increased risk for hypoglycemia and who may benefit from early glucose screening and possible therapeutic intervention include:

- Newborns of diabetic mothers
- Large for GA newborns
- Small for GA newborns
- Premature newborns
- Newborns delivered following in utero or intrapartum distress
- Family history of congenital hyperinsulinism
- Newborns with midline facial anomalies (possible marker of pituitary deficiency)

Clinical Presentation

Screening infants with risk factors is essential as those with hypoglycemia may be asymptomatic. Infants with symptomatic hypoglycemia exhibit clinical signs and symptoms including poor feeding, lethargy, hypotonia, irritability, tremor, high-pitched cry, seizure-like activity, and apnea. An infant exhibiting any of these signs should be evaluated by a medical provider and should have serum glucose checked immediately.

Differential Diagnosis

Causes of neonatal hypoglycemia—both transient and persistent—are shown in Box 16.3.

Initial Management

The treatment approach to confirm hypoglycemia depends on the glucose level and the presence of clinical signs. Newborns with symptomatic hypoglycemia require immediate intervention.[162] However, while it is apparent that severe and symptomatic hypoglycemia can result in brain injury leading to neurodevelopmental issues, the effects of less severe and asymptomatic hypoglycemia on the neonatal brain are much less clear.[163,164] Thus there is considerable debate regarding the definition of hypoglycemia and an actionable glucose level in this early transition period in asymptomatic newborns. The HypoEXIT (Hypoglycemia-Expectant Monitoring versus Intensive Treatment) trial,[165] a randomized controlled trial comparing two accepted glucose value thresholds, demonstrated that in otherwise healthy newborns with asymptomatic moderate hypoglycemia, a lower glucose treatment threshold (36 mg/dL) was noninferior to a traditional threshold (47 mg/dL) with regard to psychomotor development at 18 months. This study may provide support for less aggressive diagnostic and treatment interventions for managing asymptomatic hypoglycemia, which could be beneficial for minimizing disruptions to parental bonding and promoting the establishment of maternal breastfeeding. However, these results are not generalizable to infants at higher risk of impaired developmental outcomes or those with persistent hypoglycemia. Depending on institutional policies, glucose levels, and presence or absence of symptoms of hypoglycemia, management may include offering additional enteral feedings, supplementation of breastfeeding, buccal administration of dextrose gel,

• BOX 16.3 Causes of Hypoglycemia

Transient Hypoglycemia

Maternal conditions

- Glucose infusion in the mother
- Preeclampsia
- Drugs (tocolytic therapy, sympathomimetics)
- Infant of a diabetic mother

Neonatal conditions

- Prematurity
- Respiratory distress syndrome
- Twin gestation
- Neonatal sepsis
- Perinatal hypoxia-ischemia
- Temperature instability (hypothermia)
- Polycythemia
- Specific glucose transporter deficiency
- Isoimmune thrombocytopenia, Rh incompatibility

Persistent Hypoglycemia

Endocrine disorders

- Pituitary insufficiency
- Cortisol deficiency
- Congenital glucagon deficiency

Inborn errors of metabolism

- Carbohydrate metabolism (glycogen storage disease, galactosemia, fructose 1,6-diphosphatase deficiency)
- Amino acid metabolism (maple syrup urine disease, propionic acidemia, methylmalonic acidemia, hereditary tyrosinemia)
- Fatty acid metabolism (acyl coenzyme A dehydrogenase defect, defects in carnitine metabolism, β-oxidation defects)
- Defective glucose transport

and glucose-containing intravenous fluids. For more detail, see Chapter 87 which discusses evaluation, diagnosis, and treatment of neonatal hypoglycemia.

Respiratory Distress

The vast majority of term or late preterm newborns accomplish the transition from dependency on the placenta to the newborn cardiorespiratory system without incident. After birth, pulmonary blood flow increases, fetal shunts reverse and begin to close, spontaneous breathing effort is initiated, and fetal lung fluid is cleared. Effective cardiorespiratory function is usually established by several hours of age.[166,167]

Perinatal Risk Factors

- Preterm birth
- Prolonged rupture of membranes
- Oligohydramnios or anhydramnios
- Chorioamnionitis
- Meconium-stained amniotic fluid
- Intrapartum distress
- Delivery via unlabored cesarean section
- Exposure to maternal medications (magnesium, anesthetic agents)
- Infant of a diabetic mother

Clinical Presentation

Respiratory distress, including nasal flaring, grunting, chest wall retractions, tachypnea (respiratory rate >60 breaths/min) and hypoxia (oxygen saturation <95%), occurs in 5% to 7% of live births at term gestation and in 2% to 8% of infants born at 34 weeks' gestation or later.[168–171] The initial presenting symptoms can range from mild to severe, are relatively nonspecific, and may indicate the presence of a very common or an extremely rare condition. Therefore, any neonate with respiratory distress should be examined promptly and supportive measures implemented while completing initial studies and determining a management plan.

Differential Diagnosis

- Transient tachypnea of the newborn (TTN)
- Respiratory distress syndrome (RDS)
- Meconium aspiration syndrome
- Spontaneous pneumothorax
- Sepsis
- Congenital pneumonia
- Persistent pulmonary hypertension of the newborn (PPHN)
- Pulmonary hypoplasia
- Complex congenital heart disease
- Congenital diaphragmatic hernia
- Inherited primary lung diseases (primary ciliary dyskinesia, congenital surfactant deficiencies, alveolar capillary dysplasia)

Initial Management

In most cases, initial diagnostic efforts for a term newborn with unexpected respiratory distress should include a chest X-ray and an arterial blood gas, including an assessment of the arterial oxygen saturation. The results of these studies, in combination with the clinical exam and maternal history, should provide information helpful to (1) establish the initial management, such as the need for supplemental oxygen and/or continuous monitoring; (2) determine the need for further work-up or treatment, possibly including an echocardiogram, laboratory testing, and treatment for possible sepsis; or (3) in severe cases, refer the newborn for further specialty consultation and management. For additional information, refer to Chapter 42, Acute Neonatal Respiratory Disorders.

Cardiovascular Concerns

Congenital heart disease is a relatively common condition in newborns, with an estimated incidence of 81 per 10,000 live births.[172] The increasing accuracy of prenatal ultrasound examinations has greatly improved the early diagnosis of complex congenital heart disease (CCHD). The results of population-based reviews indicate that the sensitivity of routine prenatal ultrasound examinations in identifying selected congenital defects is as high as 70% to 85%.[173,174] For mothers at high risk of delivering a newborn with CCHD, the use of fetal echocardiography is helpful for delineating the anatomy and predicted clinical significance of specific lesions.

Perinatal Risk Factors

- Product of in-vitro fertilization
- Infants born to mothers with pregestational diabetes
- Family history of congenital heart disease
- Maternal history of autoimmune disorders (especially systemic lupus erythematosus)

Clinical Presentation and Initial Management

At birth, many babies have loud murmurs, often due to the closing ductus arteriosus or tricuspid regurgitation.[175] These murmurs are transient and not indicative of disease. Conversely, murmurs associated with VSDs may not be heard for several days, when the pulmonary vascular resistance has dropped enough to permit a significant shunting of blood from left to right across the ventricular septum. Although the ratio of pathologic to benign murmurs is higher in newborns than in older children, most of the murmurs heard during the birth hospitalization in a healthy neonate are not clinically significant. Characteristics that increase the likelihood that a murmur is pathologic include a harsh quality, auscultation during all of systole or into diastole, and loudest at the lower sternal border or right upper border.[176] In a healthy newborn the most common presentation of CCHD is a harsh systolic murmur heard best at the left lower sternal border in an asymptomatic newborn, corresponding to a VSD.

In addition to auscultation, it is helpful to assess a newborn with a murmur for dysmorphic features or other anomalies, as these findings increase the likelihood that the murmur is indicative of CCHD. It is important to evaluate the adequacy of femoral pulses to rule out coarctation of the aorta. Femoral pulses may be difficult to palpate in a neonate; if there is uncertainty, upper and lower extremity blood pressures can be measured. Chest radiographs and electrocardiograms are usually of limited value in evaluating healthy newborns with murmurs. For more detail, readers are referred to Chapter 50, Congenital Heart Disease.

Term neonates frequently have alterations in cardiac rhythm and rate. Heart rates in term newborns may be as high as 200 beats/min (particularly when agitated) or as low as 80 beats/min (when in deep sleep). These values are usually indicative of normal variation and are not clinically meaningful unless there are other signs of illness or if there is a lack of variability in rate with stimulation or attempts at calming the newborn. Arrhythmias

are relatively common, occurring in approximately 1% of newborns.[177] By far the most common arrhythmia in a well-appearing term newborn is from premature atrial contractions.[177] These are usually benign and transient, and in most cases, no further workup is indicated. If there is concern about an irregular rhythm in a newborn, an electrocardiogram can be obtained. The maternal history should be reviewed for risk factors for arrhythmia, such as autoimmune diseases, which are associated with congenital heart block.[178] For more information about the electrocardiographic features and management associated with different arrhythmias, readers are referred to Chapter 49, Perinatal Arrhythmias.

Early-Onset Sepsis

Although early-onset sepsis (EOS) is a rare occurrence in term neonates, early identification of a newborn with EOS is a central focus of newborn care and a source of considerable anxiety for clinicians. This anxiety is driven by two unique features of EOS. First, the phenomenon of an apparently healthy newborn becoming moribund from overwhelming sepsis in a matter of hours is well described. Second, the earliest signs of infection are frequently subtle and nonspecific. Fortunately, with the advent of screening mothers for group B streptococcus (GBS) colonization prenatally and providing intrapartum antibiotics for those colonized, rates of EOS have fallen dramatically.

Perinatal Risk Factors

- Prematurity
- Prolonged rupture of membranes
- Maternal fever
- Chorioamnionitis
- Maternal GBS colonization without adequate intrapartum antibiotic prophylaxis
- Sibling with a history of EOS

Clinical Presentation

Early identification of a newborn with EOS based on clinical and laboratory findings is challenging. A number of signs and symptoms have been reported to be present in neonates with EOS, including fever, hypothermia, temperature instability, hypotension, lethargy, irritability, pale or mottled skin, cyanosis, apnea, hypoglycemia, acidosis, tachycardia, tachypnea, grunting respirations, nasal flaring, and inspiratory retractions. Unfortunately, there is a paucity of information delineating the frequency of symptoms in term newborns with EOS versus those without, as well as how often infected infants demonstrate symptoms early versus later on in the disease course. Therefore, the efficacy and reliability of most clinical signs and symptoms in diagnosing EOS are unknown. Of note, while creating a predictive model to quantify the risk of sepsis in infants ≥ 34 weeks' gestation, researchers did identify four specific and objective clinical signs of sepsis: temperature of 38.0°C or greater or less than 36.4°C; heart rate of 160 or more beats per minute; respiratory rate of 60 or more breaths per minute; and respiratory distress (grunting, flaring, and retractions).[179]

Initial Management

Infants born at $\geq 35^{0/7}$ weeks' gestation can be stratified by the level of risk for EOS. Per the 2018 AAP recommendations, acceptable approaches to risk stratification include categorical algorithms in which threshold values for intrapartum risk factors are used, multivariable risk assessment based on both intrapartum risk factors and infant examinations, and serial physical examination to detect the presence of clinical signs of illness after birth.[180] These approaches provide guidance in determining which infants are at higher risk of EOS and how to decide next steps based on the infant's clinical appearance and symptoms. The Neonatal EOS Risk Calculator[181] is an example of this approach, which synthesizes the risk factors and clinical symptoms previously identified as predictive of EOS into an interactive, user-friendly calculator for use in daily clinical practice.[179,182–184] In addition, local guidelines are important for determining when to escalate to higher levels of care, such as observation in the nursery or admission to the neonatal intensive care unit (NICU). For more detail, readers are referred to Chapter 33, Neonatal Bacterial Sepsis and Meningitis.

Hyperbilirubinemia

Bilirubin is a molecule formed from the breakdown of heme, the iron-containing, nonprotein component of hemoglobin. Normal levels of bilirubin for older children and adults range from 0.3 to 1.0 mg/dL,[185] but in newborn infants, there is an expected transient elevation of these levels due to increased red blood cell turnover as the infant transitions from fetal to neonatal life. Clinically, elevated serum bilirubin levels manifest as jaundice, a yellowing of the skin, sclera, and mucous membranes. Jaundice is typically visible when the total serum bilirubin (TSB) is greater than 5 mg/dL.[186]

TSB is composed of two parts: indirect (unconjugated) bilirubin and direct (conjugated) bilirubin. Transient indirect hyperbilirubinemia is an almost universal finding in the newborn period.[187] This physiologic indirect hyperbilirubinemia is normal and benign and requires no additional intervention beyond assuring that enteral feedings are established and the infant remains hydrated with adequate elimination patterns. However, a subset of neonates will develop severe indirect hyperbilirubinemia warranting treatment. In cases of severe and untreated indirect hyperbilirubinemia, free bilirubin can cross the blood–brain barrier and cause brain injury. In its initial presentation, this injury is known as acute bilirubin encephalopathy and may be reversible or permanent; long-term injury, known as chronic bilirubin encephalopathy or kernicterus, is permanent and develops gradually, with most infants demonstrating clinical manifestations within the first year of life.

Hyperbilirubinemia is often categorized as benign or pathologic. Direct hyperbilirubinemia, also known as cholestasis, is always considered pathologic and deserves an appropriate and timely evaluation and treatment. Indirect hyperbilirubinemia is considered pathologic under the following conditions, and similarly requires timely evaluation and intervention:

- Jaundice observed within the first 24 hours of life
- Jaundice persisting beyond 2 to 3 weeks of life in a term infant
- TSB greater than 95th percentile for age based on an hour-specific nomogram
- TSB level increasing by greater than 5 mg/dL/day or greater than 0.2 mg/dL/h

Even when not classified as pathologic, indirect hyperbilirubinemia may be severe enough to require treatment, including IV hydration, medication (such as intravenous immunoglobulin), phototherapy, or exchange transfusion. Because hyperbilirubinemia evolves over time, it is important to risk stratify infants to identify those who would benefit from more frequent monitoring and prompt initiation of treatment if indicated.

Risk Factors

Perinatal and neonatal risk factors for severe hyperbilirubinemia are shown in Box 16.4.

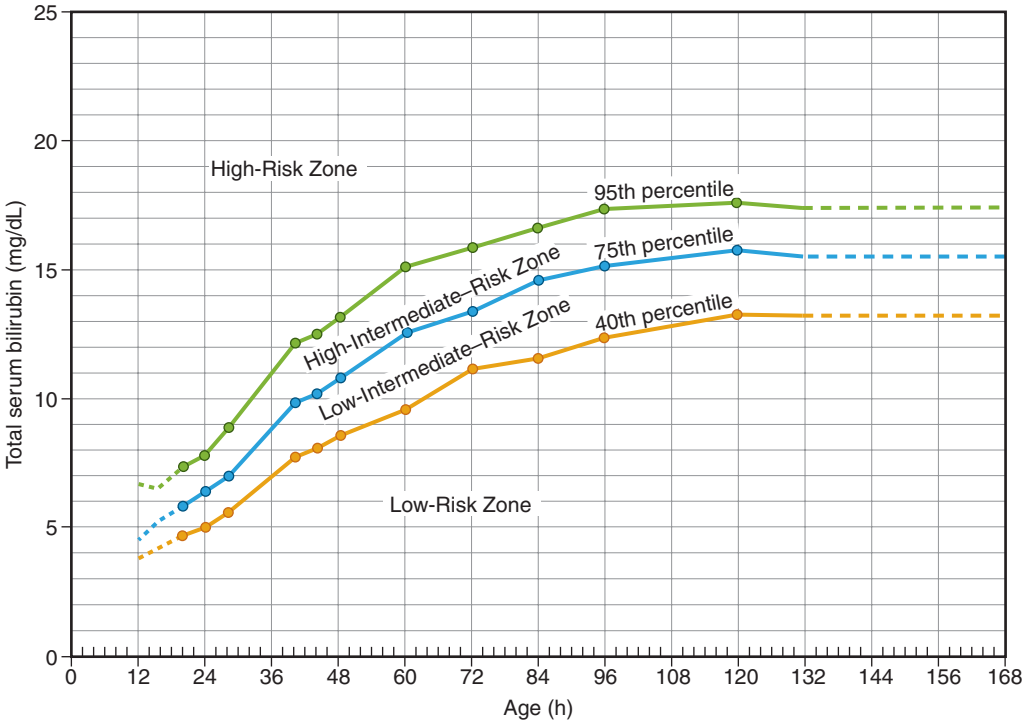
Initial Management

AAP has long recommended universal bilirubin screening in all neonates within the first 72 hours of life,[188] as well as assessment for the presence of risk factors for hyperbilirubinemia soon after birth. Even when this screening recommendation is met, newborns are often discharged from the hospital before TSB levels peak at 3 to 4 days of age. Therefore, predictive models were developed to assess the risk of severe hyperbilirubinemia in the first week of life and to help guide how closely infants should be followed after discharge. In the late 1990s, using a cohort of infants greater than 35 weeks' gestation, Bhutani and colleagues obtained serial serum bilirubin measurements and found that newborns with initial bilirubin levels above the 95th percentile at any time point (in hours) were more likely to have "significant hyperbilirubinemia" when the levels were measured subsequently than those newborns with lower initial bilirubin levels (Fig. 16.3).[189] This nomogram was subsequently used by the AAP to develop their 2004 practice guidelines for the management of hyperbilirubinemia in term and near-term infants. These practice guidelines contain hour-specific bilirubin level thresholds for initiation of phototherapy and exchange transfusions, to help guide and standardize the timing of screening and intervention for hyperbilirubinemia. For each nomogram, infants are categorized as low, medium, or high risk based on the presence of risk factors that increase the likelihood of either severe hyperbilirubinemia or neurotoxicity (see above). Based on this categorization, infants' TSB levels are plotted on their appropriate risk curve, thereby standardizing the management of hyperbilirubinemia. The 2004 AAP practice guideline[188] provides clinicians with guidance on when to initiate, continue and discontinue phototherapy or to perform an exchange transfusion, as well as assists with the identification of risk factors that may alter the serum bilirubin level at which intervention is warranted (Fig. 16.4).[190] Internet tools based on the Bhutani nomogram and the 2004 AAP guidelines are available to assist in determining the appropriate management for a newborn based on hour-specific TSB level.[191] For more detail, readers are referred to Chapter 72, Neonatal Hyperbilirubinemia and Kernicterus.

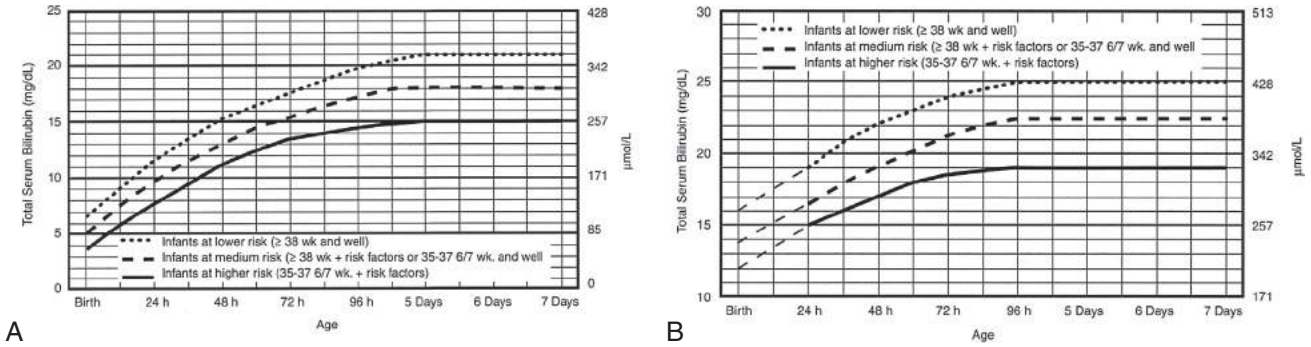
• BOX 16.4 Risk Factors for Severe Hyperbilirubinemia

- Prematurity (<38 weeks)
- Exclusive breastfeeding
- Older sibling with jaundice
- East Asian or Native American ancestry
- Cephalohematoma or other significant bruising
- Polycythemia
- Immune or non-immune mediated hemolytic disease (including ABO, Rh, minor red cell antigens)
- Glucose-6-phosphate dehydrogenase deficiency
- Predischarge bilirubin screening level in the high risk or high-intermediate risk zone for age (in hours)
- Asphyxia
- Acidosis
- Sepsis
- Albumin <3 g/dL (<30 gL)

Adapted from American Academy of Pediatrics Subcommittee on Hyperbilirubinemia. Management of hyperbilirubinemia in the newborn infant 35 or more weeks of gestation. *Pediatrics*. 2004;114(1):297–316.



• Fig. 16.3 "Bhutani nomogram" for total serum bilirubin levels in newborns born at 36 weeks' gestation or later. (Modified from Bhutani VK, Johnson L, Sivieri EM. Predictive ability of a predischarge hour-specific serum bilirubin for subsequent significant hyperbilirubinemia in healthy term and near-term newborns. *Pediatrics*. 1999;103:6–14.)



• **Fig. 16.4** American Academy of Pediatrics guidelines for phototherapy (A) and exchange transfusion (B) in hospitalized infants of 35 or more weeks' gestation. (American Academy of Pediatrics Subcommittee on Hyperbilirubinemia. Management of hyperbilirubinemia in the newborn infant 35 or more weeks of gestation. *Pediatrics*. 2004;114(1):297–316.)

Ineffective Thermoregulation

Being too cold or too hot causes metabolic stress to the newborn, so efforts to maintain a steady and neutral thermal environment should be part of routine newborn care. The best practice is to dry the baby immediately after delivery and place the newborn skin-to-skin with the mother. Although the AAP and the American College of Obstetricians and Gynecologists jointly recommend keeping newborns' core temperatures within the narrow range of 36.5°C to 37°C, in one study of healthy term newborns the average temperature was 36.5°C, with a normal range from 36.0°C to 37.9°C.[192] Thin babies tend to have lower body temperatures, and heavier babies tend to have higher body temperatures. Bathing a newborn can cause hypothermia; this is less likely when bathing is performed from trunk to head or when a bath is used versus washing with a cloth.

An elevated body temperature at birth usually reflects the intrauterine temperature and is not necessarily a sign of sepsis.[193] Isolated hyperthermia during labor is associated with neonatal encephalopathy, occurring in approximately 1 in 2000 births.[194] After the first 3 to 4 days of life, increased temperatures are most likely caused by dehydration from suboptimal breast milk supply.[195] A single increased temperature in an otherwise well-appearing newborn is not a strong predictor of infection, but has been reported as a sign of intracranial hemorrhage.[196]

Risk Factors

- Premature infants (hypothermia)
- Small for GA infants (hypothermia)
- Infants with neonatal abstinence syndrome (hyperthermia)

Initial Management

Standard practice at most nurseries is to measure axillary temperatures, probably because of reports in the 1960s and 1970s of rare perforations caused by rectal thermometers; however, axillary temperatures may not always accurately reflect core temperature.[197] Importantly, axillary temperatures are not the standard used in studies of sepsis in infants younger than 2 to 3 months of age.

Many nurseries worldwide have adopted policies to delay bathing to avoid hypothermia, allow time for initial bonding, and promote breastfeeding. Early skin-to-skin contact between the newborn and the mother is useful both to prevent and treat early hypothermia, but attention to positioning and frequent checks by nursing staff are required.[198] Hypothermia should be managed by the baby being placed skin-to-skin with a parent or under a radiant warmer. Additional information about temperature regulation in the newborn can be found in Chapter 17, Temperature Regulation.

Abnormal Voiding Patterns

Urination

Approximately 15% of healthy newborns void at the time of delivery, and 95% void by 24 hours of age. Delayed voiding is often a consequence of stress on the newborn during labor and delivery, which is a protective mechanism for the baby.[199,200] Normally, no intervention is needed once homeostatic adaption to extrauterine life has been established.

The differential diagnosis of delayed voiding (defined as no urine output by 24 to 48 hours of age) includes renal and postrenal causes. With the frequent use of prenatal ultrasound examination, it is unusual for a significant renal anomaly to be unknown before birth. In the absence of antenatal intervention, infants with bilateral renal agenesis have additional findings, such as anhydramnios, Potter sequence, and early, severe respiratory failure due to pulmonary hypoplasia and PPHN. Unilateral renal agenesis does not manifest as decreased urine output. Renal vascular thrombosis can cause anuria, and babies with this condition are usually ill. Severe cystic kidney disease can involve urinary outflow obstruction. The diagnosis of cystic kidneys is usually made after the newborn period or is found incidental to the evaluation of other anomalies and not because of delayed voiding.

Postrenal causes of delayed voiding include neuropathic bladder dysfunction and anatomic obstruction of urinary flow by anomalies in the ureters, bladder, or urethra. Persistent or recurrent bladder distention after catheterization is found with occult lower spinal cord anomalies. Presacral teratoma or other tumors can cause compression and urinary blockage as well. In male newborns, there is the possibility of posterior urethral valves. Congenital lower urinary tract obstruction occurs in 1 in 3000 births, with two-thirds of these due to posterior urethral valves. Physical findings of loose abdominal skin or musculature and a distended bladder, most often accompanied by respiratory distress, suggest this diagnosis.[201]

In a healthy-appearing newborn who has a normal physical examination and a history of a normal prenatal ultrasound, allowing up to 72 hours for a spontaneous first void will avoid excessive testing. In fussy neonates and neonates with other genitourinary

abnormalities, enlarged kidneys, or a distended bladder, testing should begin immediately. Ultrasound examination of the bladder, kidneys, and urethra is often diagnostic.

Normal newborns have decreased renal concentrating ability and excessive extracellular free water at birth. As a result, they will continue to void despite a low intake of fluids. This process is normal. Newborns maintain normal hydration despite weight loss. Conversely, delayed voiding is not indicative of dehydration in the first 72 hours after birth. When mothers undergo a cesarean section, the fluid boluses given to prevent hypotension may result in additional free water in their newborn. This can lead to greater than 7% birthweight loss with extra voiding (i.e., a physiologic diuresis).[202] Hearing about weight loss in their newborn creates stress, guilt, and anxiety in parents, which may be counterproductive to breastfeeding success. It is important for providers to emphasize the normalcy of weight loss.[76]

Stooling

The first passage of meconium occurs at an average of 7 hours of age. One-third of newborns pass meconium before their first feeding. Late preterm newborns tend to pass meconium later than term newborns, and 32% of preterm newborns do not pass meconium within 48 hours after birth. Although intake is not well correlated with meconium output, the number of wet and soiled diapers does reflect the adequacy of breast milk production by day 4. Fewer than four soiled diapers on day 4 correlate with inadequate milk production.[203] By 2 weeks of age, breastfed newborns stool more frequently than formula fed newborns; they also have larger variability in the time between bowel movements.[204,205] After the first month, breastfed and formula-fed infants have about the same rate of defecation.

Because 99.7% of healthy newborns pass meconium by 48 hours of age, those whose passing of meconium is delayed beyond that time deserve extra vigilance during the examination to avoid obstructions being missed, such as an imperforate anus. A baby with abdominal distention or vomiting and delayed stooling deserves evaluation for a possible gastrointestinal tract obstruction.

Discharge of the Newborn

Anticipatory Guidance

A primary duty of newborn care providers is to ensure that the newborn's caregivers (usually the parent or parents) have the knowledge and skills to support their baby's normal growth and development outside of the hospital setting. Anticipatory guidance should be provided to help prepare new parents for the common tasks of newborn care and to educate them about the many normal variations in newborn behavior.

Because most parents have questions about infant feeding, elimination, bathing, umbilical cord care, genital care, jaundice, and common rashes, attempts should be made to provide education about these topics during the birth hospitalization. However, addressing all of the information at once using a single format may be overwhelming and not the preferred learning style for some families. Therefore, it may be prudent for practitioners to focus on a few key points of anticipatory guidance rather than reciting a litany of instructions. There is also a philosophical choice in deciding whether to emphasize the overall health of a newborn or to concentrate on the prevention or identification of illness. There is little evidence about the efficacy of most anticipatory guidance provided to parents during the newborn nursery stay, with the notable exception of the "Back to Sleep" campaign to reduce the risk of SIDS. There is also evidence that education regarding the normality of inconsolable crying in newborns helps parents deal with this stressful situation and may reduce the risk of shaken baby syndrome.[206–208]

Postpartum depression is present in at least 10% of mothers, and depression also occurs in about 10% of fathers during early infancy. Depression is linked to sleep deprivation and has a major and long-lasting impact on infant homeostasis and development.[209] Parental depression screening should occur at all well-child visits during the first 6 to 12 months of life.

Parents who display concerning behaviors during the birth hospitalization that raise concern for future child abuse or neglect should be identified, and a plan made to assure the infant's safety after discharge. The plan may include supervision by another responsible adult, mandatory participation in community-based parenting interventions, temporary placement in foster or kinship care, or—as a last resort—termination of parental rights.[210,211]

Safe Sleep

With the exception of immunizations, no child health intervention in the past three decades has resulted in a larger decrease in postneonatal infant mortality than the "Back to Sleep" campaign. The remarkable change in the predominant sleep position of infants from prone to supine has led to a 30% to 50% reduction in the rate of SIDS in the United States.[212] A multipronged effort including brochures, public service announcements, and education provided by healthcare professionals was used to promote the recommended change in infant sleep position.[213] The education provided to parents during the newborn nursery stay is a crucial determinant of the sleep position of an infant. In addition to providing education, there is evidence that parents model sleep position for their babies after how they saw nurses and physicians place their neonate in the bassinet in the newborn nursery.[212,214] There is no evidence that placing newborns on their side during the first few hours after birth decreases the risk of aspiration.[215] Therefore it is crucial that neonates are placed on their backs to sleep in the newborn nursery. In addition, there is an additive effect from physicians and nurses both recommending and demonstrating the supine sleep position.[213]

In addition to the supine position, there are other factors related to the sleep environment that may impact the risk of SIDS. It is recommended that infants sleep on firm surfaces and without excessive bedding such as pillows, crib bumpers, or stuffed animals. Although a controversial topic, the results of a metaanalysis strongly indicate that co-sleeping increases the risk of SIDS by nearly threefold. The risk of co-sleeping is highest in infants of mothers who smoke.[216] In addition, although the use of a pacifier has been found to reduce the risk of SIDS, there is a reluctance to recommend these devices because of concerns about a negative impact on breastfeeding.[212,215]

Infant Car Seats

All states have laws that require the use of car seats for children. It is important that parents purchase only approved car seats, so bargain-hunting at used child equipment stores should be done with caution. Lists of approved car seats are available online.[217]

Pediatrician Follow-Up

In an era of early hospital discharge for healthy newborns (average length of stay 48 to 52 hours),[218–220] it is particularly

important to have in-home or clinic follow-up at 3 to 4 days of age. This is when the newborn's weight reaches its nadir, bilirubin levels peak, lactogenesis II is starting, and mothers are sleep deprived from tending to the around-the-clock needs of the newborn. Breastfeeding mothers may need extra encouragement during this time and continued vigilance to ensure that breastfeeding is established. The AAP recommends that newborns discharged before 48 hours after birth have a follow-up appointment with a provider within 48 hours.[221] This follow-up can be accomplished either by a visit to a healthcare provider or by a home nursing visit.

The risk factors for readmission following an initial hospital stay of less than 48 to 72 hours include GA less than 39 weeks (and especially <37 weeks), primiparous mother, and Asian ethnicity (presumably due to an increased risk of hyperbilirubinemia).[219,222–224] Consideration of a longer nursery stay is suggested for newborns with one or more of these risk factors. In addition, early discharge is not recommended for term newborns who have not voided, passed at least one stool, or demonstrated adequate breastfeeding.[221]

Special Considerations for Late Preterm Infants

With nearly 3.75 million live births in 2019,[225] the United States has one of the highest birth rates among industrialized countries while simultaneously having a disproportionately high rate of preterm birth. Decades of efforts to reduce preterm births have made little headway. In 2007, the AAP Committee on the Fetus and Newborn published guidelines to help standardize and optimize care for this vulnerable population, changing the term from "near term" to "late preterm" infant (those occurring between $34^{0/7}$ and $36^{6/7}$ weeks' gestation), and "early term" infants being those born between $37^{0/7}$ and $38^{0/7}$ weeks gestation. Late preterm and early term account for approximately 32% of live births.[226]

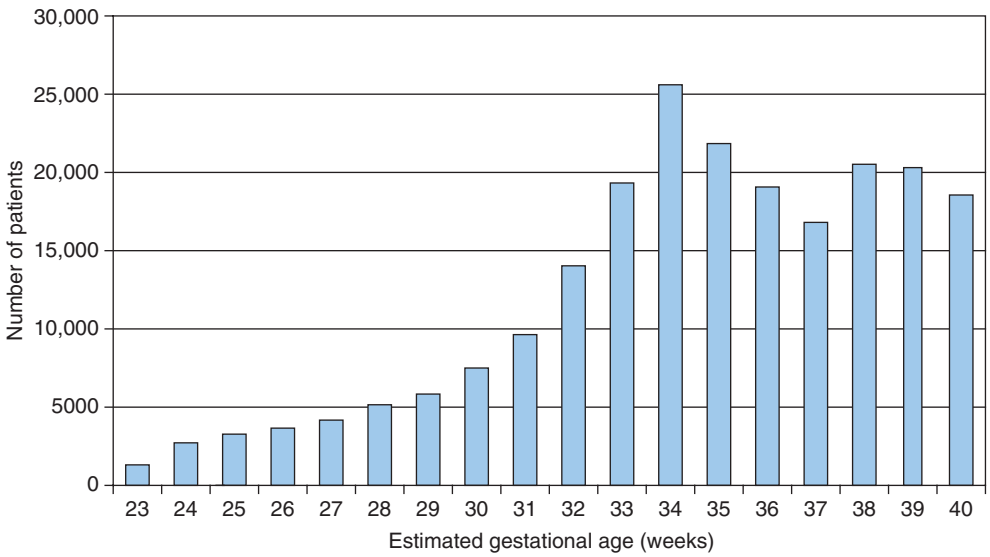
Hospitalization

As the number of late preterm infants has grown, so has the awareness of their unique set of medical challenges. In fact, late preterm newborns account for up to one-third of all NICU admissions (Fig. 16.5).[227,228] Unlike the smaller, visibly immature early preterm infant, late preterm infants appear mature because they are larger, and the tendency has been to treat them no differently than full term infants. However, they have a higher incidence of many neonatal pathologies, including TTN, RDS, PPHN, respiratory failure, prolonged physiologic jaundice, late neonatal sepsis, impaired thermoregulation, hypoglycemia, feeding difficulties, and risk of injury to the developing brain with later neurodevelopmental impairment.[229–234] This increased susceptibility to illness accounts for the increased rates of NICU admissions within the late preterm population (Fig. 16.6).

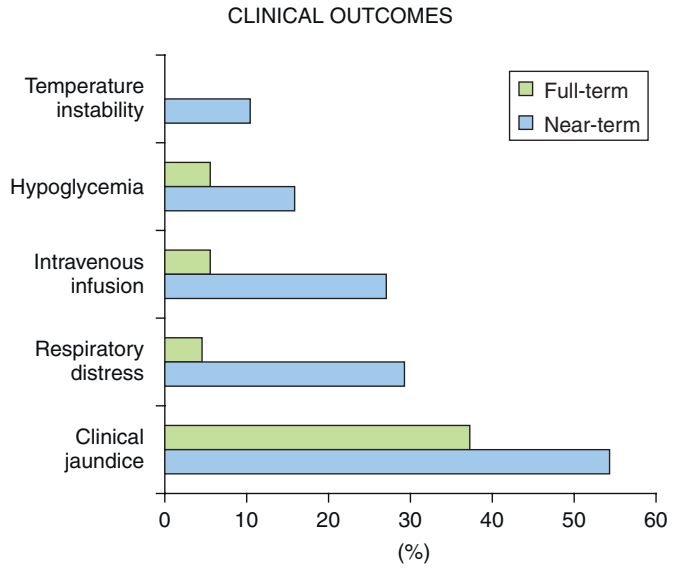
The most common reasons for admission include temperature instability, jaundice, respiratory distress, dehydration, poor feeding, and hypoglycemia.[229,235] Studies have shown that 88% of infants born at 34 weeks' gestation, 12% born at 37 weeks' gestation, and 2.6% born at 38 to 40 weeks' gestation were admitted to the NICU.[236]

In general, nutritional experts recommend that 34- and 35-week late preterm infants receive nutrient-enriched (22 kcal/oz) milk, whereas older 36- and 37-week late preterm infants with an uncomplicated neonatal course should be fed unfortified milk after discharge.[237] These nutrient-enriched formulas have a higher protein content (1.9 g/dL vs. 1.4 g/dL), increased energy (22 kcal/oz vs. 20 kcal/oz), and additional calcium, phosphorous, zinc, trace elements, and vitamins compared with standard formulas.[237]

Those late preterm infants not admitted to the NICU may require a longer hospital stay. Because of the morbidities and risk factors associated with late preterm babies, they should not be discharged before 48 hours after birth. When in the



• **Fig. 16.5** Distribution of neonatal intensive care unit admissions by gestational age, highlighting the contribution made by late preterm and early preterm infants. Data were obtained from a large consortium of neonatal intensive care units under a common management. (Modified from Clark RH. The epidemiology of respiratory failure in neonates born at an estimated gestational age of 34 weeks or more. *J Perinatol*. 2005;25:251–257.)



• **Fig. 16.6** Clinical outcomes in near-term infants (350/7 to 366/7 weeks) and full-term infants as a percentage of the patients studied. (Modified from Wang ML, Dorer DJ, Fleming MP, et al. Clinical outcomes of near-term infants. *Pediatrics*. 2004;114:372–376.)

newborn nursery, discharge criteria should reflect criteria similar to the NICU.

Newborn Car Seat Challenge

The observation that preterm newborns had episodes of hypoxia when monitored in car seats led the AAP to recommend in 1991 that preterm newborns be observed and monitored for apnea, bradycardia, or oxygen desaturation in their car safety seat prior to hospital discharge—the so-called car seat challenge.[238–240] In the United States, the car seat challenge expanded to include late preterm newborns, most of whom did not have respiratory problems during their hospital stay. It has been reported that 25% of late preterm newborns do not fit securely into standard car safety seats, and 12% of healthy late preterm newborns have apneic or bradycardic events in their car seats.[241] The rate of failure (about 4%) has been found to be about the same in small for GA babies as those born late preterm.

The authors of a Cochrane systematic review questioned whether or not car seat trials actually prevent morbidity or mortality and whether there might be adverse effects from not passing this "screen," such as prolonging the hospital stay or creating parental anxiety. Their review did not discover any randomized trials, and they concluded that "it is unclear whether undertaking a car seat challenge is beneficial or indeed whether it causes harm."[242]

Follow-Up After Discharge

After discharge from the hospital, it is recommended that the late preterm baby be brought for a checkup by its pediatrician within 24 to 48 hours. In addition, because of their increased risk of developmental delays, these infants should be monitored closely to ensure that all milestones are achieved appropriately and that early intervention (e.g., physical therapy, occupational therapy, speech therapy) is in place if needed. Early developmental testing can also be useful in determining any cognitive delays, which can then be addressed with individualized educational programs.

Primary care physicians caring for late preterm infants in an outpatient setting should also be cognizant that the higher risk of morbidities may persist beyond the neonatal period and into early childhood. One recent study showed that 30% of children younger than 2 years who were admitted to the pediatric intensive care unit for respiratory diseases were born prematurely. Of that group, 17% were former early preterm babies, and 12% were late preterm infants.[243]

Readmission to the Hospital

The late preterm infant is at increased risk of readmission to the hospital, with rates of 4.4% compared to 2% for full-term infants,[244] with common causes including dehydration, feeding problems, respiratory distress, apnea, fever, infection, and jaundice[245] In addition, 34- to 36-week infants who were never admitted to the NICU (or if admitted, their NICU stay lasted less than 24 hours) had nearly a threefold and 1.3-fold higher risk of readmission compared with full-term infants, respectively.[244,246] Because late preterm infants are at a much higher risk of rehospitalization, they need close follow-up after discharge to assess breastfeeding and nutrition and to monitor them for jaundice.

Acknowledgments

We wish to thank Valencia P. Walker, James A. Taylor, Jeffrey A. Wright, David Woodrum, Sowmya S. Mohan, and Lucky Jain for their invaluable contributions to the previous edition of this textbook, on which this chapter is based.

Suggested Readings

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17

Temperature Regulation

JANESSA B. LAW AND W. ALAN HODSON

KEY POINTS

- Providing an appropriate and stable thermal environment is important for newborns regardless of size or gestational age.
- Neutral thermal environment (NTE) refers to the ambient temperature necessary to maintain normal metabolism.
- Newborn (admission) hypothermia continues to be a global challenge, particularly in resource-limited settings.
- Radiant warmers, warm blankets, thermal mattresses, head covering, plastic wrap (without drying), delayed bathing, and skin-to-skin care have been recommended to reduce neonatal hypothermia.
- Interhospital transport, particularly for critically ill and/or low birth weight (LBW) neonates, increases the risk of thermal instability.

All newborns are at risk of experiencing physiologic instability due to aberration of their core temperature. Providing an appropriate and stable thermal environment is important for newborns regardless of size or gestational age (GA). Most of the temperature needs of the full-term infant will be associated with birth, or in rare circumstances with the development of an illness or environmental instability. It is imperative that providers involved with delivery or subsequent care understand the various causes and consequences of heat loss, the mechanisms of heat production by the infant, and the multiple management options for providing the correct thermal environment. A variety of clinical conditions present different management challenges depending upon the degree of immaturity, birth weight, and concurrent illness such as respiratory distress, sepsis, or asphyxia. Temperature regulation is an essential and important component of neonatal intensive care.

Nearly a century ago, long before the development of intensive care nurseries, research demonstrated an association between temperature control and increased survival in premature infants. Incubators first appeared in the 19th century in Europe, but it was not until the 1930s that they were incorporated into the care of premature infants at Michael Reese Hospital in Chicago. However, the concept that premature infants were unharmed by hypothermia (acting as if they were similar to a poikilothermic animal) prevailed until controlled trials demonstrated the associated morbidity and mortality of cooling in various birth weight groups.[1] Often referred to as "cold stress," a number of studies demonstrated the metabolic cost of hypothermia, resulting in a doubling or tripling of oxygen consumption, particularly in early-gestation infants.[2–5] These studies were the first attempts at defining the optimal environmental temperature for maintenance of a normal body temperature and physiology, known as the neutral thermal environment (NTE). This quest has resulted in ever-increasing refinements of protective management techniques including hybrid incubators, radiant heaters, heated gel mattresses, plastic wrapping, heat shields, a laminar flow device, clothing, caps, room temperature control, and protocols for skin-to-skin care (SSC). Appropriate incorporation of the many management options will depend upon the needs of the individual infant related to his or her birth weight, maturity, degree of illness, and postnatal age.

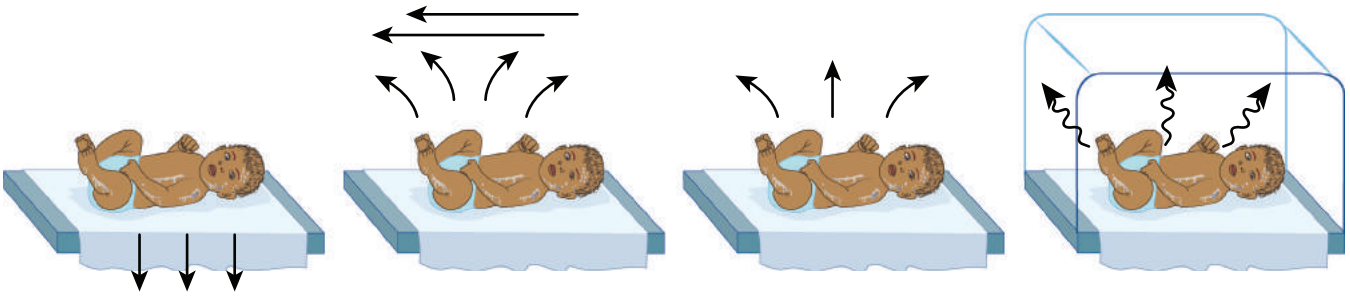
Mechanisms of Heat Loss

The most vulnerable time for heat loss occurs during the first minutes after birth. It is of interest to consider the thermal environment of the fetus and how a stable body temperature is maintained despite variations in fetal metabolic activity associated with sleep cycles, muscular activity, respiratory movements (fetal breathing), and changes in maternal temperature. Maternal-fetal heat exchange occurs primarily via the umbilical vessels. Although the fetus maintains a differential of 0.5°C above maternal temperature, the extra heat is transferred from the umbilical artery to the umbilical vein as it courses back to the placenta such that the temperature of the blood returning to the placenta is nearly equal to the temperature of the blood leaving through the umbilical vein.[6,7] Thus, the fetal environment is primarily dependent upon the maternal temperature. Significant elevations in maternal temperature have the potential to cause fetal harm, hence the standard advice given to pregnant women to avoid hot tubs. Maternal fever as a result of inflammation or infection is likely of higher risk to the fetus than moderate maternal exercise or ambient heat stress. The temperature gradient between the fetus and the mother may increase beyond 0.5°C during labor, uterine contractions, and it widens slightly with advancing GA.[8]

There are four major mechanisms of heat loss that will vary over progressive days of nursery care (depending upon the maturity of the infant, growth, illness, and environmental factors). These are evaporation, radiation, convection, and conduction, each with variable contributions to heat loss (Table 17.1; Fig. 17.1).

Evaporation

Evaporative losses at birth may result in a fall of 2°C to 3°C within the first 30 to 60 minutes after birth if the newborn is extremely premature or if no wrapping, drying, or clothing is applied in a larger newborn.[9] Delays in warming may occur if resuscitation or other medical care postpones drying. The first minutes after birth are already stressful because of the physiologic adaptions required



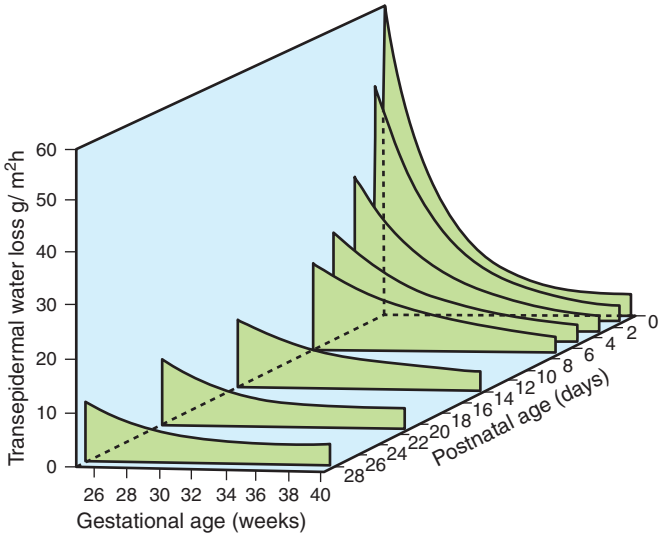
• **Fig. 17.1** Four modes of heat loss in the neonate. (Redrawn from Warren I. Thermoregulation. In: *Nursing the Neonate*. 2nd ed. Wiley-Blackwell; 2009.)

TABLE 17.1 Four Major Mechanisms of Heat Loss in Newborns: Estimated Percentages of Each, Based on the Environmental Temperature

	ENVIRONMENTAL TEMPERATURE		
	30°C (%)	33°C (%)	36°C (%)
Radiation	43	40	24
Convection	37	33	5
Evaporation	16	24	56
Conduction	4	3	1

TABLE 17.2 Relative Skin Surface Area in Adults and in LBW Infants

	Relative Skin Surface Area (cm^2/kg)
Adult	250
1500-g infant	870
1000-g infant	1000
500-g infant	1400



• **Fig. 17.2** Transepidermal water loss in relation to gestational age at birth and at different postnatal ages in appropriately grown infants. (Redrawn from Sedin G, Agren J. Water and heat—the priority for the newborn infant. *Ups J Med Sci*. 2006;111(1):45–59. doi:10.3109/2000-1967-027.)

for onset of breathing, absorption of fetal lung fluid, and circulatory changes.

The major cause of heat loss from evaporation following delivery is exposure of the infant's large surface area of skin relative to his or her body mass while wet from amniotic fluid. The more immature the newborn, the larger the relative surface area (Table 17.2). In full-term newborns, evaporation of water from the skin decreases until a body temperature of 36.6°C to 37.1°C is reached. Concomitant increase in skin blood flow does not appear to influence evaporative heat loss.

Insensible water loss through the skin continues to contribute to evaporative heat loss, but this decreases over the first few days after birth. The rate of evaporative water loss depends upon the ambient humidity and increases at humidity levels below 50%. Evaporation is somewhat greater under a radiant warmer compared to an incubator. Studies have demonstrated large losses from transepidermal water evaporation over the first few days after birth in extremely LBW newborns (Fig. 17.2). This suggests that the skin permeability of very premature neonates, especially during the first hours after birth, is very different from that of neonates born beyond a GA of 32 to 34 weeks.[10]

Radiation

Radiation is a major source of heat loss, thus it has been the focus of the majority of research studies which has led to the development of many devices to minimize that loss and to protect the infant from excessive energy expenditure. Radiant heat loss from the skin can be responsible for 40% or more of the daily heat loss (when air movement is low). Many variables can influence the degree of heat loss, including body surface area, environmental temperature, the type of external heat source, clothing, blankets, caps, heat shields, and swaddling. The full-term infant should not present ongoing concerns with radiant heat loss if certain measures of care are provided such as room temperature of 24°C to 26°C, appropriate SSC, clothing, and blankets. Premature infants and those who are growth restricted or ill with cardiorespiratory

distress, sepsis, asphyxia, or other disorders will be at continued risk of radiant heat loss. This mechanism of heat loss is influenced by the mean temperature of the skin and the mean temperature of the surrounding walls, as well as the temperature gradient to a nearby object of lesser warmth even if the object is outside but near the incubator or the warmer.

Convection

Heat loss due to convection is determined by the airflow around the infant, the mean temperature of the ambient air, the mean temperature of the skin, and the exposed surface area of the infant. Different incubators have different airflow, humidity, and wall temperature capacities that can influence the degree of convective heat loss.

Conduction

Conductive heat loss contributes minimally to energy expenditure and primarily depends upon the thermal conductivity of the mattress, which is low in incubators and under radiant warmers. Skin adjacent to a colder object such as a radiograph plate or other cold instrument will result in some conductive heat loss.

Mechanisms of Thermoregulation

Our understanding of thermoregulation in the human infant comes from translational studies in animal models. From these studies we know that temperature sensors are located throughout the surface of the neonatal body. These receptors transmit signals to a central temperature controller within the hypothalamus which in turn regulates temperature homeostasis via multiple mechanisms. Thermoneutrality in response to hypothermia in the newborn is primarily accomplished via nonshivering thermogenesis as discussed below.

The optimal temperature for an infant is defined by a range of measurable skin temperatures that indicate a central or core temperature. Measurement sites include the axilla, rectum, and skin (the small size of the ear canal precludes tympanic measurement). The recommended range for normal axillary and rectal temperatures is 36.5°C to 37.5°C for full-term infants, 35.6°C to 37.3°C for preterm infants, and 36.7°C to 37.3°C for LBW infants. Axillary temperature measurement is intermittent with minimal errors related to placement of the thermometer, while rectal temperatures have a risk of trauma and inaccuracies due to depth or duration of insertion of the thermometer as well as passage of stool. Continuous temperature monitoring with an infrared skin probe placed over the midline of the upper abdomen or on the back below the scapulae permits accurate assessment of core temperature under various external heating arrangements.

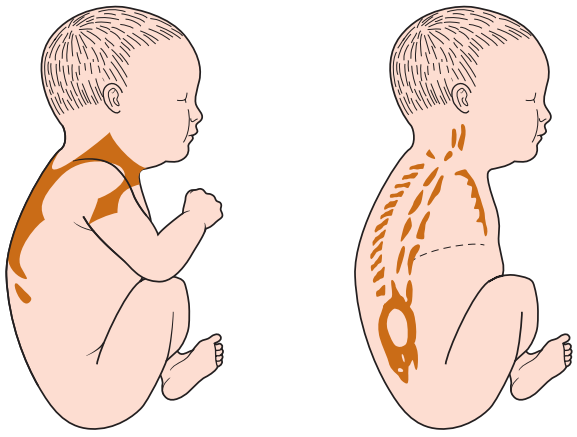
The NTE refers to the ambient temperature necessary to maintain normal metabolism. For the average naked adult it is 25°C to 27°C, and for the full-term naked newborn it is 32°C to 34°C.[11] For premature infants it will differ with GA and weight. Infants of 25 weeks' GA or less will require an ambient temperature close to that in utero (i.e., 35°C to 37°C) until they are placed in an appropriate protective environment. LBW infants are particularly susceptible to temperature instability because of the absence of subcutaneous fat for insulation, decreased levels of brown fat for heat production, increased evaporative losses from a large body surface area relative to body weight, poor vasoconstrictor control, decreased spontaneous muscle activity, and transepidermal insensible water loss. Additionally, small for GA infants have a higher metabolic rate for weight. An increase in activity and ingestion of food in growing preterm infants will also increase the metabolic rate through altered energy expenditure (see Table 17.2).

Nonshivering Thermogenesis

The newborn is unable to reduce heat loss or increase heat production by shivering or increasing voluntary muscle activity. Nonshivering thermogenesis produces energy output and heat production, largely from brown fat. Brown fat is located in six major sites near major arterials and organs, and demonstrates increased oxidation within minutes of birth (Fig. 17.3).[12] Although it appears as early as 25 weeks in the fetus, the substrates detailed below that are necessary for thermogenesis are markedly decreased in the premature infant.[13]

Temperature receptors sensing a low temperature stimulate the sympathetic nervous system to release norepinephrine and ATP adjacent to brown fat cells.[14] These in turn stimulate β-adrenergic and adenosine activated A_{2A} receptors, respectively, resulting in increased cyclic AMP (cAMP) activation of protein kinase A (PKA). PKA drives heat production by (1) release of cytoplasmic stores of fatty acids that either combust or activate uncoupling proteins (i.e., thermogenin), and (2) promoting transcription of genes involved in heat production. These genes are also regulated by thyroxine, triiodothyronine, and thyroid stimulating hormone via the hypothalamic-pituitary-thyroid axis as well as by adrenocorticoid hormone and glucocorticoids via the hypothalamic-pituitary-adrenal axis.[13,15]

During the 1960s, considerable clinical and animal research helped to define the NTE for newborns in relation to changes in environmental and core temperatures. Environmental temperatures that progressively decreased below 34°C resulted in a steady increase in oxygen consumption to values that were two to three times the resting state. Increases in oxygen consumption and metabolic output are considered important contributors to increased morbidity and mortality, especially in extremely LBW infants. Extended periods of cold stress have been associated with hypoglycemia, respiratory distress, hypoxemia, metabolic acidosis, necrotizing enterocolitis, and death. If environmental cooling



• **Fig. 17.3** Distribution of brown fat deposits in the newborn from 28 to 42 weeks' gestation. (Redrawn from Aherne W, Hull D. Brown adipose tissue and heat production in the newborn infant. *J Pathol Bacteriol*. 1966;91(1):223–234. doi:10.1002/path.1700910120.)

exceeds the infant's thermoregulatory response of energy output, the core temperature will drop along with a decrease in oxygen consumption (Fig. 17.4). This "Q_{10} effect" of decreased metabolism when the environmental temperature falls below a critical point is the basis for the use of induced hypothermia in certain clinical situations.

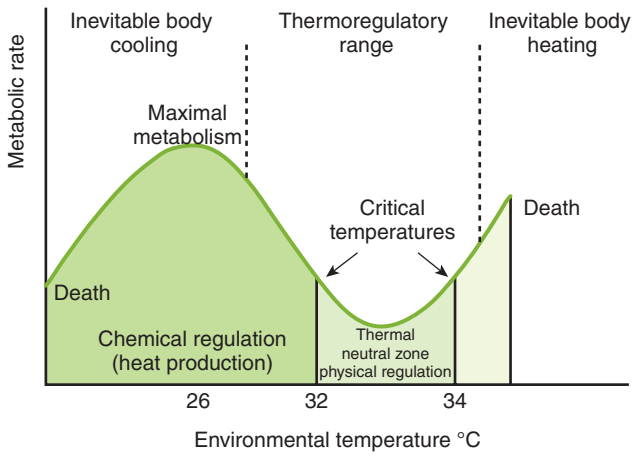
Thermal Management Strategies

Understanding heat loss, nonshivering thermogenesis, and the consequences of cold stress have led to a variety of protective and therapeutic options. Much controversy has arisen regarding various warming devices and techniques, resulting in new or modified equipment and strategies. The goal of estimating and maintaining the NTE for each infant is supported by evidenced-based trials. The indication and timing of interventions have driven evolution of management protocols applicable to various clinical settings including transport, delivery rooms, nurseries with "rooming-in" policies, and neonatal intensive care units (NICUs).

Delivery Room Environment

Hypothermia soon after birth has been associated with increased morbidity and mortality, prompting organizations such as the Neonatal Resuscitation Program and the World Health Organization (WHO) to stress the importance of preventing early postnatal hypothermia.[16] Maternal temperature at delivery should be noted on the infant's birth record along with an axillary temperature. The room temperature, including operative rooms, should be kept at 26°C. Ideally, a preheated gel mattress, a warm bassinette, warm blankets, and a radiant heat source should be in place. Immediate drying, placement of a cap, and swaddling (once stabilized) will reduce evaporative heat loss. The full-term newborn will maintain a normal body temperature with appropriate clothing and blankets in an environment of at least 24°C. Preterm newborns need additional protection from heat losses, including higher ambient temperature and the use of plastic wraps (without drying) especially in infants less than 1500 g at birth.[17] Early skin-to-skin care (SSC), once the infant has been stabilized, can be very effective in preventing hypothermia—particularly in resource-limited settings.[18]

Delayed umbilical cord clamping in very preterm newborns initially raised concern regarding thermal management. However, multiple reviews and meta-analyses of studies done in newborns of all gestational ages have found no differences in admission temperature.[19,20]



• **Fig. 17.4** Variations in metabolic rate with changes in environmental temperature in human newborns, including the neutral thermal environment and extremes of cooling and heating. (Redrawn from Baumgart S. Incubation of the human newborn infant. In: Pomerance JJ, Richardson CJ, eds. *Neonatology for the Clinician*. Norwalk: Appleton & Lange; 1993:139–150.)

Care of the Extremely LBW Infant

The neonate born before 28 weeks' gestation presents a major challenge to the prevention of heat loss. Hypothermia is reported in extremely LBW neonates upon admission to the NICU and is especially hard to prevent in neonates born at less than 25 weeks' gestation. Furthermore, it is associated with an increased risk of early and late neonatal death.[21] Studies support the use of plastic bags and caps in the delivery room with the newborn immediately wrapped in a clear plastic bag up to the neck without drying and placed under a radiant warmer.[22,23] The head is dried and covered with a plastic cap. Resuscitation can proceed with the bag in place. This provides initial thermal stability for transfer to the NICU or transport to another facility. The first 12 hours is important in preventing further heat loss. Possible contributors to cold stress are the temperature of the inspired airflow, evaporative loss under radiant heat, cold hands, weight scales, the temperature of intravenous infusions and fluid boluses within syringes, and procedural interventions such as intubation, imaging, and vessel cannulation. The duration of vessel cannulation determines the length of time the newborn is covered with a sterile drape that interferes with warming. Expediency in completing this procedure is therefore vital.

Warming Infants

Incubators and radiant warmers have been in use in the NICU for several decades and remain the focus of heat management. Each of these warming devices has undergone sophisticated evolution and remain effective in providing a NTE for infants of differing sizes and with differing illnesses. Both devices provide enhanced observation and access when needed. There are slight variations between incubators and radiant warmers in the degree of evaporative and radiant heat loss, but no significant differences in outcomes have been demonstrated.

Incubators Versus Radiant Warmers

The prototype of the modern incubator, using the principal of convective heating of air, came into widespread use in newborn nurseries in the 1960s. Since then there have been many modifications allowing precise control of air temperature, airflow, humidity, and limitation of heat loss due to convection, radiation, and conduction within the incubator.[24] There are three options to achieve a stable and desirable skin temperature of 36.0°C to 36.5°C: (1) servo control of incubator air temperature, (2) servo control of abdominal skin temperature (using an abdominal skin probe), and (3) manual control of incubator temperature with knowledge of abdominal skin temperature. The advantage of servo control with an abdominal skin probe is that it ensures maintenance of a constant body temperature despite perturbations caused by a decreased environmental temperature from open portholes or opening of the incubator top. Overheating can occur if the probe becomes detached or covered with a blanket.

Thermal blankets have been used over the infant within the incubator to reduce insensible water loss. Heat shields are employed when using single-walled incubators to reduce radiant heat loss. The rationale for creating the double-walled incubator was to reduce radiant heat loss. While double-walling incubators have become standard for contemporary NICUs, several studies demonstrate only small differences in heat loss and heat production between the two types of incubators, and they have not shown a difference in clinical outcome if skin temperature servo control is used.[25]

Overhead radiant heaters were first used in delivery rooms, particularly for support during resuscitation procedures. These evolved into the Sierracin Cradle Warmer, which fits over an open bassinet. This warmer was used to observe naked infants for signs of distress over the first few hours after birth, commonly referred to as the transition or observation period. The "observation nursery" is now a thing of the past and has been replaced by the "intermediate care nursery" for infants with a minor degree of instability such as mild respiratory distress, irritability, jaundice, or risk for sepsis. Monitoring these infants with temperature probes as well as by means of vital signs facilitated the provision of an NTE without needing a NICU admission. These devices prompted development of the current radiant warmers now in common use in NICUs. They produce overhead heat in the infrared range distributed in a uniform fashion to the infant and controlled by an abdominal skin thermistor. As expected, evaporative heat loss is large with radiant warmers and increases if humidity falls below 50%, however these losses can be minimized by swaddling the infant. Open access under the warmer is excellent and advantageous in the assessment and management of the seriously ill infant upon admission.

Comparisons of the superiority of either the incubator or the radiant warmer have shown no differences in outcome. Both methods are effective, safe, and appropriate for care of the extremely LBW newborn, with recognition of small differences in insensible water loss. The more recent development of a hybrid incubator allows the intermittent use of a vertically adjustable radiant heater as well as drop-down incubator walls to improve access. Advocates for its use suggest that the incidence of severe bronchopulmonary dysplasia may be lessened and that better fluid balance and growth velocity may be achieved.[26]

Weaning to an Open Crib

The continuing need for an incubator is relevant to discharge planning and home care. Body weight attainment serves as a useful indicator for open crib readiness. Infants weighing 1500 g transferred to an unheated open crib demonstrate satisfactory growth velocity and a stable temperature without other adverse effects.[27,28] However, earlier transfer to an open crib does not necessarily correlate with an earlier discharge. Crib nursing of full-term infants requires a room temperature of at least 24°C, while infants of 1500 g should be in an environment of 26°C to 28°C. Smaller infants should be fully dressed, including a head covering, and may require a room temperature of 30°C.

Skin-to-Skin Care

SSC, a component of "Kangaroo mother care," was originally described as an effective way for mothers to keep their full-term infants warm while breastfeeding, and subsequently as an alternative method of caring for LBW infants in resource-limited settings.[29] In these original versions, the infant is placed skin-to-skin in a vertical position between the mother's breasts and under her clothes and is exclusively (or almost exclusively) breastfed. Intermittent SSC provided by a caregiver has been introduced in resource-rich settings for infants requiring neonatal intensive care—even extremely premature infants and those on ventilators. Several guidelines regarding SSC in the NICU have been published, but because it requires intensive staffing support, resources, and parent participation, development of individualized unit guidelines has been recommended.[27,30]

When first introduced, temperature regulation during SSC was a concern for both care providers and parents; however, thermal balance even in very and extremely preterm neonates before, during, and after SSC during the first week after birth has been demonstrated to be safe.[31,32] It is likely that the conduction of heat from caregiver to infant is sufficiently high to compensate for the increases in evaporative and convective heat losses. The vast majority of studies, including a 2016 Cochrane review, have demonstrated better growth and exclusive breastfeeding rates with decreased incidence of hypothermia, hypoglycemia, sepsis, and mortality in infants who experience SSC compared to standard bassinet, radiant warmer, or incubator care.[33,34] Improvements in social and behavioral outcomes even 20 years later have been shown.[35] The Committee on Fetus and Newborn of the American Academy of Pediatrics does acknowledge, however, that NICU infants should be continuously monitored during SSC and that "any infant who requires careful temperature regulation or a high-humidity environment might have SSC delayed until he or she is more stable."[27]

Additional Considerations

Transport

Temperature instability during transport to a NICU remains a challenging problem, particularly for extremely LBW neonates. Neonates born at less than 25 weeks' gestation, even those delivered within a tertiary care center, have been noted to have a drop in core temperature within 12 hours of admission to a NICU.[36] A review of several thousand LBW infants in the National Institute of Child Health and Human Development Neonatal Research Network who were admitted directly from the delivery room to the NICU revealed an association between lower admission temperature and lower birth weights as well as the need for immediate intubation. Admission temperature was inversely related to mortality (28% increase per 1°C decrease) and the incidence of late-onset sepsis (11% increase per 1°C decrease).[37]

Interhospital transport, particularly for critically ill and/or LBW infants, increases the risk of thermal instability. Infants may be exposed to a cooler, draftier non-NICU environment at the referring hospital and during transfer to and from the transport incubator. Evaluation and stabilization efforts, often accompanied by the movement of multiple care providers and equipment, can also increase convective heat loss. Intubation equipment, vascular access instruments, cold inspired air, unheated intravenous solutions or medications, and cold hands may increase conductive heat losses. One level III referral NICU used transport risk index of physiologic stability (TRIPS) scores to evaluate the impact of transport on the physiologic stability of very LBW infants. They found that more than half the transported infants demonstrated deterioration in their TRIPS score primarily because of a decrease in temperature, and the likelihood increased with longer duration

of transport. They concluded that better temperature regulation during transport may decrease the risk of clinical deterioration in such infants.[38]

As discussed previously, the use of occlusive plastic wraps and a cap is advocated while evaluation, resuscitation, and/or stabilization measures are occurring. However, additional warming methods are often needed and may be provided by a heated gel mattress and a radiant warmer. The urgency of cardiopulmonary stabilization and other life support measures may take priority over the many details of heat loss prevention. Thus development of standard temperature care protocols and team training in their use for every neonatal transport is recommended.

Hypothermia

Induced hypothermia by controlled cooling of the body or head for the treatment of neonatal encephalopathy is discussed extensively elsewhere.

For the most part, a nontherapeutic decrease in core temperature is indicative of an infant's inability to generate sufficient heat by increasing metabolism. How best and how quickly should rewarming occur to reach a core temperature of 36°C without increasing oxygen consumption or metabolism? Few data exist to support the optimal rate of rewarming. Current protocols favor a rapid return to a NTE with extra heat provided by radiant heat and a heated mattress or an incubator. A decrease in core temperature in LBW infants, especially in the first hours of life, remains an important risk factor for increased morbidity and mortality.

The World Health Organization (WHO), concerned that neonatal hypothermia is prevalent around the world as a contributor to inadequate newborn care, has developed a classification system to underscore the importance of intervention. Three levels are defined: Mild hypothermia (aka cold stress) from 36.0°C to 36.5°C, moderate hypothermia between 32.0°C to 35.9°C, and severe hypothermia for temperatures below 32.0°C. A retrospective study assessing these criteria found that approximately 56% of very LBW newborns were moderately hypothermic in the first few hours of life and had an associated adjusted risk factor for an increase in intracranial hemorrhage (odds ratio [OR] 1.3) and death (OR 1.5).[39] Therefore, emphasis on the need for rewarming has been focused on the care of infants at risk of "moderate" hypothermia.

Where available, rewarming involves setting a skin thermistor at a desired level of 37°C or 38°C. Under a radiant warmer, heat core temperature of 37°C can be achieved in 4 hours with a set point of 37°C or 2 hours with a set point of 38°C. Although there is minimal evidence for an increase in apnea or hypoglycemia, both should be monitored. In resource-limited settings, the WHO recommends SSC for treatment of moderate hypothermia.[40]

Hyperthermia

Overheating (core temperature above 37.3°C) can result in "heat stress," manifested by tachycardia, tachypnea, and restlessness. It is important to assess whether the cause is environmental or secondary to infection. Measurement of the abdominal skin temperature and that of the foot or toe should normally reveal a difference of 2.0°C. As infection progresses, vasoconstriction of the periphery will occur in an effort to support the body's effort to increase core temperature. Therefore, a toe-to-abdominal skin temperature difference of greater than 2.5°C would indicate an infection rather than an environmental cause. If the abdomen-to-toe temperature gap is less than 1°C or reversed, this indicates excess heat is being applied. Environmental temperature should be reduced by 0.5°C at intervals of about 30 minutes, with excess layers of clothing removed. The abdomen-to-toe temperature gap in the LBW infant may be unreliable because of impaired control of microvascular perfusion, and thus monitoring for other signs of overheating is more important.

Bathing

There is a tendency for all neonates, large and small, to be bathed soon after birth. In some cultures, customs and rituals associated with birth dictate the timing of naming, cord separation, first feed, and first bath, among other events. Should early bathing be performed, it is recommended that newborns be bathed starting with the trunk and concluding with the head with its greater evaporative surface area, thus minimizing heat loss.

Although early bathing is probably of limited value and can safely be delayed, there is often a desire to rid the skin of its coating of vernix. The vernix is made up of a thick coat of mostly lipids, including triglycerides, free fatty acids, and cholesterol esters. One of the many functions of the vernix is to provide barrier function against transepidermal water movement in utero.[41] It may have a similar role after birth as well as providing mechanical protection during birth. There is no evidence that it plays a role in thermal protection, but it may provide some benefit to the premature neonate.

There is recent evidence that one benefit of a vaginal delivery is the transfer of organisms in the maternal microbiome to the infant, leading to the suggestion that smearing infants born by cesarean delivery with vaginal secretions would be of benefit to establishing a microbiome pattern akin to that of the mother.[42] Bathing may interfere with that goal.

Summary

Body temperature homeostasis is but one element of the normal adaptation to extrauterine life for all newborns. Prevention of heat loss is especially important in reducing morbidity and mortality in premature and LBW newborns. Hypothermia in critically ill infants whether due to prematurity, respiratory distress, sepsis, asphyxia, congenital anomalies, or other abnormalities will result in increased energy expenditure and oxygen consumption. Protocols and guidelines have been developed for protective management strategies based on clinical trials and physiologic rationale and are periodically revised by medical and technical staff. The use of sophisticated equipment requires constant monitoring of heat output and the neonate's core temperature with the goal of providing the appropriate NTE. It is particularly important for care providers to understand the consequences of heat loss in fragile neonates.

Several challenges remain, particularly in the maintenance of body temperature of extremely LBW newborns in the delivery room, during transport to the NICU, and in the first few hours after birth. This is due in part to resuscitative and stabilization efforts that may take priority over wrapping and other temperature control procedures. However, there is room for improvement in the coordinated and simultaneous approach to the prevention of unintended cooling without delaying or impeding necessary resuscitation and stabilization procedures.

There is also need for improvement in standards for the optimal level and duration of humidification for incubators and radiant warmers. For infants 26 weeks' GA and older, incubator humidity set at 60% to 70% during the first week of life and at 50% to 60% until 32 weeks' corrected age reduces transepidermal water loss and electrolyte disturbances.[26] However, prolonged and high levels (>80%) of humidity, often used in infants less than 26 weeks' GA, have been associated with impaired phototherapy delivery, enhanced microbial growth, and delayed maturation of the stratum corneum.[43–46] Significant practice variation persists.[25,47]

Future modifications of existing equipment will probably be directed toward improving safety and efficacy in maintaining strict and accurate control of the appropriate NTE. The use of noncontact infrared thermography to measure multiple skin sites simultaneously is being evaluated.[48,49] Different areas of the skin may demonstrate temperature fluctuations under different conditions, such as during movement from an incubator to SSC and back, phototherapy, times of vasomotor instability, and at sites of conductive heat loss. Infrared thermal imaging can provide minute-to-minute information from various sites and may be useful in studying the developmental changes in the neural control of temperature (central and peripheral receptors) with advancing gestational and postnatal age.

Perhaps the larger and more important need for future work is toward reducing newborn morbidity and mortality in lower-income and middle-income countries. While temperature management with SSC in these areas has been demonstrated to significantly reduce death in premature and LBW infants, cultural beliefs, lack of education or buy-in, and insufficient resources have all been identified as barriers to implementation of SSC.[18,50–53] Ongoing global efforts by multiple groups including Helping Babies Survive and the Kangaroo Mother Care Acceleration Partnership are focused on overcoming these barriers to improve infant outcomes in resource-limiting settings.[54,55]

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18

Newborn Screening

INDERNEEL SAHAI

KEY POINTS

- Newborn screening (NBS) provides an opportunity for early identification of newborns with disorders in which the clinical complications develop postnatally and may remain unrecognized prior to irreversible clinical damage.
- Since its inception nearly six decades ago, with screening for a single disorder, NBS has expanded substantially to more than 60 disorders in the current screening panels.
- NBS is a "screen," and individuals should not be labeled as having a disorder before diagnostic testing confirms the condition.
- Once a disorder has been confirmed, however, treatment should be initiated without delay to prevent irreversible clinical complications.
- False positives are inevitable; false negatives are possible.
- The clinical spectrum of disorders is wider than expected.
- The disorder detected is not always the one that was sought.
- Numerous methods are used in NBS, ranging from simple to complex, such as isoelectric focusing and enzymatic assays to tandem mass spectrometry and high-throughput genomic sequencing.
- Many factors will continue to transform NBS, including rapidly advancing technology and new and increasingly available therapeutic approaches to previously untreatable disorders.

Newborn screening (NBS) is directed at identifying congenital disorders in which the clinical complications develop postnatally. In metabolic and many other conditions considered appropriate for NBS, complications result from biomarker abnormalities that appear after birth, when the infant is no longer protected by fetal-maternal exchange or the fetal physiology. Initiation of treatment in the pre-symptomatic or early stages thus reduces the morbidity and mortality associated with the conditions. For example, the infant with phenylketonuria (PKU) has a normal blood phenylalanine level at birth but within a few hours demonstrates hyperphenylalaninemia. The infant with congenital hypothyroidism (CH) is also protected in utero, most likely from placental transfer of maternal thyroxine (T4). If the hyperphenylalaninemia in PKU is not controlled by diet or the hypothyroidism in CH is not corrected by supplemental T4, the infant begins to show signs of developmental delay and subsequently becomes intellectually disabled. If therapy begins during the first few weeks of life, intellectual disability in both disorders is prevented.

The association between mental retardation and PKU was initially recognized in the 1930s. Subsequently, it became evident that dietary therapy could prevent intellectual disability if initiated in the neonatal period. Detecting PKU in all affected newborns at that early age, before irreversible brain damage occurred, then became the challenge. Meeting this challenge required screening for a biochemical marker of the disease, which was accomplished in 1962 when Guthrie developed a simple bacterial assay for phenylalanine in filter paper saturated with a few drops of blood.[1] Newborns could therefore be routinely screened for PKU by measuring phenylalanine in dried blood spot specimens (DBS), obtained by lancing the heel and blotting the drops of blood onto a filter paper card, and mailed to a central laboratory for testing. An increased concentration of phenylalanine in the specimen indicated the possibility of PKU in the infant. By the mid-1960s many states had established routine NBS programs for PKU using the Guthrie method. Infants with PKU were identified in larger numbers than anticipated and showed normal development while receiving treatment.[2]

The success of PKU screening led to the addition of tests for other metabolic diseases, including galactosemia, maple syrup urine disease (MSUD), and homocystinuria. These additional tests could be performed on the same blood specimen obtained for PKU screening. Within a decade, NBS expanded to include the endocrine disorders CH and congenital adrenal hyperplasia (CAH), the hemoglobinopathies, and later biotinidase deficiency, cystic fibrosis (CF), and other diseases.

In 1990, the technology of tandem mass spectrometry (MS/MS) was applied to the DBS, opening a new era in NBS.[3] This method allowed for the accurate detection of numerous biochemical markers for metabolic disorders with a single assay, thereby replacing several assays traditionally used in screening for metabolic disorders and adding additional biomarkers not detectable by previous methods, thus greatly expanding the spectrum of conditions identifiable in the neonate.[4–6] Currently, all programs in the United States and many screening programs in Europe and elsewhere have integrated MS/MS into NBS to screen numerous biochemical disorders with high specificity and extremely low rates of false-positive results; some are screening newborns for more than 60 individual conditions.[7]

In 2006, to standardize screening panels nationwide, the American College of Medical Genetics recommended a panel of 29 core conditions for screening, and an additional 25 secondary conditions for which test results could be reported.[8] These secondary conditions are those that are identified through screening for the 29 core conditions, but either their clinical spectrum is not well-known, or effective treatment is unavailable. Currently, the task of reviewing and recommending conditions nominated for inclusion in the Recommended Uniform Screening Panel (RUSP) is performed by the Advisory Committee on Heritable Disorders in Newborns and Children.[9] The committee completes a systematic evidence-based review, deliberates on the evidence available, and votes to recommend or not recommend adding the nominated condition to the RUSP for consideration by the secretary of Health

and Human Services. The secretary makes the final decision on whether to add, or not to add, a recommended condition to the RUSP. Mucopolysaccharidosis (MPS-I), X linked adrenoleukodystrophy (X-ALD), and Spinal Muscular Atrophy (SMA) are the most recent additions to the RUSP. At the time of publication, the RUSP includes 35 core disorders and 26 secondary disorders (Tables 18.1 and 18.2).[10] In addition, the advisory committee has recently completed a review of two disorders—mucopolysaccharidosis (MPS-II) and cerebral creatine deficiency syndromes (CCDS)—and deemed that the conditions meet criteria for inclusion in the RUSP. Review of Krabbe disease to judge its suitability for inclusion in the RUSP has also begun.

TABLE 18.1 Core Disorders in the Recommended Universal Screening Panel

Condition	Acronym	Primary Biomarker
Organic Acid Disorder		
Propionic acidemia*	PA	C3
Methylmalonic acidemia (methylmalonyl-CoA mutase)*	MMA-Mut	C3
Methylmalonic acidemia (cobalamin defects: A,B)*	MMA-Cbl	C3
Isovaleric acidemia*	IVA	C5
3-Methylcrotonyl-CoA carboxylase deficiency	3 MCC	C5OH
3-Hydroxy-3-methyglutaric aciduria*	3 HMG	C5OH
Holocarboxylase synthase deficiency* (Multiple carboxylase deficiency)	MCD	C5OH, C3
β-Ketothiolase deficiency	BKT	C5:1, C5OH
Glutaric acidemia type I	GA-I	C5 DC
Fatty Acid Oxidation Defects		
Carnitine uptake defect/carnitine transport defect	CUD	C0 (Low)
Medium-chain acyl-CoA dehydrogenase deficiency*	MCAD	C8
Very long-chain acyl-CoA dehydrogenase deficiency*	VLCAD	C14:1
Long-chain L-3 hydroxyacyl-CoA dehydrogenase deficiency*	LCHAD	C16OH/C18:1OH
Trifunctional protein deficiency*	TFP	C16OH/C18:1OH
Amino Acid Disorders		
Argininosuccinic aciduria*	ASA	Argininosuccinic acid
Citrullinemia, type I*	CIT-I	Citrulline
Maple syrup urine disease*	MSUD	Leucine
Classical homocystinuria (cystathionine ß-synthase deficiency)	CBS	Methionine
Classic phenylketonuria	PKU	Phenylalanine
Tyrosinemia, type I	TYR-I	Succinylacetone
Other Inborn Errors of Metabolism		
Biotinidase deficiency	BIO	Biotinidase activity
Classic galactosemia*	GALT	Galactose1-phosphate uridyltransferase activity; Galactose (Total)
Glycogen storage disease type II (Pompe disease)	Pompe	Lysosomal acid α-glucosidase activity
Mucopolysaccharidosis type I	MPS-I	α-L-iduronidase activity
X-linked adrenoleukodystrophy	X-ALD	C 26:0 lysophosphatidylcholine
Endocrine Disorders		
Primary congenital hypothyroidism	CH	Thyroid-stimulating hormone; thyroxine
Congenital adrenal hyperplasia*	CAH	17-hydroxyprogesterone

(Continued)

TABLE 18.1 Core Disorders in the Recommended Universal Screening Panel—Cont'd

Condition	Acronym	Primary Biomarker
Hemoglobin Disorders (Sickling Disorders)		
S,S disease (Sickle cell anemia)	HbSS	Hemoglobin pattern—FS
S, β-thalassemia	HbS/β-thal	Hemoglobin pattern—FS or FSA
S, C disease	HbSC	Hemoglobin pattern—FSC
Other Genetic Disorders		
Cystic fibrosis	CF	Immunoreactive trypsinogen
Severe combined immunodeficiencies	SCID	T-cell receptor excision circles
Spinal muscular atrophy	SMA	Absence of Exon 7 of *SMN-1* gene
Other Congenital Disorders†		
Critical congenital heart disease	CCHD	Oxygen saturation by pulse oximetry
Hearing loss	HEAR	Failed hearing screen; diagnostic testing

Notes:
The markers for the organic acid disorders and fatty acid oxidation disorders are acylcarnitines; the number after the "C" represents the number of carbon atoms in the acylgroup in the acylcarnitine.
Numerous secondary markers, ratios, indices and second-tier tests employed in increasing the specificity of the primary markers are not listed.
*Disorders more likely to manifest acutely in the first week of life.
†Not screened by blood spot analysis.

TABLE 18.2 Secondary Disorders in the Recommended Universal Screening Panel

Condition	Acronym	Biomarker*
Organic Acid Disorder		
Methylmalonic acidemia with homocystinuria	Cbl C, D	C3
Malonic acidemia	MAL	C3 DC
Isobutyrylglycinuria (isobutyryl-CoA dehydrogenase deficiency)	IBG	C4
2-Methylbutyrylglycinuria (short/branched chain acyl-CoA dehydrogenase def)	2MBG SBCAD	C5
2-Methyl-3-hydroxybutyric aciduria	2M3HBA	C5OH
3-Methylglutaconic aciduria–type 1	3 MGA	C5OH
Fatty Acid Oxidation Defects		
Carnitine palmitoyltransferase IA deficiency	CPT-I	C0 (High)
Carnitine palmitoyltransferase II deficiency†	CPT-II	C16, C18:1
Carnitine acylcarnitine translocase deficiency†	CACT	C16, C18:1
2,4-Dienoyl-CoA reductase deficiency	DER	C10: 2
Glutaric aciduria II† (multiple acyl-CoA dehydrogenase deficiency)	GA II MADD	C4, C5, C5DC, C8, C14, C16
3-Hydroxyacyl-CoA dehydrogenase deficiency (medium/short-chain L-3-hydroxyacyl-CoA dehydrogenase deficiency)	HAD M/SCHAD	C4 OH
Medium-chain ketoacyl-CoA thiolase deficiency	MCKAT	C8
Short-chain acyl-CoA dehydrogenase deficiency	SCAD	C4
Amino Acid Disorders		
Arginase deficiency	ARG	Arginine

(Continued)

TABLE 18.2 Secondary Disorders in the Recommended Universal Screening Panel—Cont'd

Condition	Acronym	Biomarker*
Citrullinemia, type II (citrin deficiency)	CIT-II	Citrulline
Hypermethioninemia	H-MET	Methionine
Benign hyperphenylalaninemia	H-PHE	Phenylalanine
Biopterin defect in cofactor biosynthesis	BIOPT (BS)	Phenylalanine
Biopterin defect in cofactor regeneration	BIOPT (REG)	Phenylalanine
Tyrosinemia, type II	TYR-II	Tyrosine
Tyrosinemia, type III	TYR-III	Tyrosine
Other Disorders		
Galactose epimerase deficiency	GALE	Galactose
Galactokinase deficiency	GALK	Galactose
Various other hemoglobinopathies	—	Various hemoglobin patterns
T-cell-related lymphocyte deficiencies	—	T-cell receptor excision circles

*The markers for the organic acid disorders and fatty acid oxidation disorders are acylcarnitines; the number after the "C" represents the number of carbon atoms in the acylgroup.
†Disorders more likely to manifest acutely in the first week of life.

Molecular techniques (i.e., DNA analysis) are commonly used in NBS. At the outset, molecular assays were limited to "second-tier" testing to specify disorders, suspected because of a primary screening abnormality, such as by targeting known pathogenic mutations on the *CFTR* gene in samples with an increased concentration of immunoreactive trypsinogen (IRT), the primary biomarker for CF, or a panel of mutations in the *GALT* gene in specimens with decreased galactose1-phosphate uridyltransferase (GALT) activity in screening for classical galactosemia. Implementation of screening for severe combined immunodeficiency (SCID) and more recently for SMA, with low T-cell receptor excision circles (TREC) and absence of Exon 7 of the *SMN1* gene as the respective markers, extended the application of molecular assays into first-tier analysis in NBS. Sequencing of individual genes as second-tier testing has already been adopted by a few programs and the role of Next Generation Sequencing (NGS) in NBS is being evaluated.[11]

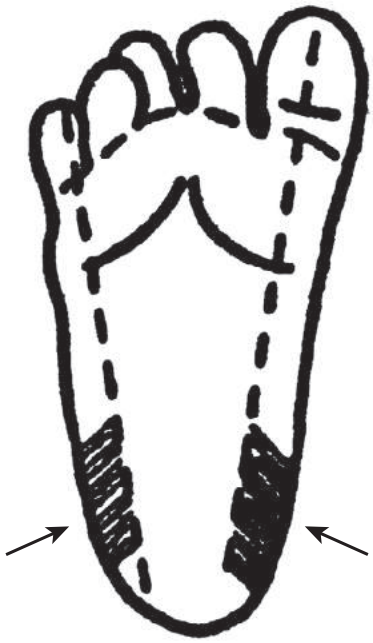
Screening Procedure

Specimen

Currently, almost all disorders on the RUSP are screened by laboratory analysis of the DBS specimen of the newborn. The two exceptions are a point-of-care hearing test and pulse oximetry that are performed directly on the newborns to screen for hearing loss and critical congenital heart disease (CCHD), respectively.

Specimen Collection Procedure

The blood specimen is generally obtained from the heel of the infant. This simple sampling method conceived and introduced by Guthrie and Susi,[1] has made NBS feasible since blood is easily obtained and can be easily and inexpensively delivered to a central testing facility by mail or courier. There are no serious complications from obtaining these newborn specimens, contrary to early fears that their collection would lead to infection or result in excessive bleeding.



• **Fig. 18.1** Hatched areas on the medial and lateral sides of the heel of the sole indicate the proper sites for a heel stick in the newborn.

The blood specimen should be obtained from the lateral or the medial side of the heel (Fig. 18.1). Blood should be applied to only one side of the filter paper card, but it should saturate each circle on the card. Contamination of the filter paper specimen

with iodine, alcohol, petroleum jelly, stool, urine, milk, or a substance such as oil from the fingers can adversely affect the results of the screening tests. In addition, exposure to heat and humidity can inactivate enzymes and produce false results. The specimen should be dried in air at room temperature for at least 3 hours before being placed in an envelope.

Specimens are sometimes collected in capillary tubes, by venipuncture of a dorsal vein or from a central line, and then spotted on filter paper. There is little or no substantial difference in analyte levels between blood collected directly from the heel and that collected by any of these other methods. However, there is the danger of introducing amino acids into the specimen in infants receiving total parenteral nutrition (TPN) if the blood is collected from a central line, resulting in a false-positive increase in the levels of amino acids or interference in some molecular assays by the heparin within the line. In general, it is preferable that a blood screening be spotted on filter paper directly from the heel.

Timing of the Collection

Specimen collection timings differ around the world. In the United States, most specimens are collected between 24 and 48 hours after birth. In Europe and Australia, screening specimens are collected within 48 to 72 hours, and in the United Kingdom the specimen is not collected until the newborn is 5 to 8 days old.[12] The specimen should be obtained from every newborn before nursery discharge or by the third day of life, whichever is first. In newborns whose initial specimen was obtained within the first 24 hours after birth, as may happen with the practice of early nursery discharge, a second blood specimen should be obtained at no later than 7 days old to be certain that a diagnosis is not missed.[13]

NBS encompasses a gamut of conditions, each with its own ideal screening period during which there is the greatest chance of diagnosing the disorder before the onset of symptoms. As a result, it is worth noting that recommendations on the timing of specimen collection, although appropriate for most conditions, may not be ideal for all conditions on the screening panel. For instance, for CAH, in which the symptoms can manifest themselves within the first week of life, the optimal time for collection of the specimen is within 24 to 48 hours after birth. Conversely, there remain concerns that with the NBS specimen collected early, often during the first day of life, some newborns with metabolic disorders or with CH might not have a sufficient degree of abnormality for identification. The MS/MS method with its increased sensitivity and specificity has considerably increased the reliability of screening for metabolic conditions in early specimens.[14] Furthermore, use of thyroid-stimulating hormone (TSH) as the primary marker for CH, or as a second-tier test when T4 is the primary marker, has similarly allowed early screening for CH to be more acceptable.

Special circumstances require specific attention to newborn blood specimen collection. Premature newborns or those with low birthweight (LBW), and newborns who are sick and those in neonatal intensive care units (NICU), are at risk of unreliable screening owing to factors such as the unique physiology of the newborn, therapeutic interventions, and a focus on critical activities in caring for the very sick neonate. Consequently, a single specimen is inadequate for screening newborns in this subpopulation, and additional specimens should be collected for retesting. Serial screening with collection of three specimens, (1) on admission to the NICU prior to interventions regardless of age, (2) between 24 and 48 hours after birth, if a prior specimen was collected at less than 24 hours of birth and, (3) at discharge or at 28 days old, whichever is sooner, has been proposed as an adequate and efficient protocol for this population.[13] In addition, some programs recommend that screening be performed every month until discharge for babies who continue to remain in the NICU.

A blood specimen should be collected from any infant who is being transferred to a different hospital or to an NICU, regardless of age. The first specimen should be collected before transfer, and a second specimen should be collected at the receiving hospital by 4 days of age. This dual collection policy covers the child from whom a newborn specimen might not have been obtained in the turmoil that frequently accompanies the transfer of neonates.

In a newborn who is to receive a blood transfusion within 24 hours of birth precluding collection of an ideal routine specimen, a screening specimen should ideally be collected before transfusion, and a second specimen should be collected 2 days after the transfusion. If a pretransfusion specimen has not been obtained, a screening specimen should be obtained 2 months after the last transfusion, when most of the donor red blood cells have been replaced, to ensure reliable testing for analytes present in red blood cells.

Screening Tests

NBS tests are usually performed in a centralized state, provincial, or regional laboratory. In a regional program, the specimens may be received by the state program and then delivered to the regional state or private laboratory, or they may be sent directly to the regional laboratory. In either case, the individual state programs serve as the state data and follow-up centers.

The testing procedure begins with punching small discs (each usually 3 mm in diameter) from the screening specimen. These small discs are then analyzed by various methods for the individual markers being sought. Amino acids (AA) and acylcarnitines (AC), the markers for a large proportion of the screened metabolic conditions, are simultaneously measured by MS/MS. MS/MS is superior in terms of accurate measurement of the individual analytes when compared with alternative methods originally used for screening the AA, such as bacterial assays or fluorometric techniques. Immunoassays, including fluoroimmunoassay and enzyme-linked immunosorbent assays, are used to test for endocrinopathies such as CH and CAH, for infectious diseases such as congenital toxoplasmosis, and for CF. Hemoglobin analysis of blood eluted from the filter paper can be performed by high-performance liquid chromatography (HPLC), capillary electrophoresis (CE), or isoelectric focusing (IEF) to identify abnormal hemoglobin variants (Hb) associated with the sickling hemoglobinopathies. An enzyme-based assay is often used to screen newborns for galactosemia and is always used to screen newborns for biotinidase deficiency and the lysosomal storage disorders (LSD).

A molecular assay, quantitative polymerase chain reaction (qPCR), is employed to identify SCID by quantifying TRECs, a marker of newly formed, antigenically naïve thymic emigrant T cells,[15] and another qPCR-based assay is used to identify homozygous absence of Exon7 of *SMN1*, expected in 95% of individuals with SMA.[16] TREC analysis was the first NBS test to use DNA as the primary analyte. Before implementation of screening for SCID, molecular assays were used only as second-tier tests to detect targeted mutations in disorders such as CF following an

out-of-range biomarker. Several platforms, DNA microarrays, and microsphere-based assays can multiplex several molecular and immunologic assays for high-throughput screening and are used by screening programs.[17–19] NGS with the high-throughput and massively parallel DNA sequencing technologies has substantially reduced the cost and time required for sequencing and made it possible to sequence the whole exome and entire coding regions of a gene/genes.[20] NGS has already been adopted by a few screening laboratories to provide supplemental genotyping information as a second-tier test,[21] and offers the prospect of becoming a first-tier test to screen newborns for genetic disorders that do not have a biomarker identifiable by current screening.

Secondary Tests

An abnormal finding on an NBS test is not diagnostic. Abnormalities in the newborn specimen can be transient or artifactual. Accordingly, when an abnormality is identified, the original specimen is retested for the primary analyte that was abnormal. In addition, secondary tests, biochemical or molecular, can be performed by the screening laboratory to substantiate the finding and increase the specificity of screening.[22] However, it should be noted that the primary analyte(s), the second-tier tests, and even the testing algorithms applied vary across screening programs.

In screening for CH, many programs have adopted protocols in which the primary analysis is for TSH, and T4 is measured as a second-tier test in specimens with high concentrations of TSH to improve specificity. Some programs initially measure T4. Specimens in which a low T4 level is found are further tested for an increased level of TSH, which would indicate CH.[23] Similarly, in screening for galactosemia, in some laboratories an elevated galactose measurement in a specimen can trigger the analysis of GALT activity as a second-tier test, while in others, a decreased GALT activity prompts galactose measurement as the second-tier test.

An out-of-range IRT, the primary marker for CF, could prompt second-tier molecular testing to identify pathogenic mutations on the *CFTR* gene.[24,25] However, the IRT concentration that prompts the molecular analysis, methods used for mutation selection and detection, and the number of mutations in the screening panel vary greatly, with some regions testing only for the most common mutation (F508del) and others for more than 400 mutations.[26] Sensitivity and specificity consequently vary across regions, but screening programs following this two-tier IRT-DNA approach can identify up to 99% of patients with CF and report a positive predictive (PPV) value ranging between 1/9.5 and 1/25.[27] Molecular assays to detect disease-causing mutations are currently used as second-tier tests for several other disorders, and their use continues to expand with advances in DNA technology. Some examples include testing for a panel of several *GALT* mutations in galactosemia screening and sequencing the relevant genes in screening for X-ALD and the LSD.

The final interpretation of the screening results is based on the primary analysis and, if available, the results of second-tier testing. The second-tier tests are used to either reduce the number of positive screens reported, or to provide supplemental information and categorize results based on probability of the disorder. However, it is important to realize that screening is not intended to be diagnostic; abnormal screening results must be supported by confirmatory investigations. These studies require additional specimens and are performed by clinical laboratories or sometimes by the screening laboratory.

Physician Contact for Abnormal Results

Tables 18.3 and 18.4 indicate disorders or other reasons for abnormal screening results, sorted according to the primary analyte usually used to screen the newborn for the condition. For example, a low T4 level together with an elevated TSH concentration indicates CH, and a marked elevation of 17-hydroxyprogesterone (17-OHP) level indicates the likelihood of CAH. An elevation of an acylcarnitine could indicate an organic acid or fatty acid oxidation disorder.

Any infant for whom an abnormal screening result is reported should be evaluated by the primary healthcare provider as soon as possible to facilitate the next steps toward the confirmation and management of the disorder. However, several conditions screened are extremely rare, and primary healthcare professionals might not have sufficient information available to direct appropriate intervention in screen-positive infants. To overcome the challenge, one-to-two-page explanations of the possible disorders, suggested follow-up action and confirmatory diagnostic investigations for the screening abnormality, known as ACTion (ACT) Sheets and Algorithms, are readily available on the website of the American College of Medical Genetics and Genomics (ACT Sheets and Algorithms [https://acmg.net]).[28] Similar explanations are often included with the screening report.

Although all screening results with a metabolite concentration that crosses its threshold are considered screen positive, all screen-positive results are not associated with the same likelihood of being associated with a disorder. Most infants with a positive screening result that is only mildly abnormal are less likely to have a disorder (see the later discussion of false-positive results) than are infants with analyte concentrations that are severalfold above the cutoff. Applying a uniform approach for all screen-positive results because of urgency of intervention or battery of tests suggested can result in unnecessary parental anxiety and medical costs. However, if recommendations for further action and workup are customized in accordance with the potential significance of the abnormality, both parental anxiety and the costs associated with false-positive results can be reduced. To achieve this goal, some programs subcategorize positive screening results and the primary care providers are supplied with category-based, customized fact sheets when a positive screening result is reported (personal communication). These sheets include information on disorders associated with the marker, the estimated likelihood of being affected, clinical presentations of likely disorders, factors contributing to false positives, and recommendations for further management. The follow-up recommendations can range from immediate admission to a hospital to simply repeating the analysis on a DBS specimen collected a few days later. Other programs approach this problem differently, but with the same goal of providing the primary care providers with the information needed to put the result in the appropriate context for the family.

When specific guidelines based on the individual results are not provided by the screening program, and the infant is ill or the likely disorder is among those that manifest acutely within the first few days of life (indicated in Tables 18.1 and 18.2), a specialist should be contacted. The infant may need to be admitted to the hospital, where further evaluation and therapy for the illness can be initiated without delay.

If the infant is clinically well on initial evaluation and the suspected disorder does not require immediate attention, a second DBS can be obtained and sent to the screening laboratory for

TABLE 18.3 Disorders and Other Factors Associated With Positive Screens for Acylcarnitines and Amino Acids

Marker	Disorders (Targeted and Incidental)	Possible Causes of False Positives
↓C0 (Free carnitine)	Carnitine uptake disorder (CUD); Other organic acid disorders	Poor feeding; Rx with valproic acid; CUD carrier; Maternal CUD/carnitine deficiency
↑C0 (Free carnitine)	Carnitine palmitoyl transferase I deficiency (CPT-I)	Carnitine supplementation
↑C3 (Propionylcarnitine)	Propionic acidemia (PA); Methylmalonyl-CoA mutase (MMA-Mut); Cobalamin disorders—A, B, C, D, F, J	Hemolysis or hyperbilirubinemia in infant; Carrier of associated disorder; Maternal cobalamin deficiency
↑C4 (Butyrylcarnitine)	Short-chain acyl-CoA dehydrogenase deficiency (SCAD); Ethylmalonic encephalopathy (EE); Glutaric aciduria (GA II); Isobutyryl-CoA dehydrogenase deficiency/isobutyrylglycinuria (IBG).	Hypoglycemia; FIGLU elevation; Carrier of associated disorder
↑C5:1 (Tigylcarnitine)	β-Ketothiolase deficiency (BKT)	VLBW neonate; Exposure of sample to heat/humidity
↑C5 (Isovalerylcarnitine)	Isovaleric acidemia (IVA); 2-Methylbutyrylglycinuria (2MBG)	VLBW neonate; TPN/HA; IVA carrier; Receiving antibiotics containing pivalic acid; FAS hemoglobin profile
↑C5-DC (Glutarylcarnitine)	Glutaric aciduria-I (GA-I)	GA-I carrier; MCAD carrier [with derivatized methods]
↑C5-3M-DC (Methylglutarylcarnitine)	3-Hydroxy-3-methyglutaric aciduria (HMG)	Severe respiratory distress; Neonates receiving ECMO
↑C5OH (Hydroxyisovalerylcarnitine)	3-Methylcrotonyl-CoA carboxylase deficiency (MCC); 3-Hydroxy-3-methyglutaric aciduria (HMG); Holocarboxylase synthase deficiency (MCD)	Carrier of associated disorder; Maternal 3MCC deficiency; Maternal biotin deficiency
↑C8 (Octanoylcarnitine)	Medium-chain acyl-CoA dehydrogenase deficiency (MCAD); Glutaric aciduria-II (GA II); Medium-chain ketoacyl-CoA thiolase deficiency (MCKAT)	MCAD carrier; GA-II carrier; MCT supplementation
↑C14:1 (Tetradecenenoylcarnitine)	Very long-chain acyl-CoA dehydrogenase deficiency (VLCAD)	VLCAD carrier
↑C16OH (Hydroxyhexadecanoylcarnitine) ↑18:1 OH (Hydroxyoctadecenoylcarnitine)	Long-chain L-3 hydroxyacyl-CoA dehydrogenase deficiency (LCHAD); Trifunctional protein deficiency (TFP)	LCHAD/TFP carrier; Renal dysfunction
↑C16 (Hexadecanoylcarnitine) ↑C18:1(Octadecenoylcarnitine)	Carnitine palmitoyl transferase II deficiency (CPT-II)	CPT-II carriers; Severe hemolysis
↑Arginine	Arginase deficiency (ARG)	TPN/HA; Arginase carrier
↑Argininosuccinic acid	Argininosuccinic aciduria (ASA)	Contamination
↓Citrulline	N-acetylglutamate synthase (NAGS) deficiency; Carbamylphosphate synthetase (CPS) deficiency; Ornithine transcarbamylase (OTC) deficiency; Pyrroline-5-carboxylate synthetase (P5CS) deficiency.	Poor feeding; Intestinal atresia or bowel resection
↑Citrulline	Citrullinemia, type I (CIT-I); Citrullinemia, type II (CIT-II); Pyruvate carboxylase deficiency (PC)	CIT I/CIT II carriers; Sample contamination (watermelon)
↑Phenylalanine	Classic phenylketonuria; Benign hyperphenylalaninemia; Biopterin defect in cofactor biosynthesis/regeneration BIOPT (BS/REG)	TPN/HA; Sample contamination (aspartame-artificial sweetener)
↑Leucine	Maple syrup urine disease	TPN/HA; Hydroxyprolinemia (benign disorder)
↓Methionine	Remethylation Defects— Methylenetetrahydrofolate reductase deficiency; Cobalamin defects—C, D, E, F, G, J	Poor feeding
↑Methionine	Classical homocystinuria (CBS); Methionine adenosyltransferase (MAT) I/III deficiency; Glycine N-methyltransferase deficiency	TPN/HA; Liver dysfunction
↑Tyrosine	Tyrosinemia, type II; Tyrosinemia, type III; Hawkinsinuria	Prematurity; Transient immaturity of enzymes; Liver dysfunction
↑Succinylacetone	Tyrosinemia, type I (TYR I)	—

HA/TPN—Hyperalimentation or Total parenteral nutrition containing amino acids. VLBW—Very Low Birth Weight —numerous secondary markers, ratios, and indices, employed for increasing the specificity of the primary markers, are not listed.

TABLE 18.4 Disorders and Factors Associated With Positive Screens for Primary Markers

Marker	Disorders (Targeted and Incidental)	Possible Causes of False Positives
↓Biotinidase activity	Biotinidase deficiency (profound or partial)	Exposure of sample to heat/humidity; Transient deficiency common in premature infants
↑Galactose (Total)	GALT deficiency (classical galactosemia and Duarte/benign variants); Galactose epimerase deficiency; Galactokinase deficiency; Citrin deficiency	Contamination with milk/cream; Portosystemic shunts
↓Galactose1-phosphate uridyltransferase (GALT) activity	GALT deficiency (classical galactosemia; Duarte/benign variants)	Exposure of sample to heat
↓Lysosomal acid α-glucosidase (GAA)	Glycogen storage disease aka Pompe	Pseudodeficiency
↓α-L-iduronidase (IDUA)	Mucopolysaccharidosis type 1	Pseudodeficiency
↑C26:0 lysophosphatidylcholine (LPC-C26:0)	X-linked adrenoleukodystrophy; Zellweger spectrum disorders	Omagaven supplementation; Carriers of associated disorders
↑Thyroid-stimulating hormone (TSH)	Primary congenital hypothyroidism	Transient elevations; Therapeutic hypothermia; Exposure to betadine
↓Thyroxine (T4)	Primary congenital hypothyroidism Secondary hypothyroidism (central); Thyroxine-binding globulin deficiency	Hypothyroxinemia of prematurity; Euthyroid sick syndrome
↑17-Hydroxyprogesterone (17 OHP)	Congenital adrenal hyperplasias	Neonatal stress (seen commonly in neonatal intensive care unit babies); very low birth weight; EDTA in specimen
Hemoglobin pattern analysis* FA [Normal in neonate]	*Sickling Disorders:* FS [HbS/S; HbS/β^0thal; HbS/HPFH]; FSC [HbS/C]; FSA [HbS/β+thal]; FSD [HbS/D^{Punjab}]; FSE [HbS/E]; FSX [HbS/OtherVariant] *Thalassemia & Other Hemoglobinopathies*: F only [β^0thal]; FABart's [HbH disease]	*Sickle Traits:* FAS[HbS]; FAC [Hb C]; FAD [Hb D^{Punjab}]; FAE[Hb E]; FAX [Other Hb Variant]
↑Immunoreactive trypsinogen (IRT)	Cystic fibrosis; CFTR-related metabolic syndrome	Cystic fibrosis carriers; Neonatal stress
↓T-cell receptor excision circles (TREC)	Severe combined immunodeficiencies; Other T-cell lymphopenias	Prematurity; Contamination (Heparin); Hydrops; Chylothorax; Gastroschisis; s/p thymectomy
Absence of Exon 7 of SMN-1	Spinal muscular atrophy	—

*The Hb variant pattern is shown outside [with the associated Hb disorder inside the brackets]. Complete listing of variants is beyond the scope of the chapter. Secondary markers, ratios and indices, and second-tier assays are employed for increasing the specificity of positive screens.

repeat testing, or confirmatory testing can be performed on a less urgent basis. In many cases, confirmatory testing or referral to a specialist is required only if the second specimen also indicates the presence of the disorder. However, the follow-up of an initial positive screening result can differ. In some cases, specific confirmatory testing is the appropriate first response to a positive newborn screen, with a less intense time frame for individuals in whom the level of suspicion is lower.

The physician should contact the screening laboratory when an infant whose screen has been reported as normal or whose screening results have not yet been reported has symptoms that suggest a disorder on the NBS panel. The screening laboratory can check the results in the infant's newborn specimen. If the testing has been completed and the newborn specimen is retained in storage, the laboratory may wish to recover the specimen and repeat the tests. The physician should also contact the screening laboratory for the repeated test results and inform the family of the results as soon as possible. If the second result is normal, the family's anxiety may be shortened.

Disorders Screened

The disorders included in the RUSP and the primary markers typically employed in NBS are listed in Tables 18.1 and 18.2. Majority of disorders on the RUSP are included in the individual state NBS panels, although implementation of screening for the newer conditions could lag behind the recommendation by a few years. Some states also screen for additional disorders. Some relevant NBS specifics related to the disorders are provided in the following sections. However, there is no attempt to describe any of the disorders in detail or their rare variants as they are covered extensively in other chapters of the book.

Every infant identified with a positive screen with a high probability of a disorder should be referred directly to the specialty center for confirmatory testing and prompt consideration of treatment. High probability positive screens, with disorders that are likely to present acutely in the first week of life, require immediate action that could include evaluation in the emergency room (ER) and admission to the NICU at a medical center with the

appropriate specialty service. Such fulminant disorders are marked in Tables 18.1 and 18.2.

Metabolic Disorders

Disorders With an Amino Acid or Acylcarnitines as the Primary Marker

The amino acid disorders, urea cycle disorders (UCD), organic acid disorders (OA), and fatty acid oxidation disorders (FAOD) detectable by NBS have a characteristic AA or AC as the primary marker. These AA and AC are analyzed simultaneously using MS/MS assays; however, the specific assays across different screening laboratories may vary in the preanalytical processing or internal standards employed. The simultaneous analysis of these numerous AA and AC in the assay has the advantage of allowing analysis of biochemical profiles in conjunction with the primary analyte, rather than the primary analyte alone. This biochemical profile, quantitated in the form of various ratios, indices, and scores by the NBS programs, can aid in differentiating the true positives from the false positives and in determining the most likely disorder when an analyte is associated with more than one disorder.[29] For instance, propionylcarnitine (C3) is the primary marker for several disorders: propionic acidemia (PA), methylmalonic acidemia (MMA), and cobalamin defects C, D (Cbl C, D). However, methionine the primary marker for homocystinuria, can be easily evaluated in an infant who screens positive for C3, and if low, would suggest Cbl C, D as the most likely disorder. Another relevant factor to be considered in analysis of AA and AC, by MS/MS based assays used in NBS is that the signal quantitated, is the combination of all ions with the same mass-to-charge ratio (m/z) as that of the analyte targeted. As an example, the concentration of an acylcarnitine (abbreviated as a "C" followed by a number indicating the number of carbon atoms in the acyl group in the carnitine ester), C4, represents the combined concentration of both butyrylcarnitine and isobutyrylcarnitine. Consequently, disorders that are not the primary target for screening are also identified in the process. Table 18.3 shows the disorders and other factors associated with positive screens for the primary AA or AC markers.

Amino Acid Disorders: The amino acid disorders are caused by an enzymatic defect in the catabolic pathway of the AA, causing an accumulation of specific AA above the block. Screening relies on the detection of these elevated AA in the DBS. The clinical manifestations may occur because of the toxic effects of the accumulating AA or metabolites produced by alternative pathways, a deficiency of products of the normal pathway, or both.

PKU is the best-known example of an AA disorder and is the paradigm for screened disorders in the newborn. In addition to PKU, MSUD and homocystinuria are historically significant in the context of screening because they are among the original metabolic conditions for which screening was performed before the expansion of NBS through the introduction of MS/MS technology. Screening for other AA disorders such as the UCD became possible only with the advent of MS/MS technology.

In PKU, the cardinal screening feature is an increased level of phenylalanine. With screening by MS/MS, PKU can reliably be identified as early as the first day after birth.[14] Not all infants with an elevation of phenylalanine level have PKU. Occasionally, an infant with elevated phenylalanine level will have one of the cofactor deficiency (pterin) disorders. Liver disease, such as that associated with galactosemia, tyrosinemia type I, or citrin deficiency, can also produce increased levels of phenylalanine.

The primary indicator for MSUD in the NBS specimen is an increase in the leucine level. Classical forms of MSUD are unlikely to be missed by NBS, but milder variants of MSUD can be missed,[30] as the increase in the leucine level may be so mild as to be below the cutoff value in the first few days of life when the NBS sample is collected. In the intermittent form of MSUD, the blood leucine concentration is normal in the newborn period, becoming elevated only in later infancy or childhood during acute metabolic episodes precipitated by febrile illness or surgery. Hydroxyproline at an increased level, as seen in a benign metabolic disorder known as hydroxyprolinemia, can be mistakenly identified as leucine in NBS. Second-tier analysis of hydroxyproline or quantitative plasma AA analysis will differentiate the condition from MSUD. To increase the specificity for MSUD of an abnormal leucine measurement, some screening programs also perform second-tier analysis of alloisoleucine, a leucine enantiomer pathognomonic for MSUD.[22] MSUD is one of the disorders that can present acutely in the first week of life and thus all high probability positive screens for MSUD require immediate action.

Majority of the characteristic metabolite for homocystinuria, homocysteine (Hcy), exists as S-linked Hcy in plasma, either bound to proteins or as disulfides. Accurate measurement of Hcy requires preanalytical deproteinization and reduction, and therefore cannot be analyzed simultaneously with the other AA and AC by the high throughput MS/MS assays utilized in NBS. Thus, NBS for classical homocystinuria, caused by cystathionine β-synthase deficiency (CBS), is based on identifying an increased concentration of methionine. The diagnosis of CBS is missed if the blood methionine concentration is not sufficiently elevated to be reportable by the screening laboratory. Adjusting the cutoff downwards has not been successful in eliminating false-negative occurrences completely, predominantly for the pyridoxine responsive forms. Some screening programs measure Hcy, methylmalonic acid and 2-methylcitric acid in a second-tier analysis to increase specificity of the primary methionine screen.[31] Although this two-tier strategy offers the advantage of substantially reducing the burden of false positives, there is a risk of missing cases of CBS with an increased concentration of methionine but without the expected increase in Hcy at the time of screening.[32] It is debatable whether the pyridoxine responsive forms of CBS is identified even when assays to measure Hcy as a primary marker are used. Strikingly high methionine levels can be seen in several other rare inborn errors of metabolism: isolated hypermethioninemia (methionine *S*-adenosyltransferase I/III deficiency), *S*-adenosylhomocysteine hydrolase deficiency,[33] glycine *N*-methyltransferase deficiency associated with liver disease,[34] and nonmetabolic causes of liver disease. Hypermethioninemia secondary to liver disease owing to tyrosinemia type I or to nonspecific liver disease is usually accompanied by increased tyrosine level.

More recently, screening programs have started reporting decreased concentrations of methionine to identify neonates with homocystinurias secondary to the remethylation defects—methylenetetrahydrofolate reductase deficiency and cobalamin defects—C, D, E, F, G, J (personal communication).[31]

Tyrosinemia type I (TYR-I) is identified in the newborn by the finding of increased levels of succinylacetone in the screening specimen using an MS/MS assay.[35] It is notable that in this eponymous disorder, caused by a deficiency of fumarylacetoacetate hydrolase the terminal enzyme in the tyrosine catabolic pathway, tyrosine is typically not increased in a well-timed NBS specimen. High concentrations of tyrosine in TYR-I are usually noted later in the neonatal period or after liver dysfunction is evident. The

other forms of tyrosinemia, type II and III, caused by deficiency of enzymes in the initial steps of the tyrosine catabolic pathway, tyrosine aminotransferase and 4-hydroxyphenylpyruvate dioxygenase respectively, are identified by an increased level of tyrosine in NBS. Another disorder caused by certain heterozygous mutations in the gene encoding the 4-hydroxyphenylpyruvate dioxygenase enzyme, hawkinsinuria,[36] has been identified in neonates with elevated tyrosine levels in the screening specimens. However, majority of positive screens for tyrosine are transient, seen in preterm newborns due to an immaturity of enzymes in the tyrosine pathway or secondary to vitamin C deficiency. Neonates with liver dysfunction with any underlying etiology often have an increased concentration of tyrosine.

Urea Cycle Disorders: The three UCD routinely screened by MS/MS analysis are citrullinemia, argininosuccinic acidemia, and arginase deficiency. All three may produce hyperammonemia in the neonate, although arginase deficiency usually presents in childhood with neurologic features such as spasticity and developmental delays in infancy and mild hyperammonemia rather than severe hyperammonemia in the neonate.[37] Severe hyperammonemia in the newborn is a medical emergency, thus high probability positive screens for these disorders should trigger prompt consultation with a metabolic specialist or referral to the ER at a metabolic center. Early identification through NBS with presymptomatic or early symptomatic therapy is critical to protecting patients with UCD. A decreased concentration of citrulline is used by the New England Newborn Screening Program, as an indicator to screen for the proximal UCDs, ornithine transcarbamylase deficiency, carbamylphosphate synthetase deficiency, and N-acetylglutamate synthase deficiency.

Fatty Acid Oxidation Disorders: The FAOD include those involving carnitine wherein the long-chain fatty acids cannot traverse the mitochondrial membranes to be oxidized within the mitochondrial matrix, and those in which there are defects in fatty acid oxidation per se. In either category the problem is the inability to fully oxidize fatty acids. The major clinical consequence of these disorders is fasting intolerance resulting in hypoketotic hypoglycemia, lethargy, hyperammonemia, metabolic acidosis, hepatomegaly, and sometimes sudden death. Each FAOD is associated with a specific or almost specific AC pattern on MS/MS analysis.

The most common FAOD is medium chain acyl CoA dehydrogenase deficiency (MCADD). Tragically, before NBS was available, this disorder was often diagnosed only retrospectively after a sudden unexplained death, usually when postmortem examination revealed a fatty liver. This devastating outcome and a frequency of 1:15,000 to 1:20,000, comparable with that of PKU, made MCADD the primary reason for the addition of MS/MS technology to NBS. The primary finding for MCADD in NBS is an elevation of octanoylcarnitine (C8) along with other medium chain ACs. Some programs pursue targeted testing of the c.985 A>G *ACADM* gene mutation as a second-tier analysis to provide supplementation information.[38] Because this mutation occurs in as many as 90% of individuals with MCADD, this additional analysis substantially increases the predictive value of the primary C8 screen, even though the absence of the mutation does not exclude the condition. Furthermore, in infants affected with a FAOD, when not metabolically stressed, the AC can be within the normal range. Thus, infants with a high probability AC pattern on the initial NBS specimen require a referral for diagnostic studies, regardless of the results on a subsequent specimen or any targeted mutation analysis. Most of these disorders are treatable, but screening enables early diagnosis and genetic counseling for the family even when early treatment may not be effective, such as in neonatal carnitine palmitoyltransferase II deficiency.[39] Short-chain acyl-CoA dehydrogenase deficiency is likely benign, although before NBS it was considered a serious disorder.[40]

Organic Acid Disorders: The OAs are a heterogenous group of disorders characterized by accumulation of organic acids. Many OA can be identified by NBS based on increased levels of their characteristic AC in the DBS. The major OAs identified in NBS are PA, MMA, the cobalamin (vitamin B_{12}) defects, and isovaleric acidemia. The classical OAs can manifest themselves in the neonatal period with a life-threatening, sepsis-like picture of feeding difficulties, lethargy, vomiting, and seizures. If a screening result suggests an OA, a metabolic specialist should be consulted immediately.

Biotinidase Deficiency

Biotin recycling is necessary for the maintenance of sufficient intracellular biotin to activate carboxylase enzymes. Biotinidase is a key enzyme in biotin recycling, and biotinidase deficiency is one form of an OA known as multiple carboxylase deficiency. NBS for the disorder is based on demonstrating deficient enzyme activity in the DBS.[41] Individuals with profound biotinidase deficiency have less than 10% of mean normal enzymatic activity, while those with the partial biotinidase deficiency variant have 10% to 30% of mean normal activity.[41] Clinical features of biotinidase deficiency, primarily seen in profound deficiency, include developmental delay, seizures, vision problems, hearing loss, and cutaneous abnormalities. Initiation of biotin therapy in the presymptomatic stage prevents all features of the condition. Occasionally, an individual with partial deficiency may develop symptoms when stressed, such as during infection. Transiently low biotinidase activity in some premature newborns results in false positive screens. Some newborns with partial biotinidase deficiency may be missed by NBS.

Galactosemias

Classical galactosemia due to GALT deficiency typically manifests itself in the neonatal period as failure to thrive, vomiting, and liver disease.[42] Death from bacterial sepsis, usually caused by *Escherichia coli*, occurs in a high percentage of untreated neonates.

Some screening programs use a metabolite assay for total galactose (galactose and galactose 1-phosphate) as the primary test to detect galactosemia. Other programs employ a semiquantitative enzyme assay in the first tier to measure the GALT activity, which is usually undetectable in classic galactosemia. A few programs use both tests as a primary screen. The enzyme assay identifies only galactosemia, whereas the metabolite assay also identifies other galactose metabolic disorders, such as deficiencies of galactokinase and epimerase. Severe neonatal liver diseases and portosystemic shunting caused by anomalies in the portal system can also increase the galactose level. NBS programs that use total galactose as the primary screen usually perform second-tier GALT testing in specimens with elevated galactose level. If the newborn specimen has markedly reduced or absent GALT activity, a few screening programs then perform targeted molecular testing for a panel of frequent mutations associated with galactosemia, particularly Gln188Arg and Asn314Asp; the Asn314Asp variant, commonly referred to as the Duarte (D or D2) allele, is associated with about half of the normal level of GALT activity, and is clinically benign. However, it is a common variant accounting for a large proportion of positive screens for GALT deficiency.

Infants having increased galactose and reduced GALT activity in NBS or those with no detectable activity when only the GALT assay is performed should immediately be seen at a metabolic center. Breastfeeding or lactose-containing formula feeding should be discontinued, and appropriate intravenous fluids with glucose should be given as needed.

Markedly increased galactose and normal GALT activity in NBS suggest the possibility of galactokinase deficiency. This disorder produces early-onset cataracts, which are prevented by removal of lactose from the diet. Moderately increased galactose with normal or somewhat reduced GALT activity could indicate uridine diphosphate (UDP)-galactose 4-epimerase deficiency, a largely benign disorder.

Lysosomal Storage Disorders

Lysosomes are organelles required for cellular turnover and contain more than 50 acid hydrolases that catabolize macromolecules. Deficiency of the individual enzyme or a combination of enzymes and transporters can result in accumulation of the substrate and progressive cellular and organ dysfunction. The disease phenotype is a consequence of the type of substrate and its sites of turnover, and severity generally correlates with the amount of residual enzyme activity. The incidence of these disorders as a group is estimated to be 1 in 7700 to 1 in 10,000 births. NBS for the LSD relies on the analysis of the specific enzyme activities by using artificial substrates in the DBS, either by fluorometry, MS/MS, or microfluidics combined with fluorometry. Screening assays are available for Fabry disease, Gaucher disease, Krabbe, MPS-I, MPS-II & IV-A, Niemann-Pick A/B disease and Pompe disease. As most laboratories screening for LSD using the MS/MS method screen for at least two LSDs simultaneously, the enzymatic activities can be evaluated in conjunction to ascertain sample quality and determine significance of a specific result and thereby risk of a disorder.[43] As indicated on the website of the Advisory Committee on Heritable Disorders in Newborns and Children,[9] until May 2021 five LSDs (Krabbe disease, Fabry disease, Niemann–Pick disease, Pompe disease, and MPS-I) had been evaluated by the Committee to be considered for inclusion in the RUSP. Only MPS-I and Pompe disease were approved for inclusion in the RUSP. The considerations underlying the decision to not approve the addition of Krabbe disease corresponded to many of the concerns expressed by the Krabbe Disease Consortium.[44] Although not included in the list of RUSP disorders, in August 2006, New York became the first state to initiate population-wide screening for early infantile Krabbe disease in response to a strong and organized advocacy. Previous high-throughput methods for the measurement of a lysosomal enzyme galactocerebrosidase (GALC) by MS/MS were adapted for NBS in blood spots.[45] Specimens with reproducibly low GALC activity were analyzed for *GALC* mutations as a second tier, particularly for homozygosity for the 30-kb deletion because of its strong association with early infantile Krabbe disease.[46] Early infantile Krabbe disease is a rare progressive neurodegenerative genetic disorder that usually leads to death in infancy. Studies had previously indicated that human stem cell transplantation (HSCT) was effective in improving survival and clinical outcomes when used before the onset of symptoms in infantile Krabbe disease. However, after more than 15 years of active screening in New York and introduction in other states, the efficacy of NBS for Krabbe disease has not been established. Some recommend placing a moratorium on mandatory NBS for Krabbe disease for now.[47] They argue that the current laboratory screening and diagnostic tools to identify infants, who are likely to develop Krabbe disease and need HSCT, are inadequate. Additional biomarkers such as psychosine that appear to be more specific than low GALC levels for diagnosing early infantile Krabbe disease, and new consensus guidelines for diagnosis and treatment following NBS have been developed that may improve screening and outcomes in the future.[48]

One challenge in screening for some LSDs is the phenomenon of pseudodeficiency that results in low activity of the specific enzyme in the in-vitro assays with no associated clinical phenotype. Pseudodeficiency is caused by certain variants in a gene, and its prevalence varies by disorder and ethnicity. Whether the presence of a pseudodeficiency variant could influence the effect of another variant is not completely understood at this time. Occurrence of pseudodeficiency could increase false-positive screens in the first-tier enzymatic assay and may complicate accurate identification of true cases. Gene sequencing is important for identification of pseudodeficiency alleles and confirmation of a diagnosis following the first-tier enzymatic assay, and thus is performed as a second-tier test for the LSDs in some screening programs.

Pompe disease, also known as *glycogen storage disorder II*, is characterized by accumulation of lysosomal glycogen, predominantly in muscles, resulting from the decreased activity of lysosomal acid α-glucosidase (GAA) due to pathogenic variations in the corresponding *GAA* gene.[49] Pompe disease exhibits a broad spectrum in regard to age of onset, cardiac involvement, and progression of skeletal muscle dysfunction. The severe infantile form manifests itself within the first few months of life and is characterized by cardiomyopathy and severe progressive muscle weakness. Without treatment, death occurs within the first year of life in the infantile forms. The overall incidence of Pompe disease is 1 in 28,000, with 28% being the infantile forms. NBS offers the opportunity to detect the infantile forms early (22 days vs. 3.6 months by clinical ascertainment) and thus justifies its inclusion in the RUSP. Decreased GAA activity in the screening blood spot should prompt molecular analysis for confirmation. Homozygosity for a "pseudodeficiency" allele c.(1726G>A; 2065 G>A) is associated with low GAA activity like that seen in patients with Pompe disease but does not cause disease. This genotype, seen in approximately 4% of individuals in the Asian population, will result in false-positive screens in enzymatic assays. Second-tier tests including GAA gene sequencing and measurement of the creatine/creatinine/α-glucosidase ratio are often utilized to improve the specificity of the first-tier enzymatic assay.[50] However, although gene sequencing can predict genotype-phenotype effects when known, the risk assessment is limited when the variants are novel or of unknown significance. Similarly, the creatine/creatinine/α-glucosidase ratio may not be reliable in accurately identifying the late onset forms of the disorder.

MPS-I is a progressive multisystem disorder with features ranging over a continuum of severity. It is caused by the deficiency of the lysosomal enzyme α-L-iduronidase (IDUA), encoded by the *IDUA* gene, which leads to an accumulation of glycosaminoglycans (or mucopolysaccharides) within lysosomes of the affected cells.[51] MPS-I is broadly categorized into Hurler syndrome (MPS I H; severe, incidence 1 in 100,000), Hurler–Scheie syndrome (MPS I H/S; attenuated; incidence 1 in 500,000), and Scheie syndrome (MPS I S). Newborns typically appear normal at birth. Decreased IDUA activity in the DBS suggests the possibility of MPS-I and needs to be confirmed by molecular analysis of the *IDUA* gene and/or confirmatory biochemical testing (urinary glycosaminoglycans, IDUA activity in leukocytes or fibroblasts). Unfortunately, the residual IDUA

activity is not predictive of the phenotype, and the presence of pseudodeficiency alleles renders interpretation of IDUA activity more difficult. Second-tier genotyping may help predict the expected phenotype if it reveals mutations with good genotype–phenotype correlation,[52] but the advantage is limited for novel variants or those of unknown significance. Second-tier analysis of glycosaminoglycans in the DBS has been incorporated by a few laboratories to improve specificity of the first-tier enzymatic assay.[53] However, the experience is limited in accurately identifying the late onset forms of the disorder.

X-linked Adrenoleukodystrophy

X-ALD is one of the most common monogenic forms of an inherited neurodegenerative disease. It is inherited in an X-linked manner, with a prevalence of approximately 1 in 21,000 in males or 1 in 16,800 with symptomatic heterozygous females included. The clinical phenotype is highly variable, but neurologic manifestations, as either the inflammatory cerebral form or adrenomyeloneuropathy only, are present in nearly all males by adulthood.[54] X-ALD can present as a primary adrenocortical insufficiency without evidence of neurologic abnormality in about 10% of males, with neurologic involvement occurring subsequently. Overall, adrenal function is abnormal in 90% of neurologically symptomatic boys and in 70% of men with adrenomyeloneuropathy. HSCT has been shown to arrest the inflammation in the early stages and thus provide an efficient treatment for the inflammatory cerebral form of X-ALD. Detection of X-ALD by NBS allows periodic reevaluation of adrenocortical function and MRI for detection of males with early cerebral disease, thereby providing an ideal window of opportunity for HCST to treat the cerebral form. NBS for X-ALD relies on measuring C26:0 lysophosphatidylcholine (C26:0LPC) using HPLC-MS/MS assays in the DBS.[55] Individuals with a positive screen require plasma very long chain fatty acid (VLCFA) analysis and sequencing of the *ABCD1* gene (performed as second-tier tests in some NBS programs) to confirm the diagnosis. However, the phenotype cannot be reliably predicted by the plasma VLCFA concentration or the *ABCD1* variants. Increased concentrations of C26:0 LPC in the screening specimen are also noted in other peroxisomal disorders, such Zellweger spectrum disorders, peroxisomal acyl-CoA oxidase 1 deficiency, and D-bifunctional enzyme deficiency.

Endocrine Disorders

Congenital Adrenal Hyperplasia

The rationale for screening for classic congenital adrenal hyperplasia (CAH) is to prevent deaths from delayed recognition and treatment of the salt-wasting form (SW-CAH), to prevent sex misassignment of affected female newborns that can occur with either the salt-wasting or simple-virilizing forms, and possibly to prevent premature epiphyseal closure in children with simple-virilizing CAH. Initial screening relies on an immunoassay for identifying increased levels of 17-OHP to detect CAH due to steroid 21-hydroxylase (CYP21) deficiency, which accounts for more than 95% of all cases.[56,57] Although screening is effective at identifying SW-CAH cases, there is a very high false-positive rate associated with the immunoassay. The finding may be due to a truly increased 17-OHP level, as in perinatal stress and early specimen collection (within the first 24 hours of life) or may be due to cross-reacting steroids present in prematurity and LBW neonates. Despite employing screening algorithms based on covariate (gestational or birth weight, gender, and age at collection) adjusted cutoffs, the PPV for the first-tier immunoassay performed on specimens collected within the first 2 days of life remains low (on average <10%).

To enhance specificity, second-tier liquid chromatography-MS/MS-based assays to measure 17-OHP and various combinations of additional steroids, is being increasingly employed by NBS programs. Most recently, PPV of 17% and reduction of false positives by 95% was demonstrated, by employing a screening algorithm including a first-tier immunoassay, second-tier multi-steroid quantification and profiling, in conjunction with stratification of population-based cutoffs by the time of specimen collection and birth weight.[58] On the other hand, the resounding success of the two-tier approach in reducing the false positives is dampened by reports of missed cases after second-tier analysis.[59]

However, the risk of missed CAH cases, whether after first or second-tier analysis, cannot be eliminated. Insufficient accumulation of 17-OHP at time of specimen collection as can occur with mild/simple virilizing forms or early collection of NBS specimens, and reduced flux through the steroidogenic pathway secondary to adrenocorticotropic hormone inhibition by exposure to glucocorticoids or maternal cortisol, and the lack of mature enzyme function, are some factors that contribute to the existence of false negatives. Several laboratories have demonstrated increased specificity after implementing molecular analysis as a second-tier test. However, thus far second-tier molecular analysis has not been implemented in routine screening. The 2018 Endocrine Society Clinical Practice Guidelines specify that NBS programs employ second-tier liquid chromatography-MS/MS (LC-MS/MS) in preference to all other methods (e.g., genotyping) to improve PPV of screening for CAH.[60] Classic CAH, caused by CYP21 deficiency, occurs in 1 in 16,000 to 20,000 births, of whom 75% are estimated to have the salt-wasting form and 25% the non-salt-wasting or simple virilizing form.[57]

Congenital Hypothyroidism

Early detection by NBS and thereby early treatment of infants with CH has essentially eliminated the neurodevelopmental disabilities associated with this disorder. The NBS strategies for CH, directed mainly towards identifying primary hypothyroidism, have evolved since its inception in the mid-1970s and vary regionally.[23,61] Currently, immunoassays are employed for analysis of both T4 and TSH. Screening programs employ algorithms in which either TSH or T4 is measured as the primary marker, and if out of range, proceed with T4 or TSH as a second-tier test to improve specificity. The primary T4 approach has the potential to detect neonates with secondary (central) CH. Only a few screening laboratories pursue simultaneous T4 and TSH testing. All approaches have similar accuracy in detecting severe primary hypothyroidism. Nevertheless, affected infants can be missed with either approach, because of lack of the identifying marker abnormality at the time of specimen collection. Specifically, the T4 level during the first 24 hours after birth in an affected newborn might not yet be sufficiently decreased for identification because of persistence of maternally transmitted T4 Moreover, in some infants it might take 2 weeks or more for a TSH level elevation to develop.[23,62] This pattern of a delayed TSH rise is particularly common in infants who are preterm, LBW, or acutely ill, and proposed mechanisms include immaturity of the hypothalamic–pituitary–thyroid axis, nonthyroidal illness, and exposure to medications that may suppress TSH secretion (e.g., dopamine or glucocorticoids). To avoid missing CH, screening programs test additional blood specimens in specific subgroup of infants a few weeks after the initial screen.

The reported false-positive rates of screening for CH range from approximately 0.05% to as high as 4%.[63] Newborns with false-positive results have transiently low T4 or elevated TSH levels. Many of those with low T4 levels are premature neonates with a normal TSH concentration or infants with perinatal stress and elevated TSH levels. Analysis of the markers adjusted for parameters such as age at collection, weight, and location may improve the performance of NBS for hypothyroidism. In addition to false-positive results, a low T4 level with a normal TSH value can result from benign T4-binding globulin deficiency or hypothyroidism secondary to pituitary deficiency.

The incidence rates of CH detected by NBS recently (1:1400 to 1:2800) are substantially higher than the incidence rates (1:3000 to 1:4000) reported in the initial years of NBS for CH.[64,65] Numerous factors, including shifting demographics and modifications in screening protocols (lowered TSH cutoffs, testing additional specimens in specific subgroups) that allow detection of mildly affected patients or those with a delayed TSH rise, probably explain the increase. The majority of increased occurrence, in addition to the mildly affected patients, is due to those with transient CH, which brings forth new dilemmas about the diagnosis and optimal management of such cases.[66]

Cystic Fibrosis

The frequency (1 in 2000 to 3000 live births in the white population) and severity of CF explains its inclusion in routine NBS. The primary screen for CF relies on detection of elevated levels of IRT, a biomarker indicative of pancreatic damage, in the first week of life. A transient increase of IRT is not uncommon in premature neonates and neonates with perinatal asphyxia or other stressors. Thus, to improve specificity of the elevated IRT on the initial screen, additional analyses are performed by NBS programs before the screen is reported as "positive" or the infant is referred for a sweat test to confirm the diagnosis. However, the approach for the subsequent analyses varies widely among NBS programs, both in regard to the specimen (initial or additional typically collected at 3 weeks of life) prompting the second-tier test, and the second-tier test performed (selected mutation analysis or sequencing of the *CFTR* gene or pancreatitis-associated protein) on the specimen, each with its own benefits and disadvantages.[67,68]

Second-tier DNA analysis on the initial specimen [IRT/DNA] protocols can reduce time to diagnosis substantially for infants carrying at least one mutation included in the panel, but a significant number of neonates who are carriers or unaffected undergo sweat testing. In other protocols, persistence of the IRT elevation on a second specimen collected, following an elevated IRT on the initial specimen [IRT/IRT], prompts a referral for sweat testing [IRT/IRT] or molecular testing [IRT/IRT/DNA]. As many screening protocols rely on mutation analysis, the impact on false negatives is determined by the number of mutations targeted and how closely the mutation panel represents the population being screened. In contrast, with the wide variety of CFTR mutations and mutation combinations identified, directly by screening or during diagnostic testing, genotype–phenotype correlations are difficult to predict and lead to increased diagnostic uncertainty. NBS commonly identifies infants who have an inconclusive diagnosis of CF following a positive screen, classified as CFTR-related metabolic syndrome or CF screen positive inconclusive diagnosis, and may need to be monitored.[69,70]

Early diagnosis and treatment of classic CF has shown to result in improved nutrition, growth, and survival compared with those diagnosed clinically (CF-related childhood mortality is lower by 5% to 10% in screened cohorts). The recent development of options that treat the underlying genetic defect bring forth the potential to substantially impact the course of the disease in individuals with specific mutations.

Sickle Cell Disease

Most programs in the United States screen newborns for the hemoglobinopathies with the objective of detecting the sickling hemoglobinopathies. Screening is based on the separation and quantification of the various hemoglobin variants (Hb), typically by IEF, CE or HPLC.[71] Other technologies using MS/MS, matrix-assisted laser desorption ionization-time of flight mass spectrometry, and DNA-based methods are also emerging. At a minimum screening assays can separate and identify the normal variants (HbF, HbA) and sickling variants (HbS, HbC). Hb Bart's, HbH and other Hb variants of clinical relevance, such as HbD, HbE, and HbO are also detected.

The typical neonatal pattern is FA, representing the Hb variants (HbF, HbA) listed in order of their fractional concentrations (highest to lowest). In NBS specimens, HbA levels range between 6% and 40% (average of 19%), showing variations based on gestational age and age at sampling, reflecting the stage of hemoglobin switch. Screening specimens showing atypical variants or profiles on the initial assay are retested using secondary assays to confirm the presence of the Hb variant or identify the abnormal Hb. Programs are increasingly adding genotyping for this purpose. Screening identifies sickling disorders (HbSS, HbSC, HbS/β^0 thalassemia), sickle cell traits, and several other hemoglobin variants. Cases identified by screening or those with unidentified Hb variants should have further diagnostic evaluation by a hematologist. Initiation of simple but effective prophylactic measures such as penicillin for pneumococcal sepsis, vaccination, and parent education, have shown to result in a significant reduction of mortality and mortality of the sickling disorders during the early years when risks are the greatest. The long-term complications are not yet preventable but, erythrocyte transfusion (for acute crises) and medications (hydroxyurea, oxbryta, L-glutamine) are some options to alleviate the morbidity and mortality. Additionally, hematopoietic stem cell transplantation and the emerging gene therapies raise the prospects of curative treatment.[72–75] Other than sickling disorders, most of the abnormalities are benign or are not impacted by early detection. It is especially important to differentiate the common and benign sickle cell trait from the much rarer sickle cell disease, to avoid the stigma attached and unnecessary medical treatment for the disease, but at the same time counseled appropriately about the risk of complications of sickle cell disease (pain crises, stroke, myolysis) with intense exercises under extreme circumstances.

The presence of a β-chain hemoglobinopathy can be missed in premature infants as they have lower percentage of HbA (and other β-chain variants) and in infants after a blood transfusion as the hemoglobinopathy may be masked by the higher proportion of the donor HbA. Screening programs thus request additional screening specimens under special circumstances.

Severe Combined Immunodeficiency

SCID is a group of at least 20 known rare genetic disorders, characterized by profound impairment in T-cell development and function and absence of antibody production. As SCID is

typified by absent or extremely low production of antigenically naïve T-cells from the thymus, a low concentration of TRECs (DNA fragments that are byproducts of thymic emigrant T-cell production) in a specimen with a valid result for an internal control (typically β-actin or RNase P gene) is used to screen newborns for SCID.[75] A high-risk positive SCID NBS result prompts further diagnostic testing, such as flow cytometry to measure specific T-cell markers and specialized immune function tests before the diagnosis is established or excluded. Majority of low-risk positive SCID NBS results are from neonates in the NICU and depending on the testing laboratory's algorithms may require only a repeat screen. Non-SCID lymphopenias and other disorders associated with T-cell impairment (e.g., DiGeorge syndrome, trisomy 21) or T-cell losses (e.g., vascular leakage, chylothorax), and infants with gastrointestinal anomalies or those who have had a thymectomy (most often during cardiac surgery) can show low TREC values.[75,76] Early treatment of SCID, using allogeneic hematopoietic cell transplantation and autologous gene therapy, has led to an improved survival with a high rate of full immune reconstitution in infants.[76,77] Genetic diagnosis is often needed for genetic counselling, prognostication, and modification of pretransplant chemotherapeutic agents. Sequencing a panel of SCID genes using NGS as a second-tier test in specimens with low TRECs is feasible and may be the norm in the future.[78]

Spinal Muscular Atrophy

Spinal muscular atrophy (SMA) is an autosomal recessive disorder, characterized by progressive degeneration of alpha motor neurons in the spinal cord leading to muscular atrophy, caused predominately by bi-allelic deletions involving exon 7 of the *SMN1* gene. Molecular assays to detect absence of Exon 7 of *SMN1* in the DBS were developed and used to screen newborns for SMA since 2016. Its inclusion in RUSP occurred subsequently in 2018, when specific treatments directed at improving functional SMN protein expression for SMA became commercially available. Neonates who screen positive for SMA require confirmatory testing for the *SMN1* deletion. Furthermore, determination of *SMN2* (a paralog of *SMN1*) gene copy number is an integral part of confirmatory testing, as SMN2 dosage is highly prognostic of disease severity and predictive of neonates who would benefit from immediate intervention.[79] Further developments of molecular assays, allowing detection of *SMN1* deletions, *SMN2* copy numbers and *SMN1* sequencing in the DBS at the NBS laboratory are ongoing. Early data shows that some SMA-affected children have remained asymptomatic and are meeting developmental milestones expected for unaffected infants.[80]

Critical Congenital Heart Disease

Congenital heart disease (CHD) is the most common cause of death in the first year of life, accounting for 3% of all infant deaths. CHD affects about 7 in 1000 to 9 in 1000 live births; a fourth of these can be classified as CCHD, a term that encompasses the heart defects that cause severe and life-threatening symptoms and require intervention within the first year of life.[81,82] Currently, pulse oximetry, in conjunction with a clinical examination, is the recommended screening approach and will usually identify CHD that results in hypoxemia. This group accounts for approximately 17% to 31% of CHD cases and includes hypoplastic left-sided heart syndrome (HLHS), pulmonary atresia with intact septum, tetralogy of Fallot, total anomalous pulmonary venous return, transposition of the great arteries, tricuspid atresia, and truncus arteriosus. Each condition has palliative and surgical options, with a spectrum of standard interventions for each. Overall, the 5-year survival rates after intervention range from 65% to 97%, with five of the seven conditions having 5-year survival rates greater than 85%. The two conditions with the lowest long-term survival rates are pulmonary atresia with intact septum and HLHS. The sensitivity of screening for CCHD is 30.8%, 47%, and 77% by pulse oximetry, clinical examination alone, and both, respectively.

Hearing Loss

Hearing loss is the most common birth defect, with 1 in 500 newborns having confirmed hearing loss. Infants with hearing loss not identified before 6 months of age have delays in speech and language development, while intervention before 6 months of age allows an infant with impaired hearing to develop normal speech and language. This rationale prompted the National Institutes of Health to recommend screening for congenital hearing loss for all newborns in 1993. Typically, automatic auditory brainstem response or otoacoustic emissions testing is performed to detect hearing loss in the neonate. To maximize the benefits, the hearing screen should be performed before 1 month of age, preferably before hospital discharge; infants who screen positive should have a diagnostic audiologic evaluation before 3 months of age, and all infants identified with hearing loss should receive appropriate early intervention services before 6 months of age (medical, audiologic, and early intervention). Approximately 1.6% of all newborns do not pass the hearing screen and are referred for an audiologic evaluation, and among those 10.3% are confirmed as having hearing loss. Enrollment in early intervention services among children diagnosed with hearing loss is estimated to be 61.7%.

Specific Issues in Newborn Screening

Criteria for Newborn Screening

In 1968, under the auspices of the World Health Organization, Wilson and Jungner published a set of criteria for screening for conditions that have generally been accepted as those required for population screening.[83] These 10 criteria state that (1) the condition must be an important health problem, (2) there must be accepted treatment, (3) facilities for diagnosis and treatment must be available, (4) there must be a recognizable latent or early symptomatic stage, (5) there should be a suitable test, (6) the test should be acceptable to the population, (7) the natural history of the condition should be understood, (8) there should be a policy prescribing whom to treat, (9) the cost of case finding should be economically balanced in relation to medical care as whole, and (10) case finding should be a continuing process.

These criteria were developed at a time when NBS was in its incipient stages and with screening for adult disorders in mind but were also germane for NBS. Regarding the first criterion, Wilson and Jungner recognized that the term *important* is relative; whereas PKU was rare, and diabetes was prevalent, PKU warranted screening in the neonatal period because of the serious consequences that would be prevented by early diagnosis and treatment. Ideally all criteria should be applied for screening; however, continuing

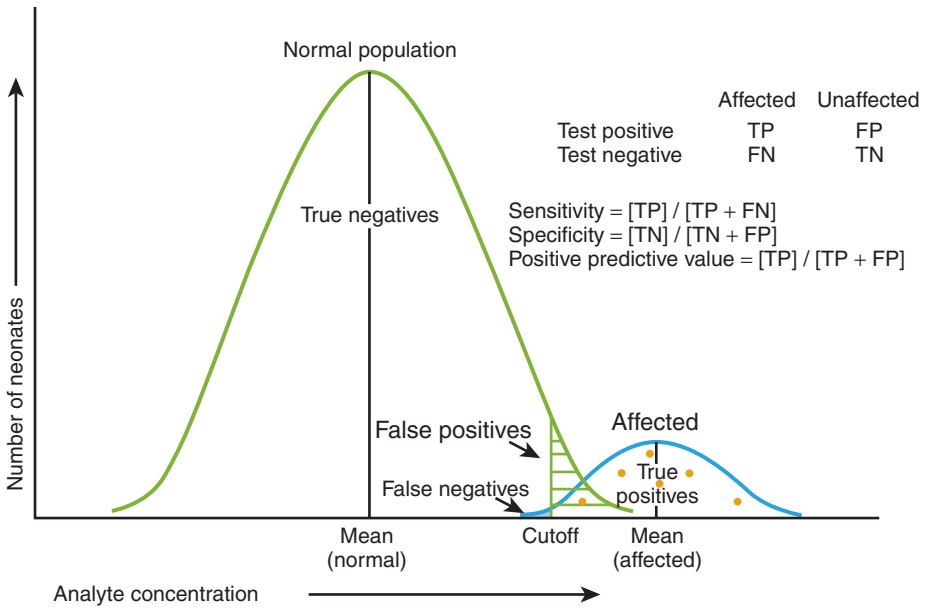
advances in technology challenge the current relevance of some Wilson and Jungner criteria for NBS.[84,85] As an example, current screening methodologies allow detection of serious disorders for which there may not be acceptable or agreed on therapy or for which the natural history is largely unknown, in conflict with the criterion that any disorder included in screening should have acceptable treatment. How is this dilemma resolved? A definitive answer is not yet available. It is hoped that the experience garnered from ongoing NBS for such disorders will be used to develop a new set of criteria that will apply to NBS. These criteria will likely retain the essence of the Wilson–Jungner compilation but with important modifications that could be applied to any new screening venture.

False-Positive Results

The majority of positive results in NBS, when the result is only mildly or moderately abnormal, are not due to a disorder. Unfortunately, such false-positive results are inevitable when relying on quantitative markers or qualitative genetic variations, for identifying all individuals at risk for a condition prior to onset of symptoms as is the essence of NBS.

The distribution of a quantitative primary metabolite concentration is expected to be different for affected individuals as compared to the unaffected population (Fig. 18.2). The primary analyte value that could distinctly differentiate individuals affected with the specified condition from unaffected individuals would be an ideal "cutoff." The cutoff is typically established by the individual screening laboratories and may differ among the laboratories, as is determined by the specific assay used and adjusted based on the marker distribution in the regional population.[86,87] Specimens in which the concentration crosses the established cutoff are considered screen positive. For disorders characterized by an increased (or decreased) metabolite concentration, the concentrations in most unaffected infants are below (or above) the cutoff value, but in a small proportion the metabolite concentration crosses this threshold. Screen-positive results in unaffected infants are considered false positives. Because of some degree of overlap in the distribution curves of the affected and unaffected populations, these false positives cannot be eliminated without compromising the sensitivity of screening. Furthermore, other physiologic factors, such as immaturity of metabolic enzymes, stress, and therapeutic interventions, can skew the concentrations of certain metabolites and lead to false-positive screening results.

Currently, the indicators measured by immunoassays (e.g., 17-OHP in CAH and T4 in CH) or enzymatic activity (low GALT activity in galactosemia) are associated with the highest false-positive rates. The false-positive results are more common in preterm and LBW infants than in term infants. For example, up to 85% of preterm infants have transiently low T4 levels.[88] Transient increases in 17-OHP level is another common abnormality in infants who are preterm or are LBW or have experienced perinatal stress. In addition, transient tyrosinemia is commonly observed in preterm and LBW infants, although it can also occur in term infants.[89] Artifacts produced in the collection or transport of the DBS account for some false-positive results. As previously mentioned in the specimen collection procedure, obtaining the specimen from a central line in infants receiving TPN can result in a false increase of the AA concentration in the specimen. Even with correct specimen collection, TPN can produce a transient AA level increase. Contamination with milk (or any drink containing milk) can result in a false elevation in galactose level and the mistaken suspicion of galactosemia. Prolonged exposure to heat can reduce the activity of GALT in the specimen and produce a false impression of galactosemia when the enzyme assay is used to screen an individual for this disorder. This error is common during the summer, especially when the specimen remains in a mailbox for some time.



• **Fig. 18.2** Distribution of quantitative markers measured in screening. For most conditions, the distribution in the unaffected or normal population overlaps that in the affected individuals. The number of false positives (*FP*), false negatives (*FN*), true positives (*TP*), and true negatives (*TN*) depends on the established cutoff.

Implementation of MS/MS has resulted in a significant reduction in false-positive screens for the AA disorders that were previously being screened using the traditional bacterial or specific assays. For PKU screening, the false-positive rate using MS/MS was reported at 0.05%, as compared to 0.23% with the earlier methods.[90] In addition, analysis of biochemical profiles instead of a single analyte, made feasible by MS/MS (e.g., phenylalanine/tyrosine ratio vs only a phenylalanine level for PKU identification) can further enhance specificity.[91] The average PPV for primary markers (AA and AC) analyzed by MS/MS reported to be 8% to 10%[92–94] can be improved substantially if the marker is evaluated in context with other metabolites that are screened or when a tiered approach is applied.[29]

Second-tier testing, either complementary quantitative metabolites (e.g., TSH testing prompted by low T4) or genetic variant analysis (e.g., sequencing the *IDUA* gene prompted by low IDUA activity), is commonly performed to reduce false-positive results. Notwithstanding, false positives will continue occur for the foreseeable future.

Some factors known to be associated with false-positive screens for the primary markers/disorders are mentioned in the previous sections and compiled in Table 18.3. The Newborn Screening Analyte Interference List (http://aphl.org) is another resource for a list of observed analyte interferences in NBS assays.[95]

Missed Cases

A few infants with one of the screened disorders, who were not identified on NBS, have been reported.[30,32,37,59] Laboratory error, suboptimal markers (primary or second-tier), specimen mix-up, and failure to collect an NBS specimen can result in missed cases. However, in screening for a multitude of disorders, each with its own biomarker that varies with time and physiological states, an occasional affected neonate may have normal biomarker concentrations in the newborn specimen simply because the timing of collection was not ideal for the particular condition that affected the baby. As examples neonates with CF-related meconium ileus may not show an elevation of IRT, or an infant with a FAOD may not have the characteristic AC pattern when not in the perinatal catabolic state.[96] Thus, physicians must exercise clinical judgment and not fall into the trap of excluding a diagnosis because an infant has presumably been screened. Clinical testing should be pursued in any infant or child with symptoms that suggest the presence of a disorder, regardless of the assumed or actual NBS result. Some conditions that could result in false-negative screens for the individual disorder are mentioned in the previous sections and found in the Newborn Screening Analyte Interference List (http://aphl.org).

Increased Detection of Cases

The number of infants identified by screening is greater than the numbers previously expected solely based on clinical identification for numerous disorders (inborn errors of metabolism, CF, CH).[66,70,93,97] Individuals who may have remained undiagnosed after a clinical presentation account for some, but the greater part of the difference represents infants with benign or milder forms of the disorders who may not have required clinical attention to have been identified prior to NBS.[98,99] Furthermore, some positive screens are not resolved despite diagnostic testing and clinical evaluation, due to inconclusive results on confirmatory metabolite testing or presence of variants of unknown significance on genetic testing or the existence of late onset forms of the disorder. Unfortunately, such individuals, although clinically asymptomatic may remain under medical surveillance for extended periods of time, and consequently experience anxiety associated with uncertainty and often interventions prompted by perceived risk of disease.[100]

The Future

Next generation sequencing (of the whole genome or exome), the dramatic technological advance allowing sequencing of nucleotides in the genome with an equally dramatic reduction in the cost for sequencing, is predicted to become an integral component in NBS in the future. Proponents envision the application of genetic screening for expansive second-tier testing, as an ideal primary modality for adding numerous genetic disorders to the screening panels, to potentially replace the assays for disorders that are currently identified reliably by primary metabolite-based screening, and as a resource that could be probed as needed throughout the individual's lifetime.

However, in an analysis of retrieved NBS specimens, primary NGS screening on its own performed poorly as compared to MS/MS-based screening for the current panel of metabolic disorders; overall sensitivity and specificity were 88% and 98.4% with NGS versus 99.0% and 99.8% with MS/MS.[101] Despite being inadequate to be a primary screen for most metabolic disorders, NGS as a second-tier test can substantially improve specificity and facilitate a timely and accurate diagnosis. Additionally, identification of genetic variants across numerous genes provides the opportunity to implement screening for a multitude of genetic disorders that do not have a biomarker identifiable by current screening.[102] Nonetheless, experience with NGS as a second-tier test augurs that primary genetic screening could be beset with a high risk of false negatives and a high false-positive rate due to the unreliable genotype-phenotype correlations and existence of numerous variants of unknown significance.[70] These challenges do not even address the medical genetic services needed that would far exceed the current availability, the greatly increased need for confirmatory tests, the increased number of infants with variants of uncertain significance in whom a diagnosis cannot be conclusively excluded and thereby would entail otherwise unnecessary follow-up or treatments, and the huge amount of uncertainty and anxiety in families that genetic screening would generate.[100,103] An overzealous implementation of primary genetic screening could thus lead to the implosion of NBS. Initial implementation of NGS in NBS is likely to be complementary to the current screening methodologies, as a second-tier test, and as the primary modality for adding on a limited number of variants for which the implications are known for some genetic disorders not identifiable by current screening.[104,105] The disorders targeted could include those in which early treatment might not be critical or even available but in which diagnosis would be important for defining the origin of illness and/or for family considerations. Other than the challenge of selecting genes and variants to detect the ethical, legal, and societal implications of sequencing in NBS need to be addressed. NGS-based screening would require informed consent that would have to be carefully constructed.

Many factors will continue to transform NBS, including rapidly advancing technology and new and increasingly available therapeutic approaches to previously untreatable disorders. Involvement

of family advocacy groups, influential citizens, or legislators who are heavily invested in individual disorders that may or may not be appropriate for NBS are additional determinants that impact NBS. Some disorders have been included in isolated state panels based on the political influences mentioned above, even though the disorders were not approved for inclusion in RUSP. Conversely, there is the issue of retention of blood spots for future study. Despite multiple safeguards in place to protect the identity and anonymity of individuals, parents and civil libertarians are concerned that retention of these blood spots poses a threat to the privacy of individuals, and therefore the specimens should be destroyed. If this view prevails, a resource of great value in the development of new and more effective tests, and one that is increasingly recognized as the avatar of personalized medicine, will be lost.

Suggested Reading

ACT Sheets and Algorithms. American College of Medical Genetics and Genomics and the National Coordinating Center for the Regional Genetics Networks Accessed May 30, 2021. ACT Sheets and Algorithms (acmg.net) [https://www.acmg.net/ACMG/Medical-Genetics-Practice-Resources/ACT_Sheets_and_Algorithms.aspx]

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Overview on Cutoff Determinations and Risk Assessment Methods_final.pdf (aphl.org) [https://www.aphl.org/programs/newborn_screening/Documents/Overview%20on%20Cutoff%20Determinations%20and%20RIsk%20Assessment%20Methods_final.pdf]

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19 Neonatal Transport

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KEY POINTS

- Regionalization of neonatal critical care improves outcomes.
- Highly effective transport systems feature centralized and standardized communications, experienced medical oversight and skilled teams trained to care for sick neonates.
- Appropriate transport care depends on the training and competency of the transport teams.
- The care delivered should not decrease in sophistication during the transport process.
- The referring provider is responsible (per Emergency Medical Treatment and Active Labor Act [EMTALA]) for ensuring the adequacy of the chosen mode of transport, but the receiving/transport teams can help inform decision-making.

Controversies

- Optimal transport team composition and configuration
- With proliferation of midlevel neonatal intensive care units, determining or anticipating which patients require transfer to higher levels of care and when
- Optimal method to achieve and monitor neonatal therapeutic hypothermia during transport
- Optimal timing and mode of transport of neonates likely to need extracorporeal membrane oxygenation (ECMO)

Regionalization of medical care enhances the ability to centralize resources and has improved patient outcomes.[1,2] For optimal coordinated care, however, an adequate medical transport infrastructure needs to be developed and continually refined to enable delivery of patients to regional centers and for specialized care to be delivered to patients in need. For centers that provide basic or specialized service, there will be times when subspecialty care is required, and for those who deliver subspecialty care, there may be times when transfer is indicated for reasons such as capacity or extraordinary care (i.e., extracorporeal membrane oxygenation [ECMO]). For hospitals that do not have birthing centers but to which neonates are brought for care, transfer may be indicated, and potential morbidity and mortality of these patients depend on high-quality and efficient neonatal transport. Although transfers to neonatal centers in the 1960s and early 1970s often occurred in an ad hoc manner, such as in a police car or a general ambulance, transfer programs today offer a more sophisticated level of care. Many transport services and centers have grown around individual center needs, without clear attention to coordination and regionalization of services. Competitive systems, often located in similar areas or vying for similar patient populations, have resulted in the duplication of services at the ground and air levels and at times increased risk and cost to patients and providers as part of the efforts to maintain or increase patient volume and revenue. Furthermore, national and international standards of care in neonatal and pediatric transport medicine are evolving. Transport guidelines and standards are available through the Commission on Accreditation Medical Transport Systems (CAMTS), Joint Commission Resources International (JCRI), and The Ground Air Medical qUality Transport (GAMUT) quality improvement collaborative.[3–6]

When considering the transport of neonatal patients, several situations can occur: *intrafacility* for services within a particular institution and *interfacility*, often between organizations with lower and higher levels of service capability, as well as between relatively equivalent levels of service because of capacity, geography, or other issues. Transported patients may be of high acuity, relatively stable, or in various stages of convalescent care. Each type of transport requires anticipatory planning, skilled, qualified, and certified transport personnel, adequate modalities (e.g., transport vehicles), and strong communication and relationships between referring and receiving providers. As noted in the next section, transfer agreements may help minimize inefficiencies and enable rapid approval and eventual transport of patients.

This chapter will review considerations and requirements for neonatal transport; discuss issues involved in transport team operation, including equipment, personnel, mode of transport, and medical legal issues; and present general and specific topics, including quality improvement opportunities, that might be encountered in a neonatal transport system.[7,8]

Regionalization of Neonatal Care, Care in the Community, and Transfer Agreements

Historical Perspective

The concept of regionalization of neonatal care and transport developed from the formation of neonatal stations, areas within hospitals, in the 1920s and 1940s[9] that had additional resources to care for premature neonates. One consequence of the formation of these stations was the development of equipment and protocols to transport premature neonates from other area hospitals to those with specialty services.

In the 1960s and 1970s, as interest in neonatal care grew, so did the number of hospitals offering services for premature

infants. To help optimize the care being delivered, the March of Dimes produced *Toward Improving the Outcome of Pregnancy: Recommendation for the Regional Development of Maternal and Perinatal Health Services* in 1976.[10] The report stratified maternal and neonatal care into levels based on complexity, and it proposed the referral of high-risk patients to centers with sufficient personnel and resources to provide care. The goal was to create standard definitions so that comparisons of health outcomes, resource utilization, and costs among regional institutions could be made. High-risk maternity patients would be able to actively participate in selecting a delivery service, and businesses would be able to select appropriate healthcare resources for their employees. The subsequent March of Dimes publication in 1993, *Toward Improving the Outcome of Pregnancy: The 90s and Beyond*, reiterated the importance of regionalized care and further delineated care levels.[11] The concepts of regional care were adopted and incorporated into the Guidelines for Perinatal Care.[12,13] While the original driving forces for regionalization in the 1970s were the shortage of centralized trained personnel to care for low birth weight (LBW) neonates and the economic expense to maintain these skills, during the late 1980s and 1990s, technology and clinical expertise disseminated outside the regional tertiary centers, resulting in proliferation of the number of intermediate-care neonatal intensive care units (NICUs). This proliferation has blurred many of the original distinctions between various care systems. Whether driven by third-party payers or other factors, with various interpretations and applications of what "regional care" means, the results have been the creation of a variety of care options[14] but without necessarily improved or optimal outcomes for all patients. For example, Kastenberg et al. found that very low birth weight (VLBW) neonates with necrotizing enterocolitis had significantly increased mortality when cared for in low-volume mid-level NICUs compared with higher-volume units and those with higher acuity status.[15] While there is limited robust evidence that demonstrates a clear causal relationship between improved outcomes and regionalized systems of neonatal care, understanding the impact of de-regionalized systems on quality of care and patient outcomes will help inform which neonates require transfer to regional, high-volume, high-acuity centers and how to optimize use of the neonatal transport system to meet these needs.[15,16] Tanem et al. present a descriptive review of the transport workforce, including transport training and team configuration in their 2016 paper.[17]

Care in the Community and Back Transport

Regionalization of care, space limitations, and longitudinal care coordination contribute to a current system in which neonates are being both transported to higher levels of care for acute and/or critical needs and also transported back ("back transport") to appropriate centers, perhaps closer to home, once their critical condition has resolved or stabilized.[18–20] Regionalization guidelines should support the return to community facilities for patients who no longer need the highest level of care. Patient selection for back transport and care in another facility should match the capabilities and expertise of the community hospital.[21] The increasing application of telemedicine and technologies that allow real-time assessment and medical guidance may enhance not only the capacities of more remote facilities and providers during initial stabilization but also assist those providing ongoing care of high-risk neonates. In addition, telemedicine applications may augment the assessment and care provided by the transport team when at the patient bedside when they are able to communicate in real time with specialty providers at a receiving facility. Kim et al. (2013) conducted a prospective study to assess the impact of telemedicine in Arkansas on regionalized care and infant mortality.[22] They found that there were fewer VLBW neonates born in hospitals without NICUs and an overall decline in infant mortality statewide. Webb et al. (2013), in a multicenter study assessing the impact of telemedicine on neonates with suspected congenital heart disease (CHD), observed improvements in the diagnosis of CHD, fewer patient transfers, reductions in the length of stay, and use of inotropes and indomethacin.[23] In a single center study on simulated newborn transport calls, Umoren et al. showed change in perception of newborn stability and increased provider confidence in recommendations made following video-based telemedicine simulations as compared to telephone only encounters.[24] Telemedicine is a rapidly growing area in medicine, with increasing presence in NICUs, and an area of continued opportunity for development for infant transport.

Transfer Agreements

Although third-party payers often drive decision-making, transport relationships can develop between various institutions by formalized transfer/preferred provider agreements and/or by historical and personal relationships.[20] Transfer agreements can help to define the roles, understanding, and expectations between institutions and the transport service; they may also help to detail reimbursement issues. These agreements set the expectations for participating facilities, with the ultimate goal of the timely movement of patients from one facility to another.[8,13] Agreements should be reviewed by legal counsel to ensure compliance with state law.[8]

A neonatal transport system must also determine if maternal transport is part of its purview. It is clear that preterm neonates who are born outside hospitals and require transfer to tertiary care centers or transfer between tertiary care centers have worse outcomes, which include increased mortality and morbidity such as intraventricular hemorrhage.[25–29]

Several investigators have shown that mortality is lowest for deliveries of VLBW neonates that occurred in hospitals with tertiary care NICUs.[30–33] In 2003, Clark supported the idea that, whenever possible, women in early preterm labor should be moved to the regional hospital rather than the neonate being transferred after birth.[34] The study was insufficiently powered, however, to make a recommendation regarding the difference between regional and large community NICUs.[34] In trying to discern which LBW neonate might be better cared for in tertiary regional care centers, Vieux et al. showed that there were factors associated with both increased and decreased need for neonatal intensive care.[35] These data can help inform guideline development for transporting women in preterm labor and optimize the use of transport and local community resources to ensure timely transfer and optimal patient outcome.

Transport Communication

When one is developing and maximizing transport capabilities, a key concept is centralized communication, often in the form of a communication center. While a call from a referring provider directly to a receiving provider might seem to be the most efficient way to initiate a clinical conversation about a potential transport, there are more effective means of communication. Those who have

transported or referred patients in systems without centralized access understand the challenges in working through operators, unit coordinators, multiple providers, and often multiple services to enable a singular transport. This process is time-consuming and often frustrating for the referring provider, and the time could be better spent in direct assessment and care of the patient. When one is communicating with the transport system, there are key elements that must be considered and appreciated on both sides to initiate and complete a successful patient transfer:

- Appreciation and recognition of the need for transfer
- Awareness of appropriate and available transport modality and options
- Identification of appropriate receiving facility and acceptance by receiving provider
- Verification of regional and local bed capacity
- Review of current medical issues
- Determination of required transport services, including personnel
- Dispatch of transport team and potential limitations

Centralized access through a communication center can allow all those functions to occur simultaneously, enabling more rapid transport response and appropriate involvement of all individuals required for the care of a particular patient (Box 19.1).[12,13]

Medical Supervision

A key requirement for any system is to have appropriately skilled and immediately available medical command physicians (MCPs) (also referred to equally as *medical control physicians*).[8,12,13] An MCP should be literate and expert in the medical area of concern, as well as up to date on transport capabilities. In most cases involving neonatal transport, this provider should be a neonatologist. There may be instances, however, when the referring or receiving physicians may request or desire additional medical expertise. For example, a cyanotic newborn with CHD may be temporarily stabilized by the referring provider and additional medical advice may be provided by the receiving MCP, as well as a partnering cardiac intensive care physician. A communication center can facilitate an initial call in which multiple providers are linked, allowing the highest level of advice to be presented and discussed among providers. These telephone calls should ideally be recorded for quality control and/or for review if verification of information is needed and should include the transport personnel so that background information and care plans are communicated directly.

BOX 19.1 Features of a Highly Functional Centralized Communication Center

- Centralized number with immediate access to transport system or center personnel 24/7
- Contact number advertised, easily recalled, monitored 24/7
- Single call or point of contact for referring provider to accomplish all transport needs
- Dedicated communication center personnel with transport-specific training
- Recorded calls for quality assurance and quality improvement activities

Data from Southard PA, Hedges JR, Hunter JG, et al. Impact of a transfer center on interhospital referrals and transfers to a tertiary care center. *Acad Emerg Med.* 2005;12:653–657.

Mode of Transport

Once the transport referral has been made and discussions have been started with the MCP, the transport process begins in earnest. A decision on mode of transport is an important consideration at this juncture and is ultimately the responsibility of the referring provider, although it can be appropriately influenced by the MCP with informational guidance from the transport team and communication center (Table 19.1).

In addition to distance from the referring and receiving facilities, which will impact the total transport time, the decision regarding the mode of transport is influenced by several additional considerations when aiming to arrange and dispatch the most appropriate team for a given patient, including:

- Available mode of transport
- Staffing and medical expertise of providers involved in each mode
- Patient's current stability and potential illness progression during the projected transport time
- Capabilities of referring facility and personnel
- Urgency of need for intervention and definitive care of patient
- Geography and weather

In a study examining the decision-making factors around the mode of transport, Quinn et al. found that the decision to activate a helicopter versus a ground unit was made in the face of not only prolonged distance (>45- to 60-minute drive time) but also the presence of perceived high-risk clinical conditions, specifically neurovascular and respiratory concerns, even more so than blood pressure or heart rate.[36]

Currently, there are guidelines but no national absolute criteria or standards to direct the choice of ground versus air transport. Each modality has its own general and more case specific benefits, constraints, and risks. First, with both air and ground transfer, concerns include potential physiologic stress and discomfort experienced by the neonate secondary to stimuli such as vibration and noise.[37–42] Therefore, adjuncts to minimize the stress and discomfort, such as gel mattresses and earmuffs, should be used as much as possible. Air transport can also present specific

TABLE 19.1 Characteristics of Mode of Transport

Transport Modality	Distance to Receiving Facility (Miles)	Features
Ground	10–20+	• Fewer weather restrictions • Door-to-door • Well-lighted care environment • Space for family, providers • Efficient in urban, short-range transfers
Rotor wing	>20–100	• More expeditious >50 miles, ideal efficacy 50–150 miles • Can access less accessible areas • Can potentially be door-to-door
Fixed wing	>100	• Expeditious over long distances, ideal efficacy >150 miles • Can circumvent weather issues • Potential space for family, provider • Ability to pressurize cabin • Requires ground transport to and from airports

stressful stimuli such as gravitational forces during acceleration and deceleration, temperature variations, and decreased humidity with altitude and introduces issues related to altitude physiology that can affect patients with respiratory issues or air trapping as well as air-containing equipment (e.g., endotracheal tube cuffs, laryngeal mask airways).[37,43–45] Dalton's law recognizes that ambient oxygen partial pressure decreases as altitude increases; therefore, there may be a need for pressurization and augmentation with increased fraction of inspired oxygen. Boyle's law states that as altitude increases, the volume of a gas also increases, as barometric pressure is inversely related to the volume of the gas. Thus, consequences of this law are potentially a serious issue for patients with an enclosed gas collection, such as a simple or developing pneumothorax or pneumatosis in suspected cases of necrotizing enterocolitis.

Second, weather and physical distance can make each mode of transport more or less accessible or reasonable at a given time. For instance, while ground transport may be more readily available than air transport because of fewer weather constraints and an increased number of vehicles, the overall transport time may be too long given the clinical needs of the patient.

Finally, each mode of transport has occupancy limitations associated with select vehicles, especially rotary air transport. These limitations can preclude extra passengers such as parents or family members or potentially crew members if weight and balance is an issue.

Transport Personnel, Education, and Team Composition

Awareness of the capabilities of the transport system and of the personnel involved is imperative in decisions regarding the mode of transport. Although it is ultimately the responsibility of the referring physician to identify the appropriate mode and personnel for transport (per the federal Emergency Medical Treatment and Active Labor Act [EMTALA]), opportunities exist for tertiary care and referral centers to help inform the referring providers regarding optimal transport and hospital planning and use.[46–48] In general, issues influencing transport decisions (as well as hospital care needs) include the patient's current level of care, urgency for a different level of medical capability or equipment, current provider capabilities, stability of the patient, options available to the provider and patient, and efficiency and quality of the transport process. Ideally, these issues are key determinants of appropriate transfer; however, referring providers are often overwhelmed, due to their lack of resources, by the severity or acuity of the patient and their primary desire may be to have the patient transferred from their facility as quickly as possible. Thus, the providers may focus on a transfer process based solely on the speed of transport rather than the quality of care. It is imperative for the receiving and tertiary care centers to educate the referring providers regarding the importance of stabilization, initiation and quality of the primary response, transport options, and definitive care to maximize patient outcome. When examining the transfer of patients, providers should ask a simple question: "Are we trying to deliver the patient to tertiary care, or are we trying to deliver tertiary care to the patient?" In most high-functioning transport and referral centers, the latter is true. The referring physician should expect to have tertiary care advice and direction delivered at the moment of the referral call and continued throughout the transport process.[12,13,47]

When considering the transport team composition, it is important to consider the quality of the personnel, their expertise and experience, and their ability to work in the transport environment.[49–51] There are many variations of transport teams in the United States and abroad.[52] These teams can be composed of a combination of physicians, nurse practitioners, nurses, respiratory therapists, paramedics, and other healthcare providers. Regardless of the formal educational background of an individual, there are several criteria that must be met to be optimally effective in the transport environment. First, the provider must have adequate certification, be licensed for the care he/she delivers, and be able to provide the assessments and interventions that a patient currently or potentially requires during the transport process. For example, a neonatal retrieval service must be able to manage acute and critical airways in the neonatal population, both at a referring hospital and during the transport. While transport team providers might not be credentialed to provide certain skills within their home hospital (i.e., intubation), they have been certified to provide them in the ambulance environment. In general, this must be done under the auspices of a physician's care, which may be from an accompanying physician or via online medical control (real-time medical advice during the transport process) or protocol-based care (off-line medical control). It is important to recognize that the transport time frame is somewhat limited; therefore, the personnel may not need to have the longitudinal or differential diagnosis expertise of a fully trained neonatologist. However, these personnel must have the acute care assessment abilities and intervention skills of an experienced neonatal caregiver.

From the transport and pediatric literature, patient outcomes are improved with specialty providers. While multiple studies have examined this issue,[53–56] the most compelling is the study by Orr et al., which examined transport by variable providers within the same system.[57] This study compared outcomes in patients whose care was delivered by specialized pediatric critical care teams with those whose care was delivered by general providers. Both teams had the same medical command oversight, equipment, and modalities. Patient outcomes were worse for those whose care was not delivered by specialized teams and were much improved for those whose care was delivered by specialized teams. While the study by Orr et al. and studies by others are compelling, a 2015 Cochrane review that focused specifically on neonatal specialty teams[58] concluded that in the absence of randomized controlled studies there is not good evidence to refute or support the use of neonatal specialized teams for high-risk neonates. Evidently, specialized teams and patient outcomes is an area for ongoing investigation.

In addition to training the transport personnel, MCPs should understand the opportunities and limitations of the transport services, the environment, and the risks and challenges that referring personnel can potentially encounter with situations and patients who exceed their own or their facility's management abilities. It is imperative that MCPs have clear and efficient communication, not only with referring providers and those from different disciplines but also within the transport team.

Quality Improvement

Throughout medicine, there is an increasing focus on measuring and improving the quality of care provided across all healthcare domains and patient experiences. Transport medicine offers an opportunity for potential quality improvement activities within the inpatient arena, in the transport system, and at the referring

facilities.[59–63] While there are guidelines of care and process recommendations such as those provided through the American Academy of Pediatrics and the Commission on Accreditation of Medical Transport Services, formal national transport benchmarks or standard quality-of-care metrics are still evolving. A 2015 consensus document on behalf of the American Academy of Pediatrics Section on Transport Medicine offered 12 core quality metrics for neonatal and pediatric transport.[64]

The authors proposed that these metrics serve as benchmarks and help guide individual program quality improvement. Details of their recommendations can be accessed at http://www.aap-sotm.org. This effort, which evolved as the GAMUT database/tools is a good place to start for of transport services and quality metrics (http://gamutqi.org/). Aspiotes et al. reviewed GAMUT's benchmarking process, noting the inclusion of more than 350 transport programs with more than 200,000 annual patient encounters.[65]

Neonatal and pediatric airway management may be one of the most important aspects of transport clinical care and historically is one of the more challenging areas for emergent and prehospital management. Examples of quality-based investigations reflect these challenges and provide information to guide improvements. Bigelow et al. retrospectively assessed first-pass intubation success for neonatal and pediatric patients across nine transport programs over a 6-month period.[66] The overall success rate was 64%, with a range of 35% to 87% but was highest for teams that had live-patient training for initial competency and lowest for those using simulation alone. Smith et al. considered risk factors for intubation failure among neonatal and pediatric specialized teams and found higher rates of intubation failure for neonates compared with pediatric patients, especially for the smallest neonates necessitating the use of tube sizes of 2.5 mm or less.[67] Additional risk factors for failure were the lack of use of sedation and neuromuscular blockade. Impact of environmental issues (altitude) on endotracheal use is reported by Orsborn et al. and Long et al.[68,69]

Quality improvement activities in the transport domain are diverse and range from assessment of the transport process by review of recorded calls to the monitoring of intubation success and vital signs in transport to improving communication through standard handovers.[70] However, this glimpse into facility/provider medical sophistication and capability is one that is privileged and should be used to identify educational opportunities for all providers involved rather than used as a judgmental review. Education by receiving physicians and transport teams can have a significant effect on the quality and outcome of patient care and the volume of future referrals. Ideally, once a referral call is made, a receiving physician or MCP will direct the care so that the job of the transport team is to verify that an appropriate working diagnosis has been made and adequate stabilization has been achieved. Systems that do not gather adequate information or offer appropriate advice, or in which the referring facilities do not follow that advice or choose not to perform needed interventions, can put the patient at risk by delaying potentially necessary interventions, prolonging the transport process, and delaying delivery of definitive care. The transport team that has invested several hours at a bedside stabilizing a newborn with medical or surgical issues may be spending time in a facility that is not ideal, has a limited number of skilled personnel, and has minimal backup, thus prolonging the transport process, delaying definitive care, and potentially putting that individual patient and the transport team at risk.[59,71] Furthermore, during this prolonged stabilization time, the entire system becomes at risk because the valuable resource of specialized neonatal transport personnel is not available for another patient.

Ideally, care delivery would be the same at referral and receiving centers, and the development of practice guidelines can help in this regard. Guidelines that are evidence-based, developed by regional and local experts, and disseminated to referring centers and transport teams will help standardize and promote consistent care across variable locations. It is necessary, however, to assess and reassess the quality of the guidelines and the competency of their use to ensure optimal results. Even in the best of hands, near-miss or realized adverse events may happen. It is clear that identification of those events, discussion with families where appropriate, and root cause analysis are imperative. Several studies have examined adverse events in transported patients. Van den Berg et al. examined adverse events that occurred over 13 years with their neonatal transport team in northern Sweden.[72] They found that such events had differing significance (53% low risk to 11% high or extreme risk), were common, and were often related to transport logistics and equipment failure. Ligtenberg et al. noted that one-third of patients had an adverse event, and 50% of adverse events resulted from the advice of the MCP not being followed.[73] Of that group, 70% of events were avoidable and 30% involved logistical issues. In a review of the London Neonatal Transfer Service, Lim and Ratnavel noted that 36% of their patients had one or more adverse events and that two-thirds of those were due to human error.[61] Half of the events occurred before the team arrived at the referral center and were due to patient preparation and communication issues.

Transport Administration

As a hospital develops and optimizes a neonatal transport program, experts in transport medicine are integral to the success of the program.[12,13] A quality medical director and program director, often a nurse or respiratory therapist, are essential for understanding the potentially complicated and challenging environment of transport medicine. These leaders should be instrumental in identifying expectations, roles, and responsibilities for the entire transport process, including oversight of the communication center and developing and disseminating referral center expectations.

The responsibilities of the referring center when transferring patients include the following:

- Stabilize and prepare the patient before transport
- Make an appropriate decision to transfer the patient
- Choose an appropriate transport process and destination
- Obtain family consent for transport, including the transport mode and the receiving facility
- Discuss and initiate a plan for stabilization with the medical command physician (MCP)
- Communicate clearly when suggested interventions are beyond the scope of the referring center or cannot be done
- Be present and participate in the transition of care to the transport team and to the receiving service.

In turn, there are also clear expectations of the receiving center and transport team:

- Be available immediately for patient care consultation
- Be able and qualified to provide clear and concise recommendations
- Have the ability to quickly determine if the receiving center is able to accept the patient for transfer
- Ensure that the receiving facility staff are prepared for both patient and transport team arrival
- Document interaction with—and recommendations to—the referring facility.

Most importantly, the team needs to ensure that appropriate skills and therapeutics are available and delivered throughout the process,

from the referral call through definitive placement, and ensure seamless transition at each point of care. The team needs to communicate well with the patient's physicians and document their advice, interventions, and activities in a clear, concise fashion to enable appropriate patient care and provide protection for the transport service.

Transport Team Safety Training and Protocols

Safety of the transport system and its providers is paramount and must be assessed and ensured before any patient is transported. Vehicles must be safe and meet the standards for air or ground transport; the personnel must be trained and skilled in the care of neonates, licensed, and competent; and the patients must be managed in the most appropriate and professional fashion. In addition, the logistics of travel must include a safe environment, including helmets and fire-retardant suits for those who fly in helicopters, three-point restraints, and appropriate ambulance seating arrangements. General equipment expectations for basic life support (BLS) and advanced life support (ALS) ambulances (often used as a platform for neonatal and pediatric critical care transport) can be found in the May 2021 multi-organizational joint position statement. These general vehicle equipment and supplies must be augmented to address the needs of the critical care transport system, providers, and patients.[74] Providers should not put themselves at risk by being unrestrained or being in an area where unsecured debris or inappropriately placed equipment may cause harm to them or the patient. Adherence to all rules and regulations of air and ground travel is essential.[75–80]

It is important to recognize that there are risks with both air and ground transport. In 2008, the air transport industry saw a spike in tragic and fatal air accidents.[78,81,82] This increase caused the industry, and the US government, to investigate these incidents and offer recommendations to improve transport safety.[83,84] Requirements such as duty hours for pilots, weather restrictions, flight under instrument flight rules with terrain avoidance equipment, and night vision goggles can help to minimize transport risk. While ground ambulances are used much more frequently and the risk of injury and death is evident, the fatality rate is lower in ambulance accidents than it is in aircraft accidents.[76,85,86] Many systems do not allow ambulances to exceed posted speed limits and allow them to use lights and sirens only as a way to identify an emergency response not to enable the vehicle to circumvent or ignore standard traffic laws.[75] The 2014 joint statement issued by the American College of Surgeons Committee on Trauma and the American Academy of Pediatrics regarding appropriate equipment for ambulances should also be reviewed by the providers and administrators of all transport systems.[87]

One challenge for transport teams is that the differentiation of medical resources, such as a neonatal specialty team, likely means that there may be a scarcity of resources and a potential need to ration those resources. It is possible to develop teams with a variety of personnel with complementary cognitive and procedural skill sets and work toward appropriate triage of transport requests to ensure the optimal level of onsite patient care and safe transport. There have been multiple attempts to develop triage tools for pediatric and neonatal care providers, including the Mortality Index for Neonatal Transport, the Modified Clinical Risk Index for Babies, the Risk Score for Transported Patients, and the Transport Risk Index of Physiologic Stability (TRIPS).[88–91] Notably, TRIPS is a validated tool that can be calculated in a single assessment and has been shown to correlate with NICU mortality. The original authors of TRIPS validated TRIPS-II, the application of TRIPS over 12 to 24 hours after NICU admission, and found it correlates with illness severity not only at admission but also up to 24 hours.[92] Given the simplicity and ease of use, TRIPS and TRIPS-II are examples of scoring tools that can be applied in both the transport environment and the hospital environment reflecting clinical deterioration or improvement over time.

Family-Centered Care

Transport team research has shown that family-oriented care, as in other areas of health care, is an important component of transport.[8,93–96] Families who have been formally surveyed appreciate the opportunity to participate in the care of their child and express increased stress and anxiety when they do not accompany their child during transport.[95,96] In neonatal transport, however, there are times when there are two patients who may require care in two disparate locations. A mother who has had a cesarean delivery and has delivered an acutely ill neonate who requires transfer to a specialty pediatric facility with neonatal intensive care capability is one such example. Transport teams should be sensitive to the challenges and opportunities for the family members and include them in the process when possible. It is evident that when parents attend or accompany transport team members during critical care transports, they are there not to assess the medical skill set of the provider but to provide support to their child. It is also a great opportunity for the transport team to demonstrate to the family that their patient is in focused, professional, caring, and capable hands.

Medical Legal Issues

There are many medical legal issues in transport medicine, just as there are elsewhere in the medical system.[8,97–100] The Health Insurance Portability and Accountability Act is a required component of transport planning and delivery. Discussion of patients should not happen in a public area or via public communication airways, where patient-specific information could be overheard. As noted earlier, a requirement of EMTALA is that the referring physician choose the appropriate mode of transport and ensure that the transport process and receiving hospital are appropriate for the patient. Patients should not be transferred if they are unstable and the ability to further stabilize them is available at the initial site of care. If a patient must be transferred for care while in an unstable condition—a frequent scenario for critically ill patients who need care not available at the referring institution—consent must be obtained from the family, which acknowledges their understanding of the potential risks and benefits of the process. In practice, there are often patients in an unstable condition who are transferred from lower to higher levels of care because the level of care that can be provided at the referring center is not optimal for the child. This reason is appropriate for transfer as compared with transfer of acutely unstable patients because of financial or other economic incentives.

The medical liability for transport is a shared process. Before the referring center contacts a receiving facility or transport team, the entire medical responsibility lies with the referring provider. Once the receiving team has accepted the patient and offered advice, medical liability becomes a shared process. The referring physician maintains most of the liability, as well as medical control of the patient, throughout the process until the transport team has left the referring hospital. It is important to recognize that most transport teams and personnel do not have privileges at referring

hospitals and are working under the guidance and supervision of the referring physician team. Transport teams that act independently or referring providers who are not available when the transport team arrives, put not only the patient but also the referring provider and transport team at risk.

There will be times, however, when there is disagreement regarding the optimal care to be delivered. This situation can be challenging, and it must be handled appropriately. It is never appropriate to have obvious provider conflict occur at a patient's bedside in front of family members. The appropriate way to handle a situation that cannot be easily mitigated is to involve the MCP with a telephone call to the referring physician in a discussion at a peer-to-peer level. Transport teams have been known to comply with the wishes of the referring providers to not perform advanced procedures at the referring hospital, only to perform those procedures in the ground or air ambulance, which is a much less desirable location. Ideally, all disagreements and considerations of different therapies should be discussed in a collegial fashion.

As noted previously, documentation of all information received, and advice offered is imperative. If there is a review or there are challenges regarding the care delivered before or during transport, clear and appropriate documentation should stand alone as an excellent defense. In addition, many centers use recorded (i.e., digital, tape, and other retrievable recording process) intake and advice lines; this is another way to review, educate, and ensure that appropriate information is delivered using an effective communication style. The use of recorded lines with frequent review, for educational and quality assurance purposes, can be invaluable. Review with legal advisors can help define the length of time the recorded materials should be maintained for quality improvement or patient record addendum.

Patient Care During Transport

The primary clinical goals for any neonatal transport include, but are not limited to, the following and should be established during the initial stabilization phase before departure from the referring facility and maintained until handover at the receiving facility:

- Secure and patent airway
- Adequate ventilation and oxygenation
- Thermoregulation, especially for premature neonates, goal 36°C to 37°C (except as indicated for hypoxic-ischemic encephalopathy)
- Normoglycemia
- Goal glucose level 50 to 200 mg/dL
- Adequate blood pressure and perfusion
- Appropriate condition-specific care such as for myelomeningocele

A team's ability to achieve these care goals may be impacted by the patient's clinical status but will also depend on the team's preparedness and skill and experience caring for critically ill neonates. Therefore, the general approach to any neonatal transport includes ensuring the availability of appropriate equipment, such as an isolette and endotracheal tubes for the smallest premature neonates, that there are skilled team members who can optimally care for a sick neonate, that there are appropriate medications for specific situations such as prostaglandin E_1 (PGE_1), and that there is smooth communication with the MCP.

Extreme Prematurity and the Limits of Viability

Of the approximately 4 million live births that occur annually in the United States, approximately 10% are preterm (<37 weeks gestation), and approximately 1.5% are VLBW (<1500 g).[101] The most effective method of transporting extremely premature neonates is before delivery with the mother serving as the transport vehicle, but sometimes this is not possible, and a very premature baby is delivered unexpectedly at a referral center. Neonates born at 23 to 26 weeks outside tertiary centers have higher mortality and morbidity than those delivered within specialty centers.[32,33,102–104] When delivered at a referral hospital, the premature neonate is exposed to all the variability of the extrauterine environment, with the added complexity of having to be transported to another facility capable of meeting the neonate's needs. Increased transport duration, lower gestational age, and severe acidosis before transfer have been associated with increased risk of clinical deterioration and mortality among transported VLBW neonates.[105,106] In one study the odds of deterioration increased 1.33 for every 5 minutes of transport time.[106] Most neonatal transport teams are regarded as extensions of the NICU. The team initiates and provides much of the same level of complex neonatal care as the receiving hospital, but in a changing environment. It is this changing environment that poses unique challenges for both the patient and the caregiver. These issues would be amplified in the case of a natural disaster with care and transfer required for premature neonates.[107,108]

Human viability is limited largely by the physiology of pulmonary development and currently appears to be at approximately 22 to 24 weeks gestation.[109,110] Unfortunately, for transport teams and providers at referring hospitals, it may be difficult to determine which neonates born at the margins of viability should be resuscitated and treated with aggressive neonatal care and which should not.[12,111] These decisions are best made collaboratively with the family, transport team members, and the referring and receiving physicians, and they may ultimately result in patient transport, even when the likelihood of survival is low. Telemedicine may be useful to help with decision making in these difficult cases.[112–114]

Thermoregulation

Problems in neonatal thermoregulation continue to be a major contributor to neonatal morbidity and mortality worldwide and can be especially problematic in neonatal transport. During transport, neonates often cross into and out of multiple different environments with wide temperature and humidity variations. Although a normal term neonate may be capable of significant homeothermic response by using its sympathetic nervous system to vasoconstrict peripherally, preterm neonates lack the subcutaneous fat insulation to protect their core temperature. Several studies using TRIPS found that temperature instability accounted for clinical deterioration during transport, with as many as 57% of neonates demonstrating clinical decline.[115,116] In one study, increased mortality was observed among those neonates with clinical deterioration.[115] Skiöld et al. identified factors associated with temperature instability before transport and found that VLBW neonates and the presence of respiratory support were predictors of temperature instability on arrival at the receiving center.[117] Clearly, steps should be taken to minimize temperature instability during transport, such as adequate isolette temperature and the use of chemical gel packs and polyethylene occlusive skin wrapping to help maintain temperature, especially of VLBW neonates.[118]

While humidity contributes to neonatal temperature control, especially for VLBW neonates, it is also important for gas delivery among those receiving mechanical ventilator support.[119] Ventilation with dry gases affects the airway epithelium in VLBW

neonates and can result in hypothermia secondary to their large surface area to body mass ratio and their relatively large respiratory minute volume.[120] While ventilator complications can be reduced, and thermoregulation can be improved by provision of exogenous heat and humidity to the gases, active heated humidification systems are used infrequently during neonatal transport. Passive hygroscopic heat and moisture exchangers have been used for short-term conventional mechanical ventilation and with some types of high-frequency ventilation.[120–122]

For all newborns, an equally important condition to avoid is hyperthermia. Although elevated temperatures in neonates occur with increased metabolic rates, prolonged seizures, dehydration, or infection, the most common cause of neonatal hyperthermia is high ambient air temperature and humidity.[123]

Surfactant

Surfactant replacement therapy has proven to be one of the most significant advances in neonatal critical care.[124–127] Benefits are achieved with both prophylactic (defined as within 10 to 30 minutes of birth) and rescue (within 12 hours of birth) surfactant administration, especially for extremely LBW neonates.[127–129] Surfactant administration can be complicated by airway obstruction, right mainstem bronchus or esophageal instillation, bradycardia, hypotension, and rarely pulmonary hemorrhage. These complications led the American Academy of Pediatrics Committee on Fetus and Newborn to recommend that "preterm and term neonates who are receiving surfactant should be managed by nursery and transport personnel with the technical and clinical expertise to administer surfactant safely and deal with multisystem illness."[130]

Given the potential for rapid physiologic changes and the risk of complications, surfactant administration should generally not be initiated en route between facilities. While investigations are still ongoing as to the optimal manner of surfactant administration, future technology might eventually allow surfactant to be delivered without tracheal intubation. The administration of surfactant via a small intratracheal catheter while the neonate breathes spontaneously while receiving continuous positive airway pressure (CPAP), a technique known as *minimally invasive surfactant therapy* (MIST),[131] has been shown to be equally effective as techniques involving tracheal intubation, and in some studies resulted in decreased morbidity.[132–135] Several studies, including NICU-based randomized controlled trials,[136,137] have shown success with the INSURE method[138,139]: *int*ubate-*sur*factant-*e*xtubate to CPAP to avoid the potential sequelae and complications of prolonged mechanical ventilation in preterm neonates. This method has not been studied in the transport environment; however, there are preliminary investigations into parameters that may serve as eligibility criteria to identify in which neonates extubation to CPAP could be could safe and successful before they depart from the referring center.[140] Aerosolized surfactant has yet to be shown to be superior to endotracheal tube administration.[141,142]

Currently, the most frequently used surfactant preparations in the United States are derived from animal lung extracts. These surfactants require refrigeration and are usually carried by transport teams in small containers cooled by gel packs. Lucinactant, a synthetic surfactant, requires a special warming cradle to convert it from a gel to a liquid before administration, adding a level of difficulty to its administration during neonatal transport.[143,144] Because smaller community hospitals may not have surfactant readily available, the transport team should carry it as part of their medical supplies.

After the surfactant has been administered, monitoring pulmonary compliance and adjusting the patient's ventilator while still at the referring facility may extend the transport time but also limit avoidable clinical complications.

Hypoxic Respiratory Failure

Hypoxic respiratory failure describes a heterogeneous group of neonatal disorders that have in common impaired oxygenation and the need for assisted ventilation, most commonly respiratory distress syndrome, meconium aspiration syndrome, and persistent pulmonary hypertension of the newborn (PPHN). Interfacility transport of the newborn with hypoxic respiratory failure is potentially hazardous, with a high risk of patient deterioration and complications of therapy.

Assisted ventilation during neonatal transport can be accomplished with a variety of devices, although not all modes of mechanical ventilators have been modified for use in a mobile setting. Newborns, including very preterm neonates born at 28 to 32 weeks' gestation with milder degrees of illness, may be managed successfully with CPAP[145] with a variety of interfaces, ranging from a face mask to nasal prongs or a nasal cannula.[146] In a systematic review of observational studies, Cheema et al. concluded that noninvasive ventilation (CPAP or high-flow nasal cannula) was relatively safe in pediatric and neonatal transport, with an in-transport adverse event rate of 1% to 4%. However, the studies included in that review did not contain information on severity of illness and were likely subject to selection bias, leading the authors to conclude that additional studies were needed.[147]

There have been a few case reports and a limited literature review that suggested a potential role for neonatal laryngeal mask airway during transport.[148] This modality is particularly interesting since the success of neonatal intubation is highly variable across transport programs, specialties, and individuals. The use of sedation and neuromuscular blockade has been shown to increase intubation success[67,149] and is recommended by the American Academy of Pediatrics.[150] However, caution is required when considering paralytic agents if the ability to establish the airway and/or ventilate the patient is at all in question, such as with a provider inexperienced in managing a neonatal or difficult airway.

Inhaled nitric oxide (iNO) is approved for use in term and near-term newborns with hypoxic respiratory failure with clinical or echocardiographic evidence of pulmonary hypertension.[151] Administration of iNO to newborns with PPHN reduces the need for ECMO.[152,153] With increasing availability in community NICUs, iNO therapy is often initiated before transport to a tertiary care center. Once iNO is administered, abrupt cessation of therapy can result in rapid clinical deterioration from rebound pulmonary hypertension, so it is essential to continue this therapy throughout the transport.[154] Transport teams may also consider initiating iNO therapy for term and near-term newborns with hypoxic respiratory failure in discussion with the MCP. If the referring facility does not have the capability to perform echocardiography, the transport staff should carefully consider the possibility of congenital heart lesions that can lead to clinical decompensation with iNO, including total anomalous venous return, and lesions with ductal-dependent systemic blood flow or severely diminished left ventricular function. While rare, some preterm infants may have PPHN despite adequate lung inflation resulting in severe hypoxemia and a need for iNO. Starting iNO in this population should be given due consideration and should be discussed with the MCP.[151]

The rationale for initiating iNO therapy for a transport is to reduce pulmonary vasoreactivity and increase stability during transport. However, there have been no prospective studies to determine whether this practice affects patient outcome. A retrospective study by Lowe and Trautwein evaluated initiation of iNO by the transport team in the field versus at the hospital following transport and showed similar death and ECMO rates, but an overall decrease in hospital length of stay at referring and receiving hospitals when iNO was started earlier.[155] During transport, iNO has been delivered by several different systems, such as the aeroNOX (Aeronox Technology Corporation, Quezon City, Philippines) iNOvent, and INOmax DS (INO Therapeutics, Hampton, New Jersey, United States) transport systems.[151,154,156–158] The use of iNO in combination with high-frequency ventilation in non-ECMO centers can complicate the transfer process. If the transport team does not have the capability to provide mobile high-frequency ventilation, it is recommended that the referring hospital perform a trial of conventional mechanical ventilation before transport to establish that the neonate can tolerate the transition.

Both high-frequency jet ventilation (Bunnell ventilators; Bunnell, South Salt Lake, Utah, United States) and high-frequency flow interrupter ventilation (Bird ventilators; CareFusion, Yorba Linda, California, United States) have been configured and used with nitric oxide during transport.[159,160] The high-frequency oscillator (SensorMedics 3100 A; CareFusion, Yorba Linda, California, United States) that is commonly used in many NICUs is impractical for ground transport and is not configured for helicopter or fixed-wing transport. However, even with these technologies, the conditions of 30% to 40% of critically ill neonates improve only temporarily with iNO therapy, and they will ultimately require a higher level of care.[157,161]

For patients with pulmonary or cardiac failure who are unresponsive to maximal medical therapy, ECMO is often used as a bridge therapy to allow either the lungs or heart, or both, to recover. Ideally, centers without ECMO capability should have prospective criteria to guide the transfer of newborns before the need for ECMO cannulation. The Extracorporeal Life Support Organization (ELSO) recommends ECMO for infants with severe hypoxemic respiratory failure on maximal medical therapy with potentially a reversible cause, if they have one or more of the following: (1) inadequate tissue oxygen delivery, (2) severe hypoxic respiratory failure (PaO_2 <40 mmHg), (3) oxygenation index with sustained elevation, and (4) severe pulmonary hypertension with evidence of right and/or left ventricular dysfunction.[162] It must be highlighted that earlier referral to an ECMO center of a patient with severe respiratory failure, may prevent significant morbidity and mortality, including the need for ECMO itself.[163] In some cases, the patient's condition is so unstable that conventional transport cannot be conducted safely. Select programs have the capability to provide mobile ECMO, during which patients are cannulated for ECMO either before transport or when the transport team arrives.[164] The ELSO provides guidelines for team composition and equipment that are based upon the need for patient cannulation at the referring hospital and on special considerations for modes of mobile ECMO transport.[165] In a recent study the most common diagnoses associated with the need for neonatal ECMO transport were meconium aspiration syndrome, persistent pulmonary hypertension, sepsis, and congenital diaphragmatic hernia.[166] Mobile ECMO can also benefit patients already receiving ECMO at a tertiary facility who are in need of transfer for advanced quaternary therapies, such as heart or heart-lung transplants.[167–169] Fixed-wing and ground ambulance transport predominate as primary modalities for ECMO transport, which is logical given the size of the equipment and the team involved. In a recent study, however, 5% of ECMO transports occurred in a helicopter.[166]

The resources and skill set necessary to perform ECMO safely and consistently in the transport environment have, by their complexity, restricted the number of transport programs with mobile ECMO capabilities,[166,169–171] and there are thus a limited number of such centers worldwide that have long-standing, relatively high-volume pediatric/neonatal ECMO transport programs. The amount of time from the decision to deploy the mobile ECMO team and the actual departure from the unit can vary depending on the mode of transport, availability of appropriate personnel, and the time of the call making mobile ECMO a lengthier process that can potentially impact patient outcome.[171] While there are no published data to set criteria for the number of ECMO transports needed to maintain competency or impact mortality, recent literature suggests that centers with at least 20 to 30 neonatal and pediatric ECMO cases annually have higher survival rates than centers with a lower volume.[166,169,171,172] These data support the regionalization of ECMO transport services to optimize transport team skills and performance as well as patient outcomes given the high-risk, low-frequency nature of these transports.

Neurologic Issues

Hypoxic-ischemic encephalopathy affects approximately 1 in 1000 to 2 in 1000 term neonates.[173,174] Therapeutic hypothermia (generally sustained core temperature of 33.5°C for 72 hours) for encephalopathic term neonates has demonstrated a reduction in the combined outcome of death and neurodevelopmental disability up to 7 years of age when neonates were cooled compared with a control population.[175–181] Clinical trials and current guidelines suggest that cooling should be initiated within 6 hours of delivery, after an acute perinatal event (Box 19.2). Several studies have demonstrated the feasibility of initiating passive (turning off radiant warmer heat) and/or active (using ice/gel packs) and device-controlled (cooling blanket with servo-control using rectal or esophageal probe) hypothermia during transport.[182–186] Passive and/or active hypothermia that is not device-controlled results in wider range of fluctuation in patient temperatures, with patients arriving at the receiving hospital outside of goal range and taking longer to reach the goal temperature.[83,186,187] Device-controlled hypothermia on transport results in patients reaching goal temperature faster which is important when considering distance of travel and time of initiation of patient transfer.[186,188]

Device-controlled hypothermia on transport is more favorable than passive and/or active hypothermia that is not device-controlled in achieving goal temperature within 6 hours of birth. This is not always achievable depending on the capabilities of the transport program and/or mode of transport. In the United Kingdom, 70% of transport teams use servo-controlled devices.[189] The US Food and Drug Administration approved servo-regulated cooling device weighs 7.2 kg and can be easily accommodated on most ground, helicopter, and fixed-wing ambulances.

Congenital Heart Disease

Transport of the neonate with CHD follows the same general approach as for the transport of any critically ill neonate. However, neonates with complex CHD often need therapeutic intervention, requiring the support of multiple subspecialty

BOX 19.2 Criteria for Neonatal Therapeutic Hypothermia on Transport

1. *Must* meet the following eligibility criteria:
   - ≥36 weeks' gestation
   - Birth weight >1800 g
   - Less than 6 hr of age
   - First-hour blood gas, if available: pH ≤ 7.15 and base deficit ≥10
   - No severe congenital anomalies
   - Plan for continued full care
2. Criteria additional to above eligibility:
   - *And* first-hour blood gas: pH ≤7.0 or base deficit or base deficit ≥16
   - Or no blood gas available or first-hour blood gas: pH 7.0–7.15 or base deficit 10–16
   - *And both:*
   - 10-minute Apgar score ≤5 or assisted ventilation required at birth ≥10 min
   - Acute perinatal event
     - Intrauterine distress
     - Cord rupture or prolapse
     - Uterine rupture
     - Maternal trauma/hemorrhage
     - Cardiopulmonary arrest
3. *And* either of the following:
   - Moderate to severe encephalopathy
   - Seizures

From McNellis E, Fisher T, Kilbride HW. Safety and effectiveness of whole body cooling therapy for neonatal encephalopathy on transport. *Air Med J.* 2015:34(4):199–206; modified from Shankaran S, Laptook AR, Ehrenkranz RA, et al. Whole-body hypothermia for neonates with hypoxic-ischemic encephalopathy. *N Engl J Med.* 2005:353:1574–1584; Jacobs SE, Berg M, Hunt R, et al. Cooling for newborns with hypoxic ischaemic encephalopathy. *Cochrane Database Syst Rev.* 2013;(1):CD003311.

TABLE 19.2 Steps Toward Optimal Transport of the Neonate With Congenital Heart Disease

All Patients	Intubated Patients
• Secure vascular access. • Ensure vascular access for volume resuscitation that is not infusing inotropes or PGE1. • Avoid interruption of PGE1 infusion. • Maintain normothermia to minimize oxygen consumption.	• Secure and record endotracheal tube position. • Place nasogastric or orogastric tube for decompression. • Maintain NPO status including fluids and medications. • Provide appropriate sedation.

NPO, Nil per os; *PGE_1*, prostaglandin E_1.

services, including a pediatric cardiothoracic surgeon and cardiologist,[190,191] which may necessitate transport to a specialized center.[192,193] Importantly, the preoperative care of these patients affects their postoperative outcomes and mortality.[194–199] In some cases, the neonate will have a prenatal diagnosis of CHD and the team will have disease-specific management plans, for instance, initiation of PGE_1 therapy on arrival for a neonate with a clinically stable ductal-dependent lesion. In other cases, CHD may be suspected in a critically ill neonate with signs of cardiovascular collapse, and the transport team must be prepared to initiate treatment, such as PGE_1 therapy, or limit others, such as treatment with fluids, oxygen and iNO, to manage a potential but not yet confirmed diagnosis. Regardless of whether the presence of CHD is known or suspected, the transport team must be prepared to manage it.

To optimize management, early communication with the receiving specialty center is vital. Whenever possible, the pediatric cardiologist, neonatologist, and cardiac intensivist at the accepting hospital should be included in formulating the transport management plan while the neonate is still at the referring hospital, thereby guiding the timing and urgency of the transport, line placement, and recommendations for airway management and supplemental oxygen therapy (Table 19.2).

The neonatal resuscitation algorithm is applicable in the presence of CHD[200] but should be modified in certain circumstances. In presentations such as hypoxemia unresponsive to supplemental oxygen, congestive heart failure, or shock, attention is devoted simultaneously to the basics of neonatal ALS and to the assurance of a patent ductus arteriosus.

A stable airway must be maintained, allowing adequate alveolar oxygenation and ventilation before transport. In critically ill neonates with known CHD presenting with severe cyanosis or circulatory collapse, intubation should be performed by a skilled provider after premedication with sedation and neuromuscular blockade. Reliable venous access is important, and arterial monitoring is helpful for ongoing assessment of blood pressure, acid–base status, and gas exchange. Volume resuscitation, inotropic support, and correction of metabolic acidosis may be required to maximize cardiac output and tissue perfusion. In neonates with suspected but not confirmed CHD, an evaluation for sepsis is typically considered simultaneously, and empiric antibiotic therapy initiated.

Supplemental Oxygen

Supplemental oxygen is a potent pulmonary vasodilator and systemic vasoconstrictor, and it can adversely affect the physiology of neonates with a single ventricle, as well as those with two ventricles with an unrestrictive ventricular septal defect or great vessel communication. The oxygen-induced pulmonary vasodilation can decrease pulmonary vascular resistance and increase pulmonary blood flow at the expense of systemic blood flow, thus reducing systemic output in some cases. For known CHD, the use of oxygen therapy and goal oxygen saturations should be determined with the use of established guidelines for specific lesions or in consultation with the MCP. Titrating oxygen via a nasal cannula or a face mask to a target peripheral saturation of 75% to 85% usually corresponds to adequate blood flow in both the pulmonary system and the systemic system. Higher oxygen saturations are typically not necessary and in fact may ultimately result in decreased oxygen delivery to the peripheral tissues. In cases of undifferentiated neonatal hypoxemia where CHD is suspected, oxygen therapy should be continued until CHD is ruled out by echocardiogram since severe pulmonary hypertension is also in the differential diagnosis for which supplemental oxygen therapy is indicated.

Prostaglandin E_1 Therapy

PGE_1 therapy is the standard of care for stabilization of known ductal-dependent CHD (DDCHD) but is often initiated in

undifferentiated hypoxic neonates when DDCHD is possible but not confirmed. In a study examining the diagnostic accuracy for CHD based on the clinical findings of a transport team, Gupta et al. found that there was no single finding predictive of DDCHD, concluding that making this diagnosis outside a specialty center was challenging.[201] In a separate study, the same authors studied the effects of PGE_1 administered to both neonates with DDCHD and those with PPHN and found that when PGE_1 was administered to neonates with persistent pulmonary hypertension, they recovered faster, and no adverse events were observed during transport.[202]

In the instance of prenatally diagnosed CHD, ductal dependency is often already determined. In these cases, maintaining ductal patency is key. Whenever possible, PGE_1 infusions should be prepared ahead of arrival at the referring center and started promptly. PGE_1 may be given via a central or peripheral intravenous catheter or in emergent situations where intravenous access cannot be obtained via an umbilical catheter or intraosseous line. For neonates with suspected CHD whose duct has begun to close, usually after the first 24 hours after birth, PGE_1 is often required, and response is immediate if ductal patency is central to the neonate's hemodynamics. In all cases, failure to respond to appropriate dosing may mean that the initial diagnosis of DDCHD is incorrect, the ductus is unresponsive to PGE_1 therapy (which may occur in older neonates), or there is no ductus arteriosus present. Often the condition of the patient with a ductal-dependent lesion will improve greatly with the initiation of PGE_1 therapy, and the patient may not need to be rushed to the cardiac referral center as an emergent case.

On rare occasions, the neonate may have progressive instability after initiation of PGE_1 therapy. This important diagnostic finding strongly suggests a congenital heart defect with obstructed blood flow out of the pulmonary veins or the left atrium.

The following are types of CHD unresponsive to PGE_1 therapy:

- Hypoplastic left-sided heart syndrome with a restrictive foramen ovale or intact atrial septum
- Variants of mitral atresia with a restrictive foramen ovale
- Transposition of the great arteries with an intact ventricular septum and restrictive foramen ovale
- Total anomalous pulmonary venous return with obstruction of the common pulmonary vein

If the neonate clinically deteriorates despite receiving PGE_1 therapy, urgent echocardiography is indicated, followed by prompt transfer to a cardiac unit.[193] Controversy exists as to whether PGE_1 therapy should be continued in these rare instances, but most importantly, the apparent lack of response is a marker for rare forms of CHD that do not respond to medical management and require urgent surgical or catheter intervention.

Although PGE_1 is critical in the management of DDCHD, there are several potential adverse effects associated with PGE_1 that must be anticipated, particularly in the premature neonate, in which they occur more commonly (Table 19.3). Apnea and hypotension usually manifest themselves during the first few hours of administration but can occur at any time during the infusion. Judicious fluid resuscitation will generally normalize the blood pressure in cases of hypotension.[203] If hypotension is refractory to fluid administration, an alternative cause of hypotension should be considered (e.g., a restrictive ductus, pericardial effusion, myocardial dysfunction, sepsis), and inotropes should be prepared. Hypotension is a late finding in neonatal cardiogenic shock and ideally should be managed before transport during the stabilization phase. Standards for septic shock management should be followed regardless of the care environment.[204]

TABLE 19.3 Adverse Effects of Prostaglandin E_1 Infusion

Most Common	Less Common
• Hypotension due to vasodilation	• Seizures
• Apnea	• Gastric outlet obstruction
• Rash	• Cortical hyperostosis
• Fever	• Leukocytosis

Data from Kramer HH, Sommer M, Rammos S, et al. Evaluation of low dose prostaglandin E_1 treatment for ductus dependent congenital heart disease. *Eur J Pediatr*. 1995;154:700–707; Lewis AB, Freed MD, Heymann MA, et al. Side effects of therapy with prostaglandin E_1 in infants with critical congenital heart disease. *Circulation*. 1981;64:893–898; Arav-Boger R, Baggett HC, Spevak PJ, et al. Leukocytosis caused by prostaglandin E_1 in neonates. *J Pediatr*. 2001;138:263–265; Teixeira OHP, Carpenter B, MacMurray SB, et al. Long-term prostaglandin E_1 therapy in congenital heart defects. *J Am Coll Cardiol*. 1984;3:838–843.

The potential side effect profile of PGE_1 mandates the need for ongoing cardiorespiratory monitoring during transport; however, it does not in and of itself usually require intubation and mechanical ventilation without the presence of significant or recurrent apnea.[203,205] Low-dose PGE_1 infusions are unlikely to cause apnea requiring mechanical ventilation, and neonates can be safely transported without an artificial airway being established in most cases.[206]

Vascular Access

Umbilical venous catheters (UVCs) and umbilical arterial catheters (UACs) are useful in the stabilization and transport of critically ill neonates, including those with CHD. Placement of these lines is not without risk. Beyond the infectious risks, newborns with CHD are at a higher risk of thromboembolic events because of their immature clotting mechanisms, small-vessel lumens, and low flow states. There is also an ongoing risk of systemic embolization of air in babies with an intracardiac right-to-left shunt. There has been much controversy surrounding the optimal placement of a UAC in premature neonates, although there are scant data regarding term neonates with CHD. Historically, high UACs have been preferred due to a presumed lower risk of vascular complications (Table 19.4).

Catheter tip positions between the high-lying and low-lying designations are associated with increased risks of complications, as are placements below the L5 level.[207] The tip of the UVC should be placed, if possible, at the inferior vena cava/right atrial junction or in the atria. It might not be necessary to obtain ideal placement of either the UAC or the UVC for stabilization and transport, and manipulation of line position and reconfirmation can delay transport to the tertiary care center. The risk-benefit relationship for the use of umbilical lines in the neonate with ductal-dependent circulation has not been well delineated. The need for central access should be judged based on the clinical status of the neonate and stability for transport.

TABLE 19.4 Features of High-Lying Versus Low-Lying Umbilical Artery Catheters

High	Low
• Catheter tip above diaphragm, below left subclavian artery, ~T6–T10 • Decreased risk of vascular complications • No significant increase in adverse sequelae	• Catheter tip below renal arteries, above aortic bifurcation, ~L3–L5

Data from Barrington KJ. Umbilical artery catheters in the newborn: effects of position of the catheter tip. *Cochrane Database Syst Rev.* 2000;1999(2):CD000505; Hermansen MC, Hermansen MG. Intravascular catheter complications in the neonatal intensive care unit. *Clin Perinatol.* 2005;32:141–156.

Surgical Emergencies

Because most births occur in hospitals without a NICU or neonatal surgical services, the need for surgical evaluation or intervention is a common reason for interfacility transport. While some surgical conditions are relatively nonurgent, there are several diagnoses that represent truly life-threatening conditions for which stabilization and transport require expertise and specialized resources.

Congenital Diaphragmatic Hernia

Congenital diaphragmatic hernia (CDH) presents along a spectrum of severity, is associated with pulmonary hypoplasia and hypertension, and can be isolated or accompanied by other congenital malformations. Prenatally diagnosed CDH are typically associated with larger size defects and have higher morbidity and mortality as compared to postnatally diagnosed CDH.[208] Protocolized management of CDH patients at high volume centers is likely to improve outcomes.[209,210]

The following are important in the transport of critically ill neonates with known or suspected CDH[211]:

- Secure the airway
  - For respiratory distress, use endotracheal intubation, not nasal CPAP or high-flow nasal cannula
- Use gentle ventilation approach:
  - Allow permissive hypercapnia
  - Limit peak airway pressures
  - Use low tidal volumes
  - Allow spontaneous breathing as much as possible with judicious use of sedation and neuromuscular agents
- Decompress the bowel with a nasogastric or orogastric tube connected to low intermittent suction
- Obtain central venous and arterial access prior to transport
- Maintain adequate systemic blood pressure with fluid and inotropes as needed

Transport to a high-volume center with ECMO capabilities is recommended since a subset of severe CDH patients will benefit from ECMO as a bridge to surgical repair while resting the lung and heart and avoiding the side effects of mechanical ventilation.[212]

Abdominal Wall Defects

The proper management of a newborn with gastroschisis or an omphalocele is critical during the first several hours after birth, and delivery in a tertiary care center has been associated with improved outcome.[213] In addition to the need for initial resuscitation and cardiorespiratory support, the correct treatment of the exposed bowel or sac may improve the newborn's chances of a successful repair and long-term intestinal function.

While current guidelines aim for at least 38 weeks estimated gestational age for elective cesarean delivery, the mean gestational age for newborns with gastroschisis in previous decades was 36 weeks.[214] Many affected neonates are small for their gestational age,[215] and as with other mildly premature and growth-restricted neonates, patients with gastroschisis are at risk for hypothermia and hypoglycemia. Heat loss is exacerbated by the large surface area of the exposed intestines, which also serve as a significant source of fluid loss. Prevention of both losses can be accomplished by placement of the lower part of the neonate's body, including the intestines, into a transport bag (i.e., bowel bag or Lahey bag) before placement of the neonate into the heated transport isolette. Significant fluid losses can still occur through the exposed mucosa, and the patient may require aggressive fluid administration (120 to 150 mL/kg/day). The use of antibiotics should be considered if risk factors for sepsis are present.

Neonates with gastroschisis are at risk for intestinal vascular compromise because the vascular pedicle containing the bowel's arterial supply and venous drainage must pass through the relatively small abdominal wall defect. Transport personnel must closely monitor the appearance of the bowel to detect signs of venous congestion or ischemia. Transporting the neonate in the lateral position, with support of the exposed intestines to avoid tension or torque, is recommended. The use of intestinal pulse oximetry has been described for monitoring the bowel for ischemia through a transparent silo but has not been studied as a tool during interfacility transport.[216] Vascular compromise of the intestine is a surgical emergency, and communication with the receiving facility is essential to coordinate urgent intervention.

The transport of a neonate with an omphalocele has similar considerations, although unless the sac has ruptured, there is minimal risk of heat and fluid loss. Neonates with an omphalocele are more likely than those with gastroschisis to have other birth defects (e.g., CHD). Furthermore, neonates with giant omphaloceles often have respiratory insufficiency caused by diaphragmatic dysfunction, pulmonary hypoplasia, or both and may require ventilatory assistance.

All neonates with gastroschisis or an omphalocele require placement of a large-bore nasogastric or orogastric tube because of the functional ileus or intestinal obstruction that may occur with associated stenoses or atresias. In general, cannulation of the umbilical vessels is not recommended unless obtaining other means of vascular access are not successful.

Esophageal Atresia and Tracheoesophageal Fistula

Esophageal atresia, with or without tracheoesophageal fistula, is typically diagnosed within the first day after birth because of increased secretions, poor feeding, and respiratory distress. General transport considerations include placing a large-bore sump-type tube for continuous aspiration of the proximal esophageal pouch; positioning prone with the head of the bed elevated; and providing respiratory support as needed. Direct aspiration of secretions into the trachea may occur with either a proximal or a distal tracheoesophageal fistula. Transport providers should be aware that neonates with a distal tracheoesophageal fistula (type C), characterized by the presence of air in the intestinal tract, are at risk of gastric and intestinal insufflation via the fistula when receiving positive pressure ventilation. Bag-mask ventilation and CPAP should be avoided. If the neonate requires endotracheal

intubation, the endotracheal tube should be positioned as close to the carina as tolerated to position the distal tip beyond the fistula and minimize direct inflation of the distal esophageal segment with pressurized gas. In extreme cases, gastric rupture with pneumoperitoneum has been reported, requiring emergency paracentesis, laparotomy, or both.[217]

Midgut Volvulus

Malrotation with midgut volvulus can be a catastrophic event resulting in intestinal ischemia and shock and represents a surgical emergency. The most common clinical presentation of midgut volvulus is bilious vomiting, which is a nonspecific sign of intestinal obstruction. Expeditious evaluation of the newborn with bilious vomiting is essential to facilitate prompt surgical intervention to prevent progression of vascular insufficiency to actual intestinal necrosis. An upper gastrointestinal tract series is the radiologic test of choice to diagnose malrotation and midgut volvulus, although some practitioners have reported success with the use of ultrasound examination to identify the relationship of the superior mesenteric vessels.[218,219]

A neonate with suspected midgut volvulus should be rapidly cared for in a facility with pediatric radiology and surgical capabilities. Care of the neonate with suspected midgut volvulus during interfacility transport is primarily supportive and includes circulatory interventions with intravenous fluid repletion, correction of metabolic abnormalities, and gastric decompression with a large-bore nasogastric or orogastric tube.

Necrotizing Enterocolitis

Necrotizing enterocolitis occurs primarily in premature neonates, and approximately 30% of affected newborns will require surgical intervention in the form of laparotomy or peritoneal drain placement.[220] Neonates with moderate or severe necrotizing enterocolitis should be transported to a facility with pediatric surgical capabilities. Care during transport is primarily supportive and includes intravenous fluids, administration of broad-spectrum antibiotics, correction of metabolic abnormalities, and gastric decompression. Respiratory failure is common because of disordered control of breathing and elevation of the diaphragm from abdominal distention. With significant free intraperitoneal air or pneumatosis and changes in altitude, neonates may be at risk of intra-abdominal hypertension and abdominal compartment syndrome. Transport providers should be aware of this and discuss potential interventions, such as a peritoneal drain, with the MCP and referring provider before departure from the referring facility. Abdominal decompression before transport has been previously recommended for the transport of neonates with pneumoperitoneum.[221]

Meningomyelocele

For purposes of transport, the newborn with myelomeningocele should be placed in the prone position, and the spinal defect covered with moist sterile dressings as well as some form of plastic wrap to maintain moisture. The lesion can be covered with a moistened nonadherent dressing and then loosely encircled with a gauze roll "donut," with the entire defect covered with a sterile drape. This dressing can be moistened as required during the transport process. The use of latex gloves should be avoided during care of these patients. If cerebrospinal fluid leakage is observed or suspected, there is an increased risk of infection, and empiric antibiotics should be considered. Neonates with a meningomyelocele may or may not have accompanying hydrocephalus at birth; approximately 25% of affected patients will require shunting in the immediate newborn period, with up to 85% eventually undergoing shunt placement.[222] In one institution, the percentage of patients necessitating shunt placement was reduced when stricter criteria were used, including permissive mild ventricular dilatation and the presence of symptomatic hydrocephalus.[223] Furthermore, the results of the Management of Myelomeningocele Study, which demonstrated reduced need for shunt placement and corresponded with improved neurologic and motor outcomes at 30 months following in utero repair of the spinal defect, may lead to additional practice changes in postnatal shunt placement.[224]

Future Directions

The field of transport medicine has grown substantially in the past several decades and will continue to grow and change, particularly in relation to its special and vulnerable patient population. The goals include rapid awareness of need, clear communications, and delivery of care that is similar to that in our level III/IV NICUs. Development of standards and guidelines will enable us to assess the impact of neonatal transport and the opportunity it provides for patients and clinicians. The role of developing technologies, such as telemedicine, will likely have an increasing presence in referral hospitals as well as for use by transport teams. In addition, there will likely be increasing attention paid to cost and value, especially of high-cost air transport, that may lead to stricter criteria and a greater push toward national transport guidelines, and potentially even drones may play a role at least in equipment and medication delivery to outlying hospitals. The transport of critically ill neonates is most often a high-risk, low-frequency event in the context of all patient transfers. Therefore, as systems move forward, it is imperative to ensure the highest level of skill and competence both in the transport team personnel and in the facilities that refer and receive these patients—be it through team specialization, regionalization of high level neonatal critical care, or support of telemedicine and outreach initiatives.

Suggested Readings

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20

Fluid, Electrolyte, and Acid-Base Balance

CLYDE J. WRIGHT, MICHAEL A. POSENCHEG, AND ISTVAN SERI

KEY POINTS

- In the neonate, many pathologic states and/or their treatment are associated with disruptions in normal body fluid, electrolyte, and acid-base balance, which at times may in themselves become life-threatening.
- Conversely, many conditions may be negatively affected by disruptions of normal body fluid, electrolyte, and acid-base balance occurring independently of the condition itself.
- The maintenance of normal fluid, electrolyte, and acid-base balance is a cornerstone of appropriate management of the sick neonate.

Fluid and Electrolyte Balance

Maintenance of fluid and electrolyte balance is essential for normal cell and organ function during intrauterine development and throughout extrauterine life. In the newborn, pathologic conditions and/or their treatment often lead to disruption of the complex regulatory mechanisms maintaining homeostasis. In addition, pathologic conditions themselves may be negatively affected by disruptions of normal body fluid, electrolyte, and acid-base balance occurring independently of the condition itself. Therefore, a thorough understanding of the physiologic changes in the neonatal period and the provision of appropriate therapies, based on the principles of developmental fluid and electrolyte physiology, are among the cornerstones of modern neonatal intensive care.

Developmental Changes Affecting Fluid and Electrolyte Balance in the Fetus and Neonate

Developmental Changes in Body Composition and Fluid Compartments

Dynamic changes occur in body composition and fluid distribution during intrauterine life, labor and delivery, and the early postnatal period. Thereafter the rate of change in body composition and fluid distribution gradually decreases, with subtler changes occurring especially after the first year of age.[1,2]

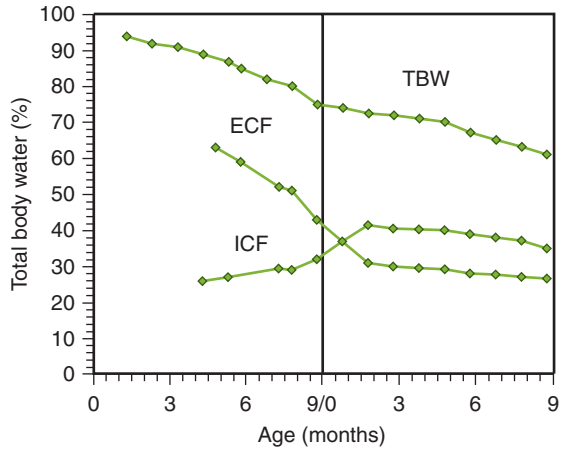
Changes During Intrauterine Development

In early gestation, body composition is characterized by a high proportion of total body water (TBW) and a large extracellular compartment.[3,4] As gestation advances, rapid cellular growth, accretion of body solids, and fat deposition and developmental changes in the production of hormones regulating body water homeostasis result in gradual reductions in TBW content and extracellular fluid (ECF) volume, while the intracellular fluid compartment increases (Fig. 20.1).[3] In the 16-week-old fetus, TBW represents approximately 94% of total body weight; approximately two-thirds and one-third of the TBW are distributed in the extracellular and intracellular compartments, respectively. After delivery, TBW decreases by 1.44% per week in preterm neonates.[2] By term gestation, TBW represents 75% of body weight, and approximately half of this volume is located in the intracellular compartment. Therefore, premature newborns have excess TBW and a larger extracellular volume compared with their term counterparts at birth, with most of the expanded extracellular volume being distributed in the interstitium.[5]

Changes During Labor and Delivery

Greater changes in TBW and its distribution occur during labor and delivery. Arterial blood pressure rises several days before delivery because of increases in plasma catecholamine, vasopressin, and cortisol levels, as well as translocation of blood from the placenta into the fetus. This rise in arterial blood pressure, along with changes in the fetal hormonal milieu and an intrapartum hypoxia-induced increase in capillary permeability, results in a shift of fluid from the intravascular to the interstitial compartment and an associated approximately 25% reduction in circulating plasma volume in the human fetus during labor and delivery.[5] If the cord is not clamped immediately after delivery of the body, placental transfusion tends to restore the circulating blood volume,[6] while the postnatal increase in oxygenation and changes in vasoactive hormone production act to restore capillary membrane integrity and favor absorption of interstitial fluid into the intravascular compartment. The return of interstitial fluid into the bloodstream aids in maintaining intravascular volume during the first 24 to 48 hours postnatally, when oral fluid intake may be limited. The return of interstitial fluid also triggers the release of atrial natriuretic peptide, which is—at least in part—responsible for the physiologic diuresis commencing on postnatal days 2 and 3.[7] However, prematurity, pathologic conditions, or both can disrupt this delicate process and interfere with the physiologic contraction of the ECF compartment in the immediate postnatal period.

In the fetus, body composition and fluid balance depend on the electrolyte and water exchange between the mother, fetus, and amniotic space.[8] Antenatal events can have significant effects on postnatal fluid balance. Maternal indomethacin treatment or excessive administration of intravenous (IV) fluids during labor



• **Fig. 20.1** Total body water content and its distribution between the extracellular fluid and intracellular fluid compartments in the human fetus, newborn, and infant from conception until 9 months of age. *ECF*, Extracellular fluid; *ICF*, intracellular fluid; *TBW*, total body water. (The data represent average values from Friis-Hansen B. Body water compartments in children: changes during growth and related changes in body composition. *Pediatrics*. 1961;28:169–181.)

can result in neonatal hyponatremia with expanded extracellular water content.[9] Placental insufficiency or maternal diuretic therapy can impair fetal extracellular volume, urine output, and amniotic fluid volume.[10]

Effect of Timing of Cord Clamping

The timing of cord clamping after delivery is another important factor significantly affecting total circulating blood volume and extracellular volume in the neonate. Immediate cord clamping does not allow placental transfusion and negatively influences hemodynamic transition, especially in preterm neonates.[11] However, if cord clamping is delayed, up to 25 to 50 mL of blood per kilogram is transfused into the neonate, representing an approximately 25% to 50% increase in the total blood volume.[6] In a metanalysis of term neonates, delayed cord clamping for at least 30 seconds was associated with increased iron stores and higher birth weight but with a higher need for phototherapy.[12] In a metanalysis in preterm neonates, delayed cord clamping was associated with significantly decreased rates of intraventricular hemorrhage (IVH) and necrotizing enterocolitis, and the need for inotropic support and blood transfusion.[13] The use of novel resuscitation trolleys[14] and a better understanding of the cardiorespiratory impact of resuscitating neonates with the cord unclamped[15] have led to a more widespread use of delaying cord clamping in the clinical practice.[16] Animal data[17] and earlier observations in term neonates[18] also suggest that the establishment of an appropriate functional residual capacity is the most relevant factor in determining the volume and rapidity of placental transfusion when cord clamping is delayed. Accordingly, neonates who do not establish effective respirations and who require resuscitation at birth may be at additional risk of lower circulating blood volumes. Although further data are still needed to understand whether increased blood volume at birth is associated with improved long-term outcomes, the American College of Obstetricians and Gynecologists now recommends delaying cord clamping for 30 to 60 seconds after birth in vigorous preterm and term neonates;[19] the World Health Organization recommends it for all newborns.[20]

Changes in the Postnatal Period

In the first few days and weeks after birth, the TBW content and distribution are affected by gestational and postnatal ages, pathologic conditions, the immediate environment (temperature, humidity), and the type of nutrition (enteral vs. parenteral). Normally, in the first few days after birth, an increase in capillary membrane integrity favors absorption of the interstitial fluid into the intravascular compartment.[21] The ensuing rise in circulating blood volume stimulates the release of atrial natriuretic peptide (ANP) from the heart, which in turn enhances renal sodium and water excretion,[22] resulting in an abrupt decrease in TBW and attendant weight loss. Although it is generally accepted that this postnatal weight loss is primarily due to the contraction of the expanded ECF compartment, some water loss from the intracellular compartment can also occur, particularly in infants with extremely low birth weight (ELBW) and increased transepidermal water losses (TEWLs).[21,23]

Healthy term newborns lose approximately 10% of their birth weight during the first 4 to 7 days of age[5] and, thereafter, they establish a pattern of steady weight gain. As preterm neonates have an increased TBW content and extracellular volume, they lose approximately 10% to 15% of their birth weight during this period[5,24] and, depending on the degree of prematurity and associated pathologic conditions, these neonates only regain their birth weight by 10 to 20 days after birth (Fig. 20.2). Neonates who have intrauterine growth retardation have a smaller initial weight loss and more rapidly regain their birth weight than their normally grown counterparts, whether term or preterm.[25] Although the mechanisms for these differences have not been well studied, they appear to be associated with less diuresis in the infant with intrauterine growth retardation.[25,26]

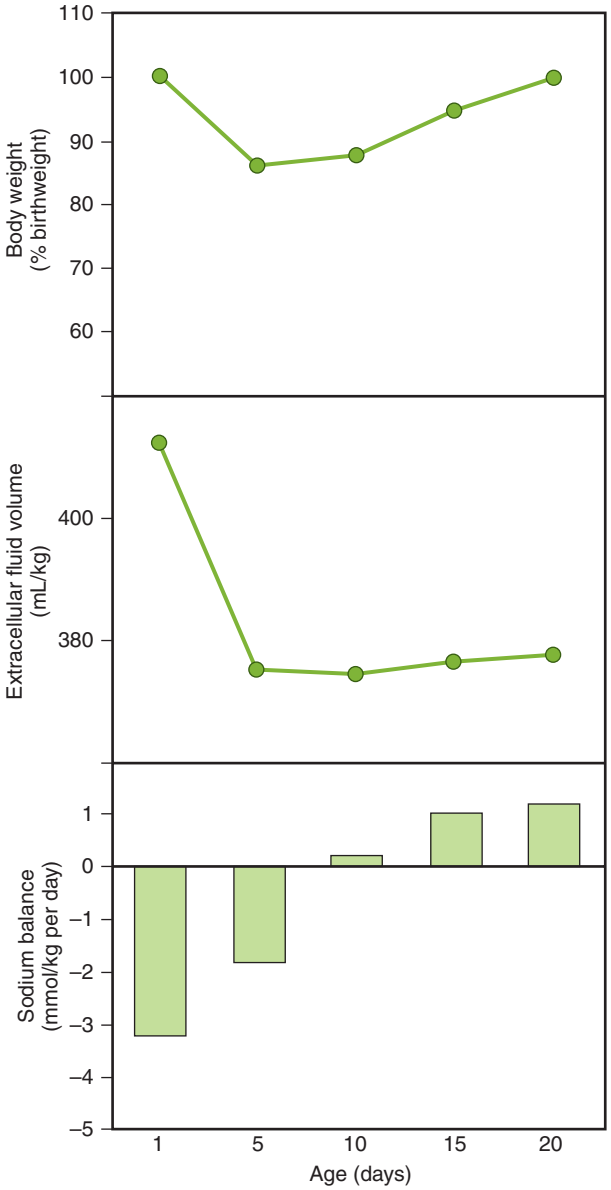
In the management of the neonate, it is important that the appropriate weight loss be anticipated and facilitated, if necessary, as the lack of the early postnatal weight loss has been associated with higher rates of persistency of patent ductus arteriosus (PDA), bronchopulmonary dysplasia (BPD), and necrotizing enterocolitis in low birthweight infants.[27–29]

Physiology of the Regulation of Body Composition and Fluid Compartments

Although human cells have the ability to adjust their intracellular composition, extracellular volume and osmolality affect intracellular conditions. If these changes are too dramatic, they may go beyond the cells' capacity to appropriately maintain the normal intracellular milieu. Therefore, monitoring and active regulation of extracellular volume and osmolality are necessary in sick infants and those born prematurely.

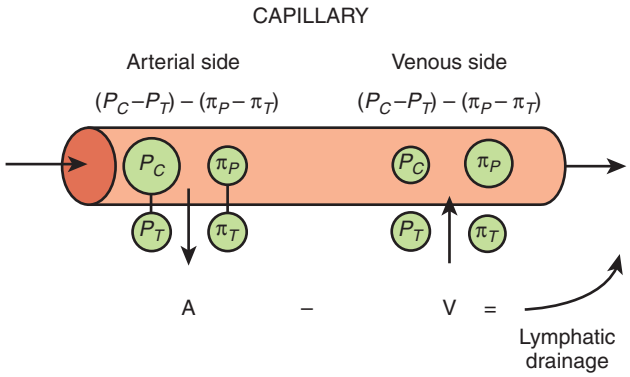
Regulation of the Intracellular Solute and Water Compartment

The major intracellular solutes include the proteins necessary for cell function, the organic phosphates associated with cellular energy production and storage, and the equivalent cations balancing the phosphate and protein anions.[30] As a consequence of the function of the cell membrane-bound sodium-potassium-ATPase enzyme, potassium is the major intracellular and sodium the major extracellular cation. The energy derived from the concentration differences for sodium and potassium between the intracellular and extracellular compartments is used for cellular work. Because changes in osmolality of the extracellular compartment are reflected as net movements of water into or out of the cell,



• **Fig. 20.2** Postnatal changes in body weight (expressed as a percentage of birth weight), extracellular fluid volume (estimated by the bromide dilution method), and sodium balance (defined as the difference between sodium intake and urinary sodium excretion). (From Shaffer SG, Weismann DN. Fluid requirements in the preterm infant. *Clin Perinatol*. 1992;19:233–250.)

regulation of ECF osmolality ultimately controls the intracellular compartment.[30] This physiologic principle must be kept in mind when managing sick term and preterm neonates with disturbances of sodium homeostasis. Rapid changes in serum sodium concentration, and thus in extracellular osmolality, directly affect the osmolality and size of the intracellular compartment and can lead to irreversible cell damage, especially in the central nervous system (CNS). Hyponatremia in the neonatal period has been associated with adverse long-term outcomes, especially in preterm neonates, while hypernatremia has been associated with short-term morbidities, including seizures and thrombosis.[31–33] These associations underscore the importance of maintaining appropriate fluid and electrolyte homeostasis in the neonatal period.



• **Fig. 20.3** Filtration and reabsorption of fluid along the capillary under physiologic conditions. *A*, Arterial; P_C, capillary hydrostatic pressure; P_T, tissue/interstitial hydrostatic pressure; *V*, venous; π_P, plasma oncotic pressure; π_T, tissue/interstitial oncotic pressure.

Regulation of the Intracellular–Extracellular Interface: The Interstitial Compartment

In the healthy term neonate, hydrostatic (P_C, capillary hydrostatic pressure; P_T, tissue/interstitial hydrostatic pressure) and oncotic (π_P, plasma oncotic pressure; π_T, tissue/interstitial oncotic pressure) pressures are well-balanced, with both being approximately half of those in the adult.[34] Under normal physiologic conditions, movement of fluid across the capillary is determined by the direction of the net driving pressure ($[P_C - P_T] - [\pi_P - \pi_T]$), the water permeability and the protein permeability, as well as transport characteristics of the capillary wall (Fig. 20.3). At the arterial end of the capillary, intracapillary hydrostatic pressure (P_C) is high and plasma oncotic pressure (π_P) is relatively low, resulting in a net movement of fluid out of the capillary. As filtration of relatively protein-poor fluid continues along the capillary, plasma oncotic pressure rises and intracapillary hydrostatic pressure drops; therefore on the venous side, fluid moves from the interstitium into the capillary and thus the majority (85% to 90%) of the filtered fluid is reabsorbed at the end of the capillary bed. The fluid remaining in the interstitium (arterial–venous side of the capillary) is drained by the lymphatic system. Interstitial hydrostatic (P_T) and oncotic (π_T) pressures remain virtually unchanged along the capillary bed. However, pathologic conditions readily disturb the delicate balance between the hydrostatic and oncotic forces, leading to an expansion of the interstitial compartment at the expense of the intravascular compartment. The increased interstitial fluid volume (edema) then further affects tissue perfusion by altering the normal function of the extracellular–intracellular interface. Box 20.1 summarizes the mechanisms for conditions resulting in interstitial edema formation in the neonate. There are also some important developmentally regulated differences between the newborn and the adult relating to the pathogenesis of edema formation. Capillary permeability to proteins is increased during the early stages of development.[5,35] Because neonatal capillary permeability is further increased under pathologic conditions (see Box 20.1), protein concentration in the interstitial compartment may approach that of the intravascular space, favoring further intravascular volume depletion and interstitial volume expansion. The findings of most[36,37] but not all[38] clinical studies suggest that 0.9% saline administration for suspected hypovolemia is associated with less fluid retention and similar improvements in the cardiovascular

• BOX 20.1 Mechanisms for Conditions Causing Interstitial Edema in the Neonate

Conditions Favoring Fluid Accumulation in Interstitial Space by Causing Disequilibrium Between Filtration and Reabsorption of Fluid by Capillaries

Increased hydrostatic pressure
Elevated capillary hydrostatic pressure
Increased cardiac output
Venous obstruction
Decreased tissue hydrostatic pressure
Conditions associated with changes in the properties of the interstitial gel (edematous states, effects of hormones including prolactin)
Decreased oncotic pressure gradient
Decreased capillary oncotic pressure
Prematurity, hyaline membrane disease
Malnutrition, liver dysfunction
Nephrotic syndrome
Increased interstitial oncotic pressure is usually the result of increased capillary permeability.
Elevation of the filtration coefficient
Increased capillary permeability
Organs with large-pore capillary endothelium (liver, spleen)
State of maturity (preterm infants > term newborns > adults)
Production of proinflammatory cytokines (sepsis, anaphylaxis, hypoxic tissue injury, tissue ischemia, ischemia–reperfusion, soft tissue trauma, extracorporeal membrane oxygenation)
Increased capillary surface area
Vasodilation

Conditions Associated With Decreased Lymphatic Drainage

Decreased muscle movement
Neuromuscular blockade and/or heavy sedation
Central and/or peripheral nervous system disease
Obstruction of lymphatic flow
Increased central venous pressure
Scar tissue formation (bronchopulmonary dysplasia)
Mechanical obstruction (dressings, high mean airway pressure in mechanically ventilated newborns)

status compared with 4.5% or 5% albumin infusion; however, the topic requires further investigation.

Even in the presence of hypoalbuminemia, when sick neonates are treated with frequent albumin boluses, much of the infused albumin rapidly leaks into the interstitium. This creates a vicious cycle of intravascular volume depletion and edema formation, resulting in vasoconstriction and disturbances in tissue perfusion and cellular function, exacerbating impairments in the regulation of extracellular volume distribution. If the cycle is not interrupted, anasarca develops, which is usually associated with an extremely poor prognosis. In summary, the sick neonate has limited capacity to maintain appropriate intravascular volume and to regulate the volume and composition of the interstitium, and high vigilance is thus required by the caretaker in appropriately managing intravascular volume, including avoiding routine use of albumin in the critically ill neonate.[39]

Regulation of the Extracellular Solute and Water Compartment

The osmolality of the extracellular compartment is tightly maintained within 2% of the osmolar set point, which lies between 275 and 290 milliosmoles (mOsm).[40] Blood pressure and serum sodium concentration (i.e., the main contributor to osmolality under homeostatic conditions) are monitored by baroreceptors and osmoreceptors, respectively. The effector limb of the regulatory system consists of the heart, vascular bed, kidneys, and intake of fluid in response to thirst. The inability of critically ill term and preterm neonates to maintain fluid balance by responding to thirst places increased importance on caregiver management of fluid administration. By regulating the function of the effector organs, several hormones play a role in the control of the extracellular compartment, including the renin–angiotensin–aldosterone system (RAAS), vasopressin, ANP, brain (B-type) natriuretic peptide (BNP), bradykinin, prostaglandins, and catecholamines. Effective regulation of the extracellular compartment and intravascular volume also depends on intact cardiovascular function and capillary endothelium integrity.[40] For example, under physiologic conditions, an increase in the extracellular volume is reflected by an increase in the circulating plasma volume, leading to increased blood pressure and renal blood flow. The ensuing increase in glomerular filtration and the hormonally mediated inhibition of renal tubular sodium and water reabsorption result in increased urine output returning the extracellular volume to a normal level. In critically ill neonates, however, the capillary leak and reduced myocardial responsiveness resulting from immaturity and underlying pathologic conditions limit the increase in the circulating blood volume when extracellular volume expands. Thus, especially in sick preterm neonates, blood pressure may rise only transiently,[41] and renal blood flow may remain low after volume boluses as fluid rapidly leaks into the interstitium. Inappropriate central regulation of vascular tone results in vasodilatation, further decreasing effective circulating blood volume and compromising tissue perfusion; this leads to impaired gas exchange in the lungs, resulting in hypoxia with further increases in capillary leak. Unless it is interrupted by appropriate therapeutic measures, a vicious cycle with further deterioration readily occurs in the sick neonate.

Maturation of Organs Regulating Body Composition and Fluid Compartments

The heart, kidneys, skin, and endocrine system play the most important roles in the regulation of ECF (and thus intracellular fluid) and electrolyte balance in the neonate. Immaturity of these organ systems, especially in infants with very low birth weight (VLBW), results in a compromised regulatory capacity, which must be noted in the estimation of daily fluid and electrolyte requirements in these patients.

Maturation of the Cardiovascular System

There is a direct relationship between gestational maturity and the ability of the neonatal heart to respond to acute volume loading.[42] The blunted Starling response of the immature myocardium primarily results from its lower content of contractile elements, immature intracellular calcium handling, and incomplete sympathetic innervations.[43] Because central vasoregulation and endothelial integrity are also developmentally regulated,[5,35] an appropriate intravascular volume is seldom maintained in the critically ill preterm neonate. Since regulation of the extracellular volume requires the maintenance of an adequate effective circulating blood volume, the immaturity of the cardiovascular system contributes to the limited capacity of sick preterm neonates to effectively regulate the total volume of their extracellular compartment.

Maturation of Renal Function

The kidney has a crucial role in the physiologic control of fluid and electrolyte balance. It regulates extracellular volume and osmolality through the selective reabsorption of sodium and water, respectively. Immaturity of renal function renders preterm neonates susceptible to excessive sodium and bicarbonate losses.[21,44] As described earlier, the inability of the preterm neonate to respond promptly to a sodium or volume load also results in a tendency toward extracellular volume expansion with edema formation. Because prenatal steroid administration accelerates maturation of renal function,[45] preterm neonates exposed to steroids in utero have a better capacity to regulate their postnatal ECF contractions. During the first few weeks postnatally, hemodynamically stable but extremely immature infants produce dilute urine and may develop polyuria because of their renal tubular immaturity. As tubular functions mature, their concentrating capacity gradually increases from the second to the fourth postnatal week. However, it takes years for the developing kidney to reach the concentrating capacity of the adult kidney.[46]

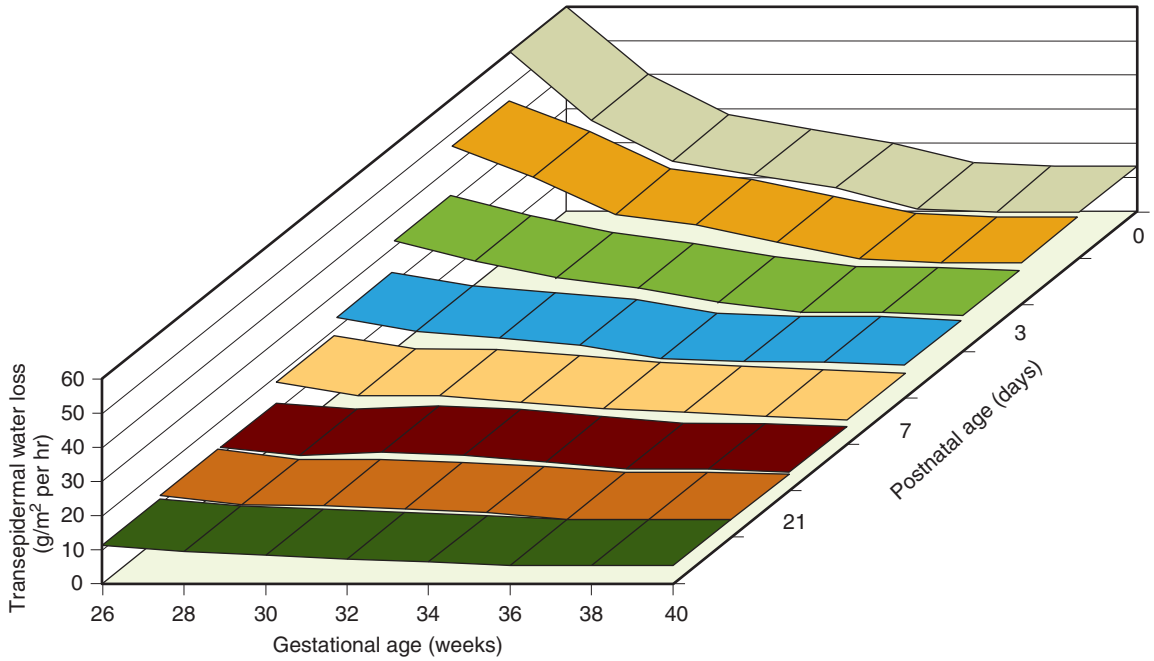
Maturation of the Skin

Although the epidermis of term neonates is well developed and cornified, in extremely immature neonates it consists of only two or three cell layers.[47] The absence of an effective barrier to the diffusion of water increases TEWL in the immature neonate. TEWL through immature skin can result in early postnatal hypertonic dehydration, with rapid changes in intracellular volume and osmolality. In many organs, especially the brain, these abrupt changes can result in cellular dysfunction and ultimately cell death. Gestational age, postnatal age, the pattern of intrauterine growth, and environmental factors (e.g., humidity and temperature) affect transepidermal free water loss (Fig. 20.4). Postnatal skin cornification occurs rapidly, but full maturation of the epidermis does not occur until 2 to 3 weeks of age.[48] Chronic intrauterine stress[49] and prenatal steroid treatment[50] also enhance maturation of the skin.

Maturation of End-Organ Responsiveness to Hormones Involved in the Regulation of Fluid and Electrolyte Balance

Several hormones directly regulate the volume and composition of the extracellular compartment by altering renal sodium and water excretion, as well as by inducing changes in systemic vascular resistance and myocardial contractility. These include the RAAS, vasopressin, ANP, and BNP. Other hormones, including the prostaglandins, bradykinin, and prolactin, acting via endocrine, paracrine, or autocrine mechanisms modulate the actions of many of the regulatory hormones.

Renin–Angiotensin–Aldosterone System. Decreases in renal capillary blood flow stimulate renin secretion from the juxtaglomerular cells of the kidney, which in turn catalyzes the conversion of angiotensinogen to angiotensin I. Angiotensin-converting enzyme hydrolyzes angiotensin I to angiotensin II, which can then bind to the cell membrane–bound receptors AT_1 and AT_2.[51] Angiotensin induces vasoconstriction, increased tubular sodium and water reabsorption, and the release of aldosterone.[51] Aldosterone increases potassium secretion and further enhances sodium reabsorption in the distal tubule; therefore the primary function of this system is to protect the volume of the extracellular compartment, maintain adequate tissue perfusion and mitigate the impact of the hypovolemia-induced metabolic acidosis on potassium homeostasis.[52] However, its effectiveness in the neonate is somewhat limited by the decreased responsiveness of the immature kidney to the sodium- and water-retaining effects of these hormones.[53] Vasodilatory and natriuretic prostaglandins generated



• **Fig. 20.4** Transepidermal water loss in relation to gestational age during the first 28 postnatal days in newborns who are appropriate for their gestational age. There is an exponential relationship between transepidermal water loss and gestational age, the water loss being higher in preterm newborns than in term newborns. Transepidermal water loss is also significantly affected by postnatal age, especially in the immature preterm newborn. The measurements were performed at ambient air humidity of 50% and with the newborns calm and quiet. (From Hammarlund K, Sedin G, Stromberg B. Transepidermal water loss in newborn infants. VIII: relation to gestational age and post-natal age in appropriate and small for gestational age infants. *Acta Paediatr Scand*. 1983;72:721–728.)

in the kidney[54] are the main counterregulatory hormones balancing the renal actions of the RAAS. Therefore, when prostaglandin production is inhibited by indomethacin, the unopposed vasoconstrictive and sodium-retentive actions of the RAAS contribute to the development of the drug-induced renal failure in the preterm neonate.[54,55]

Vasopressin. Vasopressin (antidiuretic hormone) regulates the osmolality of the extracellular compartment and directly affects vascular tone through the V_{1a} and V_2 receptors. Vasopressin selectively raises free water reabsorption through the upregulation of aquaporin-2 water channels in the collecting duct, resulting in blood pressure elevation.[46,56] Plasma levels of vasopressin are markedly elevated in the neonate, especially after vaginal delivery, and its cardiovascular actions facilitate neonatal adaptation.[46,57] Although the developing kidney is less sensitive to circulating vasopressin, the high vasopressin levels are in part also responsible for the diminished urine output of the healthy term neonate during the first day after birth as their renal response to vasopressin is less immature than that of their preterm counterparts. However, under certain pathologic conditions, the dysregulated release of and/or the end-organ unresponsiveness to vasopressin significantly affects renal and cardiovascular functions and electrolyte and fluid status in the sick preterm and term neonate.[58] In the syndrome of inappropriate secretion of antidiuretic hormone (SIADH), an uncontrolled release of vasopressin occurs in sick preterm and term neonates, with resulting water retention, hyponatremia, and oliguria. In the syndrome of diabetes insipidus, the lack of pituitary production of vasopressin or renal unresponsiveness to the hormone results in polyuria and hypernatremia.

Atrial Natriuretic Peptide. Via its direct vasodilatory and renal natriuretic actions, the hormone ANP regulates the volume of the extracellular compartment in the fetus and neonate in a fashion opposite to that of the renin–angiotensin–aldosterone system.[59–61] ANP also has a direct inhibitory effect on renin production and aldosterone release.[62]

The stretch of the atrial wall caused by an increase in the circulating blood volume is the most potent stimulus for ANP release in the cardiovascular system. Plasma levels are high in the fetus[63] and, along with BNP, ANP likely plays a role in cardiac development.[64] There are a few specific conditions in which the actions of ANP are directly relevant for the neonatologist. For example, the hormone is involved in the regulation of both the fluid shifts during labor[5] and the extracellular volume contraction during postnatal transition.[7,65,66] Furthermore, the oliguric effects of positive end-expiratory pressure ventilation are due, in part, to a decrease in ANP secretion[62] along with the enhanced release of vasopressin.[67]

Brain (or B-Type) Natriuretic Peptide. BNP is released from the ventricular myocardium in response to increases in wall tension. Similarly to ANP, BNP causes natriuresis, diuresis, and vasodilatation, while inhibiting the RAAS.[68,69] Compared with ANP, BNP and its inactive N-terminal fragment (NT-proBNP) demonstrate longer half-lives and may thus be more useful clinical biomarkers as their levels are relatively more stable over time.[70] NT-proBNP is renally excreted, and renal function should be considered when interpreting its serum levels.[71]

BNP levels increase by up to 20-fold during the first postnatal day,[72,73] followed by a declining trend during the first postnatal week.[73,74] Not unexpectedly and unlike serum levels of ANP, decreasing BNP levels after the first postnatal day correlate with the downward trend in pulmonary arterial pressure.[75] By causing vasodilatation and diuresis, high levels of BNP play a critical role in the hemodynamic transition of the fetus. The usefulness of measuring BNP levels is limited by the variability of levels in the first few days after birth and, to a certain extent, by the variety of assays available to measure BNP levels. In summary, its clinical utility remains at best uncertain, especially since trending requires repeated measurements in the same patient over time with the same assay.

Prostaglandins. Prostaglandins have a well-documented, counterregulatory role for the renal vascular and tubular effects of renin–angiotensin–aldosterone and vasopressin.[76] The inhibition of prostaglandin synthesis by indomethacin results in clinically important and sometimes detrimental renal vascular and tubular effects especially in the preterm neonate managed with restricted fluid intake.[77] Interestingly, ibuprofen demonstrates fewer side effects than indomethacin on renal and mesenteric blood flow.[77] How prostaglandins modulate the effects of the other regulatory hormones of neonatal fluid and electrolyte homeostasis is less well studied.

Prolactin. Prolactin plays a permissive role in the regulation of fetal and neonatal water homeostasis.[78,79] High fetal plasma prolactin levels contribute to the increased tissue water content of the fetus. Postnatal prolactin levels remain high in the preterm neonate until approximately the 40th postconceptional week.[80] Low levels have been associated with increased risk of developing respiratory distress syndrome (RDS).[81,82] However, the clinical relevance of this observation is unclear.

Management of Fluid and Electrolyte Homeostasis

General Principles of Fluid and Electrolyte Management

Fluid and electrolyte management is one of the cornerstones of neonatal intensive care, and appropriate management requires an understanding of the previously outlined physiologic principles and careful monitoring of key clinical data. Requirements vary substantially from infant to infant and in the same infant over time; therefore, fluid prescription must be individualized and frequently reassessed. The primary goals are to maintain the appropriate ECF volume, extra- and intracellular fluid osmolality, and ionic concentrations.

Assessment of Fluid and Electrolyte Status

Maternal conditions during pregnancy, drugs and fluids administered to the mother during labor and delivery, and specific fetal and neonatal conditions all affect early fluid and electrolyte balance. Excessive administration of free water or oxytocin use in the mother can result in hyponatremia in the neonate. Maternal therapy with indomethacin, angiotensin-converting enzyme inhibitors, furosemide, and aminoglycosides can all adversely affect neonatal renal function. A newborn's history of oligohydramnios or birth asphyxia may also alert the clinician to the possibility of abnormal renal function. In young neonates, altered skin turgor, sunken anterior fontanel, and dry mucous membrane are not sensitive indicators of dehydration, but tachycardia and oliguria present relatively early and hypotension and metabolic acidosis somewhat later during the course of intravascular volume depletion. On the other hand, when there is volume overload or illness, edema usually occurs early. Serial measurements of body weight, intake and output, and serum electrolyte levels will usually provide the most precise and accurate information regarding overall fluid status.

Normally, both term and preterm neonates will void within the first 24 hours after birth.[83] In most neonates without hemodynamic compromise, urine output increases from 1 to 2 mL/kg/h on the first postnatal day to 3 to 5 mL/kg/h by the third to fifth postnatal day and is associated with a weight loss of 5% to 10% in term infants and 10% to 15% in preterm neonates.[84] Frequently, the onset of diuresis heralds the resolution of RDS.[85] However, this phenomenon has become less noticeable after the introduction of early surfactant administration. In critically ill newborns and in situations of altered homeostasis, additional clinical data that may help in diagnosis and management include blood urea nitrogen (BUN) levels, serum and urine osmolarity or specific gravity, and urine electrolyte and serum bicarbonate levels, along with close monitoring of cardiac output and organ blood flow, blood pressure, and heart rate. The frequency of monitoring depends on the extent of immaturity, the underlying pathologic condition, and the severity of the fluid and electrolyte disturbance.

Water Homeostasis and Management

Water Losses

Free water loss can be categorized as either *insensible* (skin and respiratory track) or *sensible* (urine and feces). Urine output is the most important source of sensible water loss. Extremely preterm neonates without systemic hypotension or renal failure usually lose 30 to 40 mL of water per kilogram per day in the urine on the first postnatal day and approximately 120 mL/kg/day by the third day. In stable, more mature preterm neonates born after 28 weeks' gestation, urinary water loss is approximately 90 mL/kg/day on the first postnatal day and 150 mL/kg/day by the third day.[86] Because of their renal immaturity, preterm neonates have a tendency to produce dilute urine, thereby increasing their obligatory free water losses. In term neonates, urinary water loss is considerably less, approximating 40 to 60 mL/kg/day by the third day.

Normal water losses in the stool are less significant, amounting to approximately 10 mL/kg/day in term neonates and 7 mL/kg/day in preterm neonates during the first postnatal week.[23] Water losses in the stool increase thereafter and are influenced by the type of feeding and the frequency of stooling, which is usually higher in breastfed neonates.

In the preterm neonate, consideration of daily insensible water losses (IWLs) through the skin is critical (see Fig. 20.4). During the first few postnatal days, in a nonhumidified environment, TEWLs may be 15-fold higher in extremely premature neonates born at 23 to 26 weeks' gestation than in term neonates.[23] Although the skin matures rapidly after birth, even in extremely immature neonates, insensible losses are still somewhat higher at the end of the first month than in the term counterparts. Prenatal steroid exposure is associated with substantially less IWL in preterm neonates.[50,87]

Appropriate management of the neonate's immediate environment most effectively counteracts the high degree of IWL through immature skin. Among environmental factors, ambient humidity has the greatest effect on TEWL. In extremely immature neonates, a rise in the ambient humidity in the incubator from 20% to 80% decreases the TEWL by approximately 75%.[23] However, the use of an open radiant warmer more than doubles TEWLs,[88] and it is now standard of care to maintain extremely immature neonates in humidified incubators. Other factors that increase transepidermal IWL include phototherapy, especially when the neonate is nursed under low humidity, activity, airflow, elevated body, and environmental temperature as well as skin breakdown and skin or mucosal defects (e.g., gastroschisis, epidermolysis bullosa).

IWLs from the respiratory tract depend mainly on the temperature and humidity of the inspired gas mixture and on the respiratory rate, tidal volume, and dead space ventilation. In a healthy term newborn, the water loss through the respiratory tract is approximately half the total IWL if the ambient air temperature is 32.5°C and the humidity is 50%.[23] However, in neonates undergoing mechanical ventilation, there will be no IWL through the respiratory tract if the ventilator gas mixture is humidified at body temperature.

Extraordinary water losses are also seen in the neonate requiring intensive care. The most commonly encountered extraordinary losses occur when a nasogastric tube is placed under continuous suction (discussed in Surgical Conditions). Large losses may also occur in association with phlebotomy, chest tubes, surgical drains, ostomies, and fistulas, as well as with emesis or diarrhea.

Management of Water Requirements

When managing the fluid status of the neonate, the clinician must consider fluid requirement dictated by three broad categories. First, any existing deficits or surpluses must be estimated. Secondly, ongoing maintenance needs to replace usual sensible and insensible losses, and support for growth must be calculated. Finally, additional needs as a result of extraordinary losses should be anticipated. Importantly, while administered together, the composition of each of these fluids is unique and must be considered individually. For example, fluids given to replace ongoing losses from a chest tube or ostomy will require a different electrolyte composition than simple maintenance fluids to maintain hydration, and these will differ again from fluids given to support growth. The neonate's prenatal history, birth weight, gestational age, and need for mechanical ventilation and the environment in which the neonate is to be cared for should be considered when initial fluid and electrolyte needs are being determined. Frequent reevaluations are necessary. The most useful parameter for monitoring fluid balance is the weight of the baby, as rapid changes in weight will reflect changes in water balance. Serial weights can be used to estimate the IWL with use of the following formulas:

$$\text{IWL} = \text{Fluid Intake} - \text{Urine Output} + \text{Weight Loss}$$

or

$$\text{IWL} = \text{Fluid Intake} - \text{Urine Output} - \text{Weight Gain}$$

It is reasonable to initiate fluid volume on the basis of the sum of an allowance for sensible water loss of 30 to 60 mL/kg/day and the estimated IWL. Fig. 20.4 shows usual IWL ranges by gestational and postnatal age. Factors previously outlined that predictably affect IWL should be considered when fluids are being prescribed. However, prevention of excessive IWL rather than replacement of increased IWL is associated with fewer complications in the preterm neonate and can usually be achieved by modification of the neonate's environment. See Table 20.1 for usual maintenance fluid administration based on birth weight. These numbers are guidelines for initial management only; the approach must subsequently be individualized on the basis of laboratory values and other clinical data.

It is important to remember that the TBW excess and extracellular volume expansion of preterm neonates imply that their negative water and sodium balance during the first 5 to 10 postnatal days (see Fig. 20.2)[24] represents an appropriate adaptation to extrauterine life and should not be compensated for by increased fluid administration and sodium supplementation. If this

TABLE 20.1 Estimated Maintenance Fluid Requirements

Birth Weight (g)	DAILY FLUID REQUIREMENTS (ML/KG)			
	Day 1	Day 2	Days 3–6	Day 7+
<750	100–140	120–160	140–200	140–160
750–1000	100–120	100–140	130–180	140–160
1000–1500	80–100	100–120	120–160	150
>1500	60–80	80–120	120–160	150

principle is not followed, and a positive fluid balance (i.e., weight gain) is achieved during the transitional period, preterm neonates have been shown to be at higher risk of a more severe course of RDS[89] and a higher incidence of PDA,[90] congestive heart failure,[90] pulmonary edema,[89] necrotizing enterocolitis,[27] and BPD.[29,91] However, most of the published studies on outcomes related to fluid balance were performed in the presurfactant era and before the widespread use of prenatal corticosteroids.

Neonates, especially very preterm infants with anticipated fluid problems should be weighed daily or twice daily. Serum sodium levels should be measured every 4 to 6 hours until they have stabilized, usually by 3 to 4 days after birth, and urine output should be recorded and reviewed every 6 to 8 hours. In ELBW infants, electrolyte levels should be checked by 12 hours of age to help guide fluid management. Once data are available, fluids should be increased if weight loss is greater than 1% to 2% per day in term neonates and 2% to 3% per day in preterm neonates if urine output is inappropriately low; if urine specific gravity is rising; and/or if serum sodium concentration is rising. Overall, expected and appropriate weight loss during the first week postnatally is up to 10% in term neonates and up to 15% in preterm neonates. Conversely, fluids should be decreased if weight is not falling appropriately and/or serum sodium concentration is decreasing. The goal is to reach 140 to 160 mL of fluids per kilogram per day by 5 to 10 days to allow adequate caloric intake.

Treatment of Fluid Overload

Fluid overload commonly occurs in sick neonates, often because of the use of fluid bolus administration for hypotension and/or poor perfusion. The diagnosis is based on weight gain, edema, and often hyponatremia. Overhydration can sometimes be prevented by the use of blood transfusions and/or careful vasopressor-inotrope (dopamine, epinephrine) administration instead of colloid or crystalloid, if appropriate, for blood circulatory support. Once overhydration has occurred, management is usually affected by 10% to 20% decrements of total daily fluid intake and with careful monitoring of clinical and laboratory signs to ensure maintenance of adequate intravascular volume as well as normal glucose and electrolyte status while the ECF contraction occurs. While administration of albumin followed by a diuretic (e.g., furosemide) may be used in an attempt to mobilize interstitial fluid, there is little evidence to support this practice, and it may be counterproductive because of the albumin leak into the interstitial space.[39]

Treatment of Dehydration

Dehydration in the neonate may be suspected on the basis of history or clinical signs and confirmed by laboratory studies. One may estimate the total water deficit by using weight changes, calculating total inputs and outputs, and following serial sodium levels. Appropriate treatment requires consideration of sodium status. For additional details of fluid correction and sodium management, see the discussion in Treatment of Hypernatremia.

Sodium Homeostasis and Management

Serum sodium values should generally be kept between 135 and 145 milliequivalent (mEq)/L.[92] As sodium urinary losses are both gestational and postnatal age-specific, recommended intakes vary depending on these two factors and range from 1 mEq/kg/day to 12 mEq/kg/day.[93] Sodium chloride supplementation should be started in preterm and sick term neonates only after completion of the postnatal extracellular volume contraction, usually after the first few days of age or after more than 5% of birth weight has been lost.[94] Appropriate supplementation ensures a positive sodium balance that is necessary for adequate growth.

Hyponatremia

Hyponatremia (serum sodium <130 mEq/L) represents a deficit of sodium in relation to body water content and may be caused by either total body sodium (TBS) deficit or free water excess. In either situation, TBW may be decreased (hyponatremia with volume contraction), normal, or increased (hyponatremia with volume expansion). While hyponatremia has been associated with various morbidities, it may primarily be a marker of severity of illness. Regardless, sodium supplementation in preterm neonates is associated with improved long-term outcomes, thus underscoring the importance of appropriate sodium management.[93,95]

It is important to determine the primary cause and duration of the hyponatremia in order to initiate appropriate therapy. Fig. 20.5 summarizes the clinical evaluation and therapy of neonates with hyponatremia. The most common cause of hyponatremia in the sick neonate is excessive administration and/or retention of free water. In these situations, the total body sodium content is normal, and the appropriate treatment is restriction of free water intake and not administration of sodium. In situations of true sodium deficit, one can estimate the deficits by assuming 70% of total body weight as the distribution space of sodium. The formula for calculating sodium (Na^+) deficit is:

$$Na^+ \text{ Deficit (or Excess) (mEq)} \approx 0.7 \times \text{Body Weight (kg)} \times ([Na^+]_{\text{desired}} - [Na^+]_{\text{current}}).$$

In this formula, (0.7 × BW) is the estimation of TBW.

In most situations of depletional hyponatremia (i.e., true sodium deficit), two-thirds of the replacement sodium should be provided in addition to maintenance sodium needs in the first 24 hours, and the remainder is to be provided over the next 24 hours. Frequent measurements of serum levels of electrolytes are needed to ensure that the correction is occurring appropriately. With severe hyponatremia (serum sodium concentration Na <120 mEq/L), regardless of whether the hyponatremia is due to free water overload or TBS deficit, the correction of the serum sodium concentration up to 120 mEq/L is recommended with administration of 3% saline solution (513 mEq of sodium per liter). This recommendation is due to concern of increased risk of neurologic complications associated with severe hyponatremia, although this relationship has not been explicitly demonstrated in large cohorts of neonatal patients.[96] This correction should be done over 4 to 6 hours, depending on the severity of

hyponatremia,[97] with the use of the preceding formula and with close monitoring of serum sodium changes. Although rapid IV bolus administration of 4 to 6 mL/kg of 3% saline solution has been effective in children with seizures or coma,[98] rapid and complete correction of low serum sodium concentration in adults with chronic hyponatremia have been shown to be associated with pontine and extrapontine myelinolysis.[99] Although this association has not been demonstrated in neonates, the potential risk of injury to the immature central nervous system necessitates caution with rapid correction of long-standing hyponatremia. Once the serum sodium concentration has reached 120 mEq/L, complete correction of hyponatremia should be performed more slowly over the next 48 hours. In patients with asymptomatic hyponatremia whose serum sodium concentration exceeds 120 mEq/L, hypertonic infusions are not indicated. Additional therapy should be directed toward fluid restriction if the hyponatremia is dilutional or sodium repletion if the hyponatremia is depletional. More stable infants with chronic sodium losses can also be corrected with enteral sodium chloride.

Hypernatremia

Hypernatremia (serum sodium concentration >150 mEq/L) reflects a deficiency of water relative to TBS and is most often a disorder of water rather than sodium homeostasis. The presence of hypernatremia does not reflect the TBS content, which can be high, normal, or low depending on the cause of the condition. Hypernatremia can also be associated with hypovolemia, normovolemia, or hypervolemia (Box 20.2). If hypernatremia is primarily due to changes in sodium balance, it can result from pure sodium gain or, more commonly, sodium gain coupled with a lesser degree of water accumulation or, rarely, water loss. It is important to recognize that neonates with hypernatremic dehydration often do not demonstrate overt clinical signs of intravascular depletion and dehydration until late in the course of the condition. The hypernatremia-induced hypertonicity causes water to shift from the intracellular to the extracellular compartment, resulting in intracellular dehydration but with relative preservation of the extracellular compartment. This fluid shift is the main reason that neonates with chronic hypernatremic dehydration often do not demonstrate overt clinical signs of intravascular depletion and dehydration until late in the course of the condition.

Compared with other organs, the CNS has a unique and more effective adaptive capacity to respond to the hypernatremia-induced hypertonicity, leading to a relative preservation of neuronal cell volume. The shrinkage of the brain stimulates the uptake of electrolytes such as sodium, potassium, and chloride (immediate effect). However, these electrolytes, at higher-than-normal intracellular concentrations, have severe adverse effects on intracellular enzyme functions. The synthesis of osmoprotective amino acids and organic solutes (delayed response, starting perhaps around 4 to 6 hours into the process) serves as a defensive mechanism to protect cellular functions. These idiogenic osmols, such as taurine, glycine, glutamine, sorbitol, and inositol aid in attenuating the volume loss in the cells of the central nervous system during longer periods of hyperosmolar stress and limit the accumulation of intracellular sodium and chloride.[100] As long as hypernatremia develops rapidly (within hours), as in accidental sodium loading, a relatively rapid correction of the condition is usually safe. Intracellular fluid accumulation does not occur because the accumulated electrolytes (sodium, potassium, and chloride) are rapidly extruded from the brain cells, and the development of cerebral edema is unlikely. In these cases, reducing serum sodium concentration by 1 mEq/L/h (24 mEq/L/day) is appropriate.[101]

In contrast to rapidly developing hypernatremia, in cases of chronic hypernatremia, the dissipation of idiogenic osmols in response to correction of the hypernatremia occurs slowly over several days.[101] Thus in these chronic cases, or in cases in which the time frame over which hypernatremia developed is unknown, the hypernatremia should be corrected more slowly, at a maximum rate of 0.5 mEq/L/h (12 mEq/L/day). If correction is performed more rapidly, the abrupt fall in the extracellular tonicity results in the movement of water into the brain cells, which have a relatively fixed hypertonicity because of the presence of the osmoprotective molecules. The result is the development of brain edema with potentially deleterious consequences.[101,102] A large population-based study, published in 2007, showed that neonates admitted to the hospital with dehydration (weight loss >12% of birth weight) and hypernatremia (serum sodium concentration ≥150 mEq/L), but without shock, respiratory failure, infarct, or gangrene and in a managed care setting, did not have increased rates of adverse neurodevelopmental outcome at 5 years.[31] The authors noted that these favorable outcomes may not be generalizable to neonates presenting with more severe symptoms.

In the breastfed term neonate, hypernatremia most commonly develops in association with dehydration secondary to inadequate breast milk intake.[102] In addition, it has also been associated with high sodium levels in maternal breast milk, especially from mothers of neonates who are not successfully lactating.[103,104] Reduction in breastfeeding frequency has been associated with a marked rise in the sodium concentration of breast milk.[104] Thus, the initial clinical presentation of neonates with breastfeeding-associated hypernatremia is a bodyweight loss of 10% or more, poor hydration state, lethargy, and poor feeding. Recognition may be delayed because these neonates may appear quiet, content, and without overt clinical signs of dehydration initially as the extracellular water loss is supplemented from the intracellular compartment. During this process, idiogenic osmols are being produced in the cells to follow the rise of osmolality in the extracellular compartment and protect the cells from a rapid

• BOX 20.2 Conditions Causing Hypernatremia

Hypovolemic Hypernatremia

Inadequate breast milk intake
Diarrhea
Radiant warmers
Excessive sweating
Renal dysplasia
Osmotic diuresis

Euvolemic Hypernatremia

Decreased Production of Antidiuretic Hormone

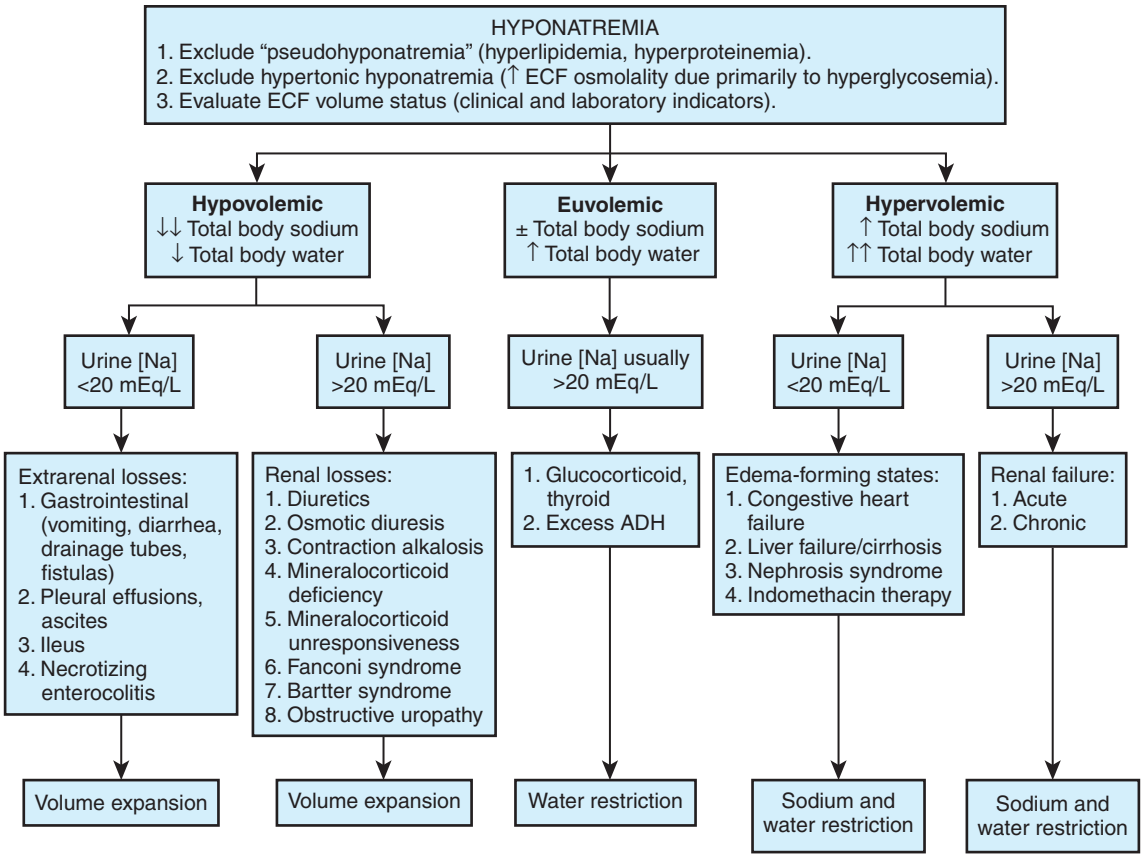
Central diabetes insipidus, head trauma, central nervous system tumors (craniopharyngioma), meningitis, or encephalitis

Decreased or Absence of Renal Responsiveness

Nephrogenic diabetes insipidus, extreme immaturity, renal insult, and medications such as amphotericin, hydantoin, and aminoglycosides

Hypervolemic Hypernatremia

Improperly mixed formula
Sodium bicarbonate administration
Sodium chloride administration
Primary hyperaldosteronism



• **Fig. 20.5** Flow diagram for the clinical evaluation of and therapy for neonates with hyponatremia. *ADH*, Antidiuretic hormone; *ECF*, extracellular fluid. (Modified from Avner ED. Clinical disorders of water metabolism: hyponatremia and hypernatremia. *Pediatr Ann*. 1995;24:23–30.)

and unsustainable water loss. However, once this compensatory capacity is overwhelmed, the full clinical presentation develops with oliguria, lethargy, irritability, and hypotonia. Finally, at the most advanced stages, seizures, and cardiovascular collapse with renal failure present. In general, once the clinical presentation of full-blown shock develops, treatment efforts usually fail as cellular volume contraction has reached an irreversible stage. Even before the irreversible phase, the presentation can be associated with serious CNS morbidity from both the hypertonicity (sagittal or other venous sinus thrombosis) and/or inappropriately rapid rehydration therapy (brain edema).[104] Although thorough follow-up studies of neonates with breastfeeding-associated severe hypernatremia are not available, observational studies suggest that up to 5% of neonates experience brain damage (cerebral hemorrhage, edema, thrombosis, or infarction).[103]

In the extremely immature neonate, early hypernatremia most commonly occurs from excessive transepidermal free water losses. The condition usually develops rapidly, within 24 to 72 hours after birth. The diagnosis is supported by a decrease in body weight, an increase in serum sodium concentration, and clinical signs of extracellular volume contraction. Appropriate adjustments of free water intake, the use of humidified incubators and frequent monitoring of serum electrolyte levels prevents this condition.[21] A recent retrospective single center study of VLBW neonates using multiple linear regression analysis with generalized estimating equations found that a peak serum sodium value over149 mmol/L is associated with poor cognitive outcomes at 24 months of age.[105] Further studies are needed to determine if this association is casual rather than a consequence of disease severity.

The central and nephrogenic forms of diabetes insipidus (DI) are much less common causes of hypernatremia and occur because of the lack of production of and renal responsiveness to vasopressin, respectively. Central diabetes insipidus can be congenital or can be acquired secondary to a neurologic insult.[106] Iatrogenic hypernatremia can also develop in response to excessive sodium supplementation, mainly in the sick neonate receiving repeated volume boluses for cardiovascular support or sodium bicarbonate for metabolic acidosis. In these cases, clinical signs of edema, increased body weight, and the history of volume boluses help to establish the diagnosis.

Treatment of Hypernatremia. Thorough analysis of the medical history and the changes in clinical signs, laboratory findings, and body weight are necessary to determine the major etiologic factor in hypernatremia and thus the appropriate treatment. In the critically ill neonate, the cause of the serum sodium abnormality may be multifactorial, making the treatment strategy less straightforward. Although some cases of hypernatremia are a result of sodium excess with normal or high TBW, most cases in neonates are due to hypernatremic dehydration. Treatment of this condition is generally divided into two phases: the *emergent phase*, where the intravascular volume is restored, usually by administration of a solution with a sodium concentration of 10 to 15 points lower than the that of the patient, and the *rehydration phase*, where the sum of the remaining free water deficit and the usual maintenance needs are administered evenly over at least 48 hours. To resuscitate the patient using a solution with a sodium concentration of 10 to 15 points lower than the that of the patient is especially important when serum sodium levels are greater than 175 mEq/L.[107]

The free water deficit can be calculated as follows:

$$\mathrm{H_2O\ Deficit\,(or\ Excess)\,(L)} \approx 0.7 \times \mathrm{Body\ Weight\,(kg)} \times \left(\frac{[\mathrm{Na^+}]_{\mathrm{current}}\ (\mathrm{mEq/L})}{[\mathrm{Na^+}]_{\mathrm{desired}}\ (\mathrm{mEq/L})} - 1 \right).$$

In this formula, (0.7 × body weight) is the estimation of TBW. When dehydration is diagnosed, correction should generally occur over 24 hours, with half of the correction occurring over the first 8 hours and the remainder over the next 16 hours. It is important to note that longer correction times are indicated when dehydration is accompanied by moderate (serum sodium concentration >160 mEq/L) to severe (serum sodium concentration ≥175 mEq/L) hypernatremia, particularly in cases with a more chronic presentation of hypernatremia.

Alternatively, one can consider the amount of free water required to decrease the serum sodium concentration by a desired amount. The amount of free water required to decrease the serum sodium concentration by 1 mEq/L is 4 mL/kg with moderate hypernatremia but only 3 mL/kg when the serum sodium concentration is as high as 195 mEq/L.[102] Therefore, the amount of free water required to decrease the serum sodium concentration by 12 mEq/L over a 24-hour period when hypernatremia is moderate (serum sodium concentration >160 mEq/L) is calculated as follows:

$$\mathrm{Free\ Water\ Required} = \mathrm{Current\ Weight\,(kg)} \times 4\,\frac{\mathrm{mL}}{\mathrm{kg}} \times 12\,\frac{\mathrm{mEq}}{\mathrm{L}}$$

or

$$\mathrm{Free\ Water\ Required} = \mathrm{Current\ Weight\,(kg)} \times 48\,\frac{\mathrm{mL}}{\mathrm{kg}}\,\mathrm{per\ Day}.$$

When hypernatremia is severe (serum sodium concentration >175 mEq/L), the amount of free water required to decrease the serum sodium concentration by 12 mEq/L over a 24-hour period is calculated as follows:

$$\mathrm{Free\ Water\ Required} = \mathrm{Current\ Weight\,(kg)} \times 36\,\frac{\mathrm{mL}}{\mathrm{kg\ per\ Day}}.$$

The free water contents of the common IV fluids are listed in Table 20.2. The selection of the correct replacement fluid requires consideration of the sodium content of the replacement fluid in relation to the serum sodium concentration. It is important to note that sodium must be delivered with the free water replacement to avoid the hypernatremia being corrected too rapidly. The relative free water content of an IV solution for a specific patient with sodium perturbations can be calculated with the formula:

$$\mathrm{Percentage\ of\ Free\ Water} = 1 - (\mathrm{Intravenous\ Fluid\ Sodium} \,/\, \mathrm{Serum\ Sodium}).$$

Serum electrolyte levels should be monitored every 2 to 4 hours until the desired rate of decline in serum sodium concentration is established. At this point, the frequency of the laboratory measurements can be relaxed to every 4 to 6 hours until the serum sodium concentration is less than 150 mEq/L. The speed of correction of hypernatremia also depends on the rate of its development. This approach provides a reasonable chance that the serum sodium concentration will gradually decrease to the normal range over 2 to 4 days. Except in cases of acute massive sodium overload, the goal should be to lower the serum sodium concentration at a rate of maximum 1 mEq/L/h. A slower pace of correction of 0.5 mEq/L/h is prudent in patients with severe hypernatremia of chronic or unknown duration to minimize the chances of iatrogenic CNS sequelae.

While free water deficits are being corrected, the usual maintenance fluids and electrolytes must also be provided. Of note is that appropriate supplementation of calcium and magnesium is of especially great importance in neonates with hypernatremic dehydration. Ongoing urine losses should be replaced volume for volume every 4 to 6 hours with a solution tailored to the urine's electrolyte concentration (usually 0.225% to 0.45% normal saline). Extraordinary losses caused by open wounds, tubes, drains, ostomies, emesis, and/or diarrhea should always be considered in the dehydrated or hypernatremic infant and also accounted for in fluid management. The composition of this latter replacement solution depends on the electrolyte concentration of the fluid loss. The most common extraordinary loss, gastric fluid, contains significant amounts of sodium and chloride. See Table 20.3 for approximate electrolyte compositions of body fluids. Because

TABLE 20.2 Free Water Content (as Volume Percent) of Common Intravenous Solutions at Normal and High Serum Sodium Concentrations*

	SERUM SODIUM CONCENTRATION			
	145 MEQ/L		195 MEQ/L	
Intravenous Fluid	Isotonic (%)	Water (%)	Isotonic (%)	Water (%)
5% dextrose in water	0	100	0	100
0.2% saline	22	78	17	83
0.45% saline	50	50	39	61
0.9% saline	100	0	79	21
Lactated Ringer's solution	86	14	68	32

*Isotonic saline provides 21% free water when given to a patient with a serum sodium concentration of 195 mEq/L and therefore will induce undesirable decreases in serum sodium concentration when used for volume resuscitation in the severely dehydrated hypernatremic neonate.

Modified from Molteni KH. Initial management of hypernatremic dehydration in the breastfed infant. *Clin Pediatr*. 1994;33:731–740.

TABLE 20.3 Approximate Electrolyte Composition of Body Fluids (mEq/L)

Body Fluid	Sodium	Potassium	Chloride
Gastric	20–80	5–20	100–150
Small intestine	100–140	5–15	90–130
Bile	120–140	5–15	80–120
Ileostomy	45–135	3–15	20–115
Diarrhea	10–90	10–80	10–110

of the association between hyponatremia and neurologic injury in hospitalized pediatric patients,[108] thoughtful consideration of fluid tonicity must be considered when the replacement fluid composition is being determined in the treatment of hypernatremia. Some have advocated routine administration of isotonic ("normal" saline) fluids regardless of sodium requirement to avoid "hospital-acquired hyponatremia" caused by overadministration of free water.[108,109] This approach is not without risk, given the overdose of sodium that may occur with administration of normal saline.[110,111] A reasonable approach may be to base the appropriate fluid prescription on accurately assessed fluid deficits and ongoing requirements, with thoughtful consideration of sodium requirements of each compartment, as well as frequent monitoring of serum sodium changes.[110,111]

Once serum sodium concentration, urine output, and renal function are normal, the patient should receive standard maintenance fluids, either intravenously or orally, depending on his or her condition. Potassium replacement (usually by addition of 20 to 40 mEq of potassium per liter of replacement fluid) should not begin until adequate urine output has been established. At this time, electrolyte status must still be monitored for an additional 24 hours to ensure that complete recovery has occurred. Hyperglycemia and hypocalcemia commonly accompany hypernatremia. The use of insulin to treat the hyperglycemia is not recommended because it can increase brain idiogenic osmol content. Hypocalcemia should be corrected with appropriate calcium supplementation.

Potassium Homeostasis and Management

Serum potassium concentration should be kept between 3.5 and 5 mEq/L. Neonates, especially the immature preterm infant, can experience a relative hyperkalemia in the early postnatal period due to developmentally regulated differences in renal function in general and renal potassium handling in particular, decreased Na^+, K^+-ATPase activity,[112] and hormonal milieu. Exposure to steroids prenatally in premature neonates is associated with a decreased incidence of hyperkalemia, believed to be due to improved renal function.[113]

In general, potassium supplementation should be started only after urine output has been well established, usually by the third postnatal day. Supplementation should be started at 1 to 2 mEq/kg/day and increased over 1 to 2 days to the usual maintenance requirement of 2 to 3 mEq/kg/day. Some preterm neonates may need more potassium supplementation after the completion of their postnatal volume contraction because of their increased plasma aldosterone concentrations, prostaglandin excretion, and disproportionately high urine flow rates. Most neonates will require additional potassium supplementation if they are receiving diuretics.

Hypokalemia. Hypokalemia in the neonate is usually defined as a serum potassium level of less than 3.5 mEq/L. Hypokalemia can occur from (1) inadequate potassium intake; (2) potassium loss due to diuretics, diarrhea, renal dysfunction, or nasogastric drainage; or (3) shift of potassium into the intracellular compartment in the presence of alkalosis. Electrocardiogram (ECG) manifestations of hypokalemia include flattened T waves, prolongation of the QT interval, or the appearance of U waves. Except in patients receiving digoxin, hypokalemia is rarely symptomatic until the serum potassium concentration is less than 2.5 mEq/L. This degree of hypokalemia can result in cardiac arrhythmias, ileus, and lethargy.

Treatment of Hypokalemia. Hypokalemia is treated by slow enteral of parenteral replacement. Rapid administration of IV potassium chloride is not recommended because it may be associated with life-threatening cardiac dysfunction. In extreme emergencies, potassium can be given as an infusion over 30 to 60 minutes of not more than 0.3 mEq of potassium chloride per kilogram. If hypokalemia is secondary to alkalosis, the total body potassium content is usually normal, and the alkalosis should be corrected before considering potassium replacement.

Hyperkalemia. Hyperkalemia in the neonate is defined as a serum potassium level greater than 6 mEq/L in a nonhemolyzed specimen. Hyperkalemia often presents without oliguria in the very preterm infant and occurs in more than 50% of neonates weighing less than 1000 g ("non-oliguric hyperkalemia of prematurity").[114] A common cause of hyperkalemia is renal dysfunction, of particular concern in very preterm neonates, and in neonates whose course is complicated by asphyxia or hypotension. In addition, hyperkalemia secondary to release of potassium from dying cells often complicates IVH, tissue ischemia (i.e., volvulus or necrotizing enterocolitis) and intravascular hemolysis. Less commonly, hyperkalemia may be one of the earliest manifestations of congenital adrenal hyperplasia or may occur because of other causes of neonatal acute adrenal insufficiency. Furthermore, because pH affects the distribution of potassium between the intracellular and the extracellular space, serum potassium levels may rise acutely during acidosis. The clinician should be aware of the potential for life-threatening arrhythmias to occur in infants with chronic lung disease on diuretics and potassium supplements who develop a sudden respiratory deterioration with acidosis.

It is important to understand that most of the body's potassium is contained within cells; therefore serum potassium levels do not accurately reflect total body stores. Even if total body stores are normal or low, serum potassium greater than 6.5 to 7 mEq/L can be life-threatening due to the vital role played by potassium in the electrophysiologic function of the heart. ECG manifestations of hyperkalemia include peaked T waves (the earliest sign), a widened QRS configuration, bradycardia, tachycardia, supraventricular tachycardia, ventricular tachycardia, and ventricular fibrillation.

Treatment of Hyperkalemia. When hyperkalemia is diagnosed, all potassium intake should be discontinued, and the ECG should be monitored. Table 20.4 presents medications used in the management of significant hyperkalemia. Calcium gluconate stabilizes cardiac membranes, and alkali therapy (sodium bicarbonate, tromethamine), insulin/glucose, and inhaled albuterol[115] all rapidly enhance cellular uptake of potassium and can cause a sharp drop in serum potassium levels in life-threatening situations but will not decrease total body potassium content. It is important to recognize that none of these therapies will decrease total body potassium and their effect will be temporary. Intravenously administered furosemide and rectally administered sodium polystyrene sulfonate (Kayexalate) enhance potassium excretion and will lower total body stores but require at least several hours to take effect. Furthermore, use of polystyrene sulfonate to treat hyperkalemia in preterm neonates (<29 weeks' gestational age or <1250 g birth weight) has been associated with intestinal complications, including hematochezia and necrotizing enterocolitis.[116,117] Dialysis or exchange transfusion may be used when the hyperkalemia is life-threatening and other measures do not result in relief.

TABLE 20.4 Medications Used for Treatment of Hyperkalemia

Medication	Dosage	Route	Onset	Duration of Effects	Mechanism of Action	Comments and Cautions
Calcium gluconate	100 mg/kg	Intravenously over 2-5 min	Immediate	30 min	Protects the myocardium from toxic effects of potassium; no effect on total body potassium	• Risk of precipitation when administered with sodium bicarbonate • Administer with caution when using peripheral venous access due to the risk of tissue injury with extravasation
Sodium bicarbonate	1-2 mEq/kg	Intravenously over 2-5 min	Immediate	Variable	By increasing pH, $NaHCO_3$ shifts potassium intracellularly; no effect on total body potassium	• Consider lower dose and infusion rate for very preterm infants in the first postnatal days • Risk of precipitation when administered with calcium gluconate
Tromethamine (THAM)	3-5 mL/kg	Intravenously over 1 hr	Immediate	Variable	By capturing H^+, THAM increases pH without increasing CO_2 and thus shifts potassium intracellularly; no effect on total body potassium	—
Insulin plus dextrose	*Insulin:* 0.1-0.15 U/kg *Dextrose:* 0.5 g/kg	Intravenously over 30 min	15-30 min	2-6 hr	By stimulating the Na^+,K^+-ATPase enzyme, it shifts potassium intracellularly; no effect on total body potassium	Monitor for hypoglycemia
Albuterol	0.4 mg (in 2 mL of NS)	Inhalation, up to every 2 hr	15-30 min	2-3 hr	By stimulating the Na^+,K^+-ATPase enzyme, it shifts potassium intracellularly; no effect on total body potassium	Should not be used as sole agent
Furosemide	1-4 mg/kg per dose Intravenously: 1-2 mg/kg per dose	Orally Intravenously	15 min to 1 hr (sooner with IV admin)	4 hr	Increases renal excretion of potassium	—
Sodium polystyrene	1 g/kg every 6 hr	Rectally or orally	1-2 hr (sooner with rectal admin)	4-6 hr	Removes potassium from gut in exchange for sodium	• Use with extreme caution in neonates, especially preterm neonates • Contains sorbitol • May be associated with bowel necrosis and sodium retention

Clinical Conditions Associated With Fluid and Electrolyte Disturbances

Extreme Prematurity

Infants born between 22 and 27 weeks' gestation, or with a birth weight of less than 1000 g (i.e., ELBW), are at particular risk of acute abnormalities of both fluid and electrolyte status in the immediate postnatal period. TEWL is much higher than in more mature preterm neonates (see Fig. 20.4), and thus it is difficult for water balance to be maintained unless this water loss is prevented. Neonates exposed to prenatal glucocorticoids often have fewer problems because prenatally administered glucocorticoids enhance maturation of the epidermis and cardiovascular and renal development and function, resulting in increases in urine output and fractional excretion of sodium.[87,118]

Table 20.1 shows suggested maintenance fluid requirements by birth weight and postnatal age. Critically ill, extremely immature neonates often receive excess sodium with volume boluses, medications, and the maintenance infusion of their arterial lines. Therefore, extra sodium supplementation should usually not be started during the first few postnatal days, to prevent a rise in TBS concentration and thus extracellular volume, which will hinder the appropriate postnatal diuresis. However, one must be careful as a positive sodium balance is a prerequisite for appropriate growth and, thus, after the transitional period, it must be ensured. Extremely premature neonates are at risk of the development of both oliguric and nonoliguric hyperkalemia, so the serum potassium concentration should be monitored closely, and supplementation should be discontinued if warranted by changes in serum potassium values or in renal function.

Many critically ill preterm neonates retain their originally high extracellular volumes, even when sodium and water intakes are restricted, and such neonates also tend to lose more bicarbonate in the urine. Proximal tubular bicarbonate reabsorption

is gestation-age appropriate even in the VLBW neonate despite the immaturity of its renal function, as long as extracellular volume contraction occurs.[119] Therefore, the presence of the extracellular volume expansion appears to be an important factor in the renal bicarbonate wasting in these neonates. The diagnosis of functional proximal tubular acidosis in such cases should not rely solely on the finding of an alkaline urine pH because the distal tubular function is usually mature enough to acidify the urine once the serum bicarbonate concentration has decreased to its new threshold. Provided that liver function is normal, daily supplementation of bicarbonate, in the form of sodium acetate, potassium acetate, or both, rapidly begins to normalize blood pH and serum bicarbonate concentration in these neonates and also increases urine pH, aiding in the diagnosis. Once extracellular volume contraction occurs, these neonates generally achieve a positive bicarbonate balance,[119] and further supplementation becomes unnecessary.

Other general guidelines in the fluid and electrolyte management of the extremely immature preterm neonate during the first postnatal week are daily calculation of fluid balance and measurements of body weight. The need to measure serum electrolyte levels, plasma glucose levels, urine glucose, osmolality, and specific gravity should be considered based on the severity of illness. The frequency of testing and the addition of other tests, including the measurement of serum albumin concentration and osmolality, depend on the clinical status, the severity of the underlying disease, and the fluid and electrolyte disturbance of the individual patient. Serum creatinine and especially BUN levels are not accurate measures of fluid status in the first postnatal weeks but following their trend can be helpful. There is little to no association between BUN levels and protein intake,[120] even when changes in renal function are taken into account.[121] In the stable infant, the information gained by these studies often do not justify the potential harms inflicted by overstimulation and blood draws, and testing must be tailored for individual patients.

Transient Tachypnea of the Newborn

Transient tachypnea of the newborn (TTN) is a self-limited respiratory complication in term and late preterm neonates caused by delayed clearance of fetal lung fluid during labor and in the immediate postdelivery period. Infants born by cesarian section or to mothers with diabetes are at higher risk for TTN. The maternal diabetes-associated delayed fetal maturation, the lack of labor-induced stress and the absence of the mechanical effects of vaginal delivery explain, at least in part, these findings.[122] These neonates present with mild to moderate respiratory distress frequently requiring respiratory support including supplemental oxygen, high-flow nasal cannula, or continuous positive end-expiratory pressure for a duration ranging from 24 to 96 hours. Fluid management is thought to impact the duration of illness in neonates with TTN. Although multiple studies have been performed evaluating whether fluid restriction is beneficial for neonates with TTN, a definitive answer is lacking.[123] However, maintaining adequate hydration is indicated for neonates with TTN.

Bronchopulmonary Dysplasia

BPD is a multifactorial disease. Many risk factors have been associated with the development of BPD, including a high fluid and salt intake during the first few days to weeks of postnatal life. Specifically, higher fluid intake and lack of appropriate weight loss during the first 10 postnatal days are associated with significantly higher risk of BPD, even after other known risk factors have been controlled for.[29] Furthermore, there is an association between cumulative fluid intake in neonates with an intake of more than 345 mL/kg in the second through fourth postnatal days and an increased incidence of severe BPD.[124] Therefore, careful fluid and electrolyte management during the first few weeks of life, allowing the appropriate degree of weight loss, are of great importance in decreasing the incidence and severity of this condition.

Patent Ductus Arteriosus and Treatment With Indomethacin/Ibuprofen

Fluid, electrolyte, and acid-base management can affect the closure of the ductus arteriosus and the hemodynamic impact of a PDA, as increased fluid administration, increases in extracellular volume, and metabolic acidosis prolong patency of the ductus arteriosus[125] and worsen the hemodynamic impact of left-to-right shunting. Accordingly, clinical management aimed at preventing the occurrence of ductal patency and mitigating its hemodynamic effects involve thoughtful management of fluid and electrolyte balance.[126] For more detail, see Chapter 48.

In the preterm neonate, indomethacin administration has been shown to have clinically significant, although mostly transient, renal side effects because of decreased prostaglandin production through inhibition of cyclooxygenase. In the indomethacin-treated neonate, the unopposed increased catecholamine and angiotensin production associated renal vasoconstriction and sodium and water reabsorption lead to decreases in renal blood flow and glomerular filtration rate and to increases in sodium and free water reabsorption. These side effects occur despite the diminishing left-to-right shunt through the closing ductus. Characteristic clinical findings include a rise in serum creatinine level, oliguria, and hyponatremia.[127] Hyponatremia occurs because the free water retention caused by the unopposed renal actions of high plasma vasopressin levels is out of proportion to the sodium retention induced by angiotensin and norepinephrine. Fluid management of the preterm neonate receiving indomethacin must focus on maintaining an appropriately restricted fluid intake and avoiding extra sodium supplementation until urine output increases and renal function recovers. As the prostaglandin inhibitory effects of indomethacin diminish following the last dose, renal prostaglandin production returns to normal, and the retained sodium and excess free water are usually rapidly excreted, especially with the improvement in cardiovascular status as the ductal shunt decreases.

Ibuprofen is an alternative inhibitor of cyclooxygenase for the treatment of PDA and appears to have equivalent efficacy in closing the symptomatic PDA, with fewer adverse effects.[128] Ibuprofen administration is specifically associated with less renal and gastrointestinal (GI) dysfunction[129] and no apparent effect on cerebral perfusion.[130,131] The lack of an effect on cerebral perfusion likely explains why prophylactic ibuprofen does not reduce the incidence of severe IVH.[128]

Oral or intravenous acetaminophen has also been recently used in the management of PDA in neonates either resistant to one of the COX-inhibitors or as a primary agent.[132] Acetaminophen blocks prostaglandin production by inhibiting the peroxidase segment of the prostaglandin synthetase enzyme. Interestingly, it appears to have less renal side effects than indomethacin or even ibuprofen,[133] but further studies are required for a more in-depth investigation of this topic.

Syndrome of Inappropriate Antidiuretic Hormone Secretion

In the preterm and term newborn, SIADH may be associated with neurologic (birth asphyxia, intracerebral hemorrhage, meningitis) and pulmonary (RDS, pneumothorax, bronchiolitis, and pneumonia) insults.[67] The syndrome is characterized by oliguria, free water retention, decreased serum sodium concentration and serum osmolality, increased urine concentration, and weight gain caused by edema formation. However, because the urinary concentrating capacity of the newborn is limited, a less than maximally diluted urine satisfies the diagnosis of SIADH in the presence of the other symptoms. As a result of their more immature renal function, during the first few weeks of postnatal life, ELBW neonates do not usually exhibit the full-blown syndrome despite their sometimes excessively high plasma vasopressin levels.[134] Appropriate treatment requires fluid and sodium restriction despite the oliguria and hyponatremia, as well as on appropriate circulatory and ventilatory support. The clinician must remember that TBS content is normal, but TBW content is elevated, making treatment of the hyponatremia with large amounts of sodium dangerous.

Diminished vasopressin secretion or complete unresponsiveness of the renal tubules to vasopressin results in polyuria, dilute urine production, and increased serum osmolality,[135] otherwise known as *diabetes insipidus*. This condition is not common in neonates but can occur in association with CNS injury or disease, such as in meningitis, in cerebral hemorrhage affecting the pituitary gland (central diabetes insipidus), or in an inherited form (nephrogenic diabetes insipidus). In addition, diabetes insipidus is one of the features of congenital developmental abnormalities also affecting the pituitary gland, such as septo-optic dysplasia (de Morsier syndrome). The treatment of neonates with this condition consists of facilitating adequate free water intake and the use of desmopressin with or without chlorothiazide.[136]

Surgical Conditions

Surgery has a major effect on metabolism, fluid balance, and electrolyte balance in the newborn. Preterm neonates with acute or chronic lung disease are especially sensitive and respond to the procedure with significant catabolic responses, increases in capillary permeability, with the attendant shift of fluid into the interstitial space, and retention of sodium and free water.[137] The retention of sodium and free water is secondary to the decrease in effective circulating blood volume and to the increased plasma levels of sodium-retaining and water-retaining hormones, including catecholamines, renin–angiotensin–aldosterone, and vasopressin.

Preoperative management has a significant effect on outcome and should aim at maintaining adequate effective circulating blood volume as well as cardiovascular and renal function. In preterm neonates who have evidence of absolute or relative adrenal insufficiency,[138] as well as those who have received prolonged steroid therapy for hypotension or lung disease, the provision of stress doses of steroids may be necessary (see Chapter 84). In the postoperative period, close monitoring and maintenance of the integrity of the cardiovascular system is required. The judicious use of volume expanders and vasopressor–inotropic support, as well as meticulous replacement of ongoing surgical and nonsurgical fluid and electrolyte losses in addition to maintenance needs are essential to ensure a successful outcome. As capillary integrity improves, reabsorption and excretion of the expanded interstitial fluid volume occurs, with normalization in the secretion of hormones regulating fluid and electrolyte balance.

Carefully monitored post-surgical fluid management necessitates consideration of various sources of surgical water losses. These include fluid losses from chest tubes, ostomies, and nasogastric tubes placed under continuous suction to provide relief for the GI tract. Because these losses may be substantial, they should be monitored and a portion of the losses should be replaced every 6 to 12 hours to maintain appropriate water and electrolyte balance. However, free water retention often develops after surgery; therefore, full replacement of these losses is not usually recommended. One of the approaches to the fluid–electrolyte management of postoperative neonates may be to replace half of the removed/lost volume or increase the total fluid limit for a period to account for these loses. The composition of the replacement solution depends on the electrolyte concentration of the fluid loss. Gastric fluid usually contains 50 to 60 mEq of sodium chloride per liter, and therefore 0.45% sodium chloride with potassium is normally used as the fluid of choice for replacement. See Table 20.3 for estimated electrolyte compositions of body fluids.

Acid-Base Balance

Physiology of Acid-Base Balance Regulation

Like adults, newborns must maintain their extracellular pH, or hydrogen ion concentration, within a narrow range. A normal pH is essential for intact functioning of all enzymatic processes and therefore the intact functioning of all organ systems of the body. Newborns are subjected to many stresses that can affect their acid-base balance. In addition, neonates, especially if they are premature, have a limited ability to compensate for acid-base alterations; therefore acid-base disturbances are common in the neonatal period. An understanding of the principles of acid-base regulation is essential for proper diagnosis and treatment of these disturbances.

In healthy humans, the normal range of ECF hydrogen ion (H^+) concentration is 35 to 45 mEq/L. Because pH is defined as the negative logarithm of hydrogen ion concentration ($pH = -\log[H^+]$), these hydrogen ion concentrations correspond to a pH range of 7.35 to 7.45. Acidosis is a downward shift in pH to less than 7.35, and alkalosis is an upward shift in pH to more than 7.45. Alterations in normal pH are resisted by complex physiologic regulatory mechanisms. The main systems that maintain pH are the body's buffer systems, the respiratory system, and the kidneys. Some of these systems respond immediately to sudden alterations in hydrogen ion concentration, whereas others respond more slowly to changes but maintain the overall balance between acid and base production, intake, metabolism, and excretion over the long term.

The physiologic regulatory systems that respond immediately to changes in acid-base balance include the various intracellular and extracellular buffers as well as the lungs. A buffer is a substance that can minimize changes in pH when acid or base is added to the system. The extracellular buffers, which include the bicarbonate–carbonic acid system, phosphates, and plasma proteins, act rapidly to return the extracellular pH toward normal. The intracellular buffers, which include hemoglobin, organic phosphates, and bone apatite, act more slowly and require several hours to reach maximal capacity.

The most important extracellular buffer is the plasma bicarbonate–carbonic acid buffer system, in which the acid component (carbonic acid [H_2CO_3]) is regulated by the lungs, and the base component (bicarbonate [HCO_3^-]) is regulated by the kidneys. The buffer equation is as follows:

$$H^+ + HCO_3^- \leftrightarrow H_2CO_3 + H_2O + (CO_2)_d$$

where $(CO_2)_d$ represents the dissolved carbon dioxide. At equilibrium, the amount of dissolved CO_2 exceeds that of H_2CO_3 by a factor of 800:1; therefore, for practical purposes, dissolved CO_2 and H_2CO_3 can be treated interchangeably. The fact that CO_2 excretion can be controlled by the respiratory system markedly increases the efficiency of this buffer system at physiologic pH. The enzyme carbonic anhydrase allows rapid interconversion of H_2CO_3 to H_2O and CO_2. If the hydrogen ion (H^+) concentration increases for any reason, hydrogen combines with HCO_3^-, driving the buffer reaction toward greater production of H_2CO_3 and CO_2. CO_2 crosses the blood–brain barrier and stimulates CNS chemoreceptors, leading to increased alveolar ventilation and decreased concentration of extracellular CO_2. This respiratory compensation begins within minutes after a pH change and is complete within 12 to 24 hours. A similar compensation occurs in response to a decrease in H^+ concentration, leading to decreased alveolar ventilation and a resultant increase in extracellular CO_2 concentration.

The relationship between the two components of the bicarbonate–carbonic acid buffer system and pH is expressed by the Henderson–Hasselbalch equation:

$$pH = pK_a + \log\left(\frac{[HCO_3^-]}{[H_2CO_3]}\right).$$

Because H_2CO_3 is in equilibrium with the dissolved CO_2 in the plasma, and because the amount of dissolved CO_2 depends on the partial pressure of CO_2, the equation can be modified as follows:

$$pH = pK_a + \log\left(\frac{[HCO_3^-]}{0.03}\right) \times PaCO_2.$$

Both the original equation and the modified equation are clinically difficult to use; therefore the modified Henderson–Hasselbalch equation can be rewritten as the Henderson equation without logarithms for easier clinical use:

$$[H^+] = 24 \times \frac{PaCO_2}{[HCO_3^-]}.$$

This last equation clearly describes the clinically most important aspect of acid-base regulation by the bicarbonate–carbonic acid buffer system, that the change in the ratio of $PaCO_2$ to HCO_3^- concentration, and not in their absolute values, determines the direction of change in H^+ concentration and thus in pH. The status of the plasma bicarbonate–carbonic acid buffer system can be monitored easily by serial blood gas measurements, making understanding of this buffer system important in clinical care.

The physiologic regulation system that responds more slowly to changes in acid-base balance is the renal system. There must be a long-term balance between net acid increase caused by intake and production and net acid decrease caused by excretion and metabolism. Although infant formula and protein-containing IV fluids have small amounts of preformed acid, most of the daily acid load is derived from metabolism. A large amount of the acid produced is in the form of the volatile H_2CO_3, which can be excreted in the lungs. Nonvolatile or fixed acids are also produced and must be excreted through the kidneys. The nonvolatile acids are normally sulfuric acid produced in the metabolism of the amino acids methionine and cysteine and, to a lesser extent, phosphoric acid, lactic acid, hydrochloric acid, and incompletely oxidized organic acids. In addition to the excretion of nonvolatile acids, however, the kidneys have a role in long-term acid-base regulation by controlling renal HCO_3^- excretion.

Two regions of the kidney act to achieve urinary acidification: the proximal tubule and the collecting tubule. The proximal tubule acidifies the urine by two mechanisms. The first mechanism is by the reabsorption of any HCO_3^- already present in the blood that is being constantly filtered through the glomeruli. The proximal tubule reabsorbs 60% to 80% of all filtered HCO_3^- and performs this role through the exchange of Na^+ for H^+ across the luminal membrane of the proximal tubular cells via the Na^+/H^+ exchanger. The excreted H^+ combines with filtered HCO_3^-, producing H_2CO_3 through the activity of carbonic anhydrase in the cellular brush border. The H_2CO_3 is then quickly converted to CO_2, which crosses into the tubular cell, where HCO_3^- is regenerated and reabsorbed back into the bloodstream, mostly in exchange for chloride (Cl^-). The regenerated H^+ ion reenters the cycle at the Na^+/H^+ exchanger.

The second mechanism by which the proximal tubule acidifies urine is by the production of ammonia (NH_3). Inside the tubular cell, NH_3 is produced by the deamination of glutamine. The NH_3 is secreted into the tubular lumen where it combines with and traps free H^+ to form ammonium (NH_4^+).

The remaining urinary acidification occurs mostly in the collecting tubule. H^+ secreted in this region of the kidney is sufficient to combine with or titrate any remaining filtered HCO_3^- or any filtered anions, such as phosphate and sulfate. Hydrogenated phosphate and sulfate anions produce the titratable acid of the urine. The collecting tubule also takes up NH_3 from the medullary interstitium and secretes it into the urine where, again, it can combine with and trap H^+ as NH_4^+. This urinary NH_4^+ can act as a cation and can be excreted with urinary anions such as Cl^-, phosphate (PO_4^-), and sulphate (SO_4^-), thereby preventing loss of cations such as Na^+, Ca^{2+}, and K^+. Total acid secretion in the kidney can be represented by

$$\text{Titratable Acid} + NH_4^+ - HCO_3^-$$

and under normal conditions should equal the net production of acid from the diet and metabolism that is not excreted in the form of CO_2 through the lungs.

In adults, the steady state for renal compensation for respiratory alkalosis is reached within 1 to 2 days and that for respiratory acidosis is reached within 3 to 5 days. Newborns are able to compensate for acidemia through the previously described renal mechanisms, although the renal response to acid loads is limited, especially in premature neonates born before 34 weeks' gestation. Reabsorption of HCO_3^- in the proximal tubule and distal tubular acidification are also decreased, with a fairly rapid gestational age-dependent maturation of these functions after birth.[139]

To accomplish the tight regulation of pH necessary for survival, H^+ ions generated in the form of the volatile acid H_2CO_3 are excreted by the lungs as CO_2. H^+ ions generated in the form of nonvolatile acids are buffered rapidly by extracellular HCO_3^- and more slowly by intracellular buffers. HCO_3^- is then replenished by the kidneys via the reabsorption of much of the filtered HCO_3^- and by the excretion of H^+ in the urine as NH_4^+ and titratable acids.

Disturbances of Acid-Base Balance in the Newborn

General Principles

The evaluation of the acid-base status in a newborn is one of the most common laboratory assessments made in the neonatal intensive care unit (NICU). The status of this system can be monitored with blood gas measurements and should be the starting point for the evaluation of any acid-base disorder. In the blood gas measurement, the pH and $PaCO_2$ levels are directly measured; from these, the HCO_3^- level and base excess or deficit are calculated.

The whole blood buffer base, defined as the sum of the HCO_3^- and non-HCO_3^- buffer systems, is another important blood gas value used in evaluating acid-base disturbances. The difference between the observed whole blood buffer base of any blood gas sample and the expected normal buffer base of that sample is called the base excess or base deficit. The base excess and base deficit give an accurate measure of the amount of strong acid or base respectively that would be needed to titrate the pH back to normal once the respiratory contribution of the acid-base disturbance is also corrected. For example, a base excess of 10 mEq/L indicates that there is an additional 10 mEq of base per liter (or loss of 10 mEq of H^+ per liter) that is contributing to the acid-base abnormality. Conversely, a base deficit of 10 mEq/L indicates there is relatively more acid (or less base) in the ECF than expected after the effect of $PaCO_2$ on pH has been accounted for.

Acid-base disorders are classified according to their cause as being either metabolic or respiratory. Metabolic acidosis occurs as a result of the accumulation of increased amounts of nonvolatile acid or decreased amounts of HCO_3^- in the ECF. Metabolic alkalosis occurs as a result of increased amounts of HCO_3^- in the ECF. Respiratory acidosis is caused by hypoventilation and decreased excretion of volatile acid (CO_2), whereas respiratory alkalosis is caused by hyperventilation and increased excretion of CO_2.

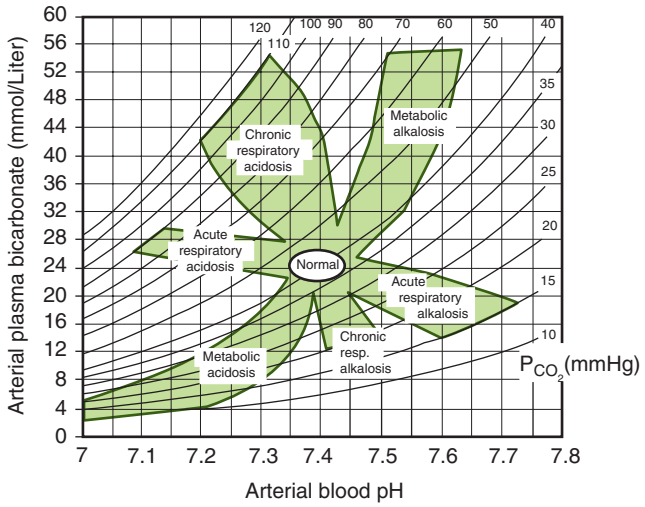
Acid-base disorders are also classified according to the number of conditions causing the disorder. When only one primary acid-base abnormality and its compensatory mechanisms occur, the disorder is classified as a *simple acid-base disorder*. When a combination of simple acid-base disturbances occurs, the patient has a mixed (or complex) acid-base disorder. Because secondary physiologic regulatory mechanisms often compensate for the alteration in pH caused by primary disturbances, it is sometimes difficult to differentiate simple from mixed disorders or even a simple disorder from its resulting compensation. One important principle that allows the determination of primary acid-base disturbance is that the compensatory regulatory mechanisms do not completely normalize the pH.

Nomograms, such as the one shown in Fig. 20.6, can help in the diagnosis of the primary disturbance. The nomogram describes the 95% confidence limits of the expected compensatory response to a primary abnormality in either $PaCO_2$ or HCO_3^- concentration. Table 20.5 summarizes the expected respiratory and metabolic compensatory mechanisms for primary acid-base disorders.[140] If the compensation in a given patient differs from that predicted in Fig. 20.6 or Table 20.5, the patient either has not had enough time to compensate for a simple acid-base disturbance or has a mixed acid-base disorder. Furthermore, the complete correction of an acid-base disturbance occurs only when the underlying process responsible for the abnormality has been treated effectively.

For identification of the primary disturbance, the analysis of blood gas values must be considered in light of the patient's history and physical findings and with an understanding of expected compensatory responses. Further laboratory evaluation is indicated if the problem is not immediately obvious or if the response to therapy is not as expected. The evaluation of the acid-base disturbance should always involve efforts to determine the underlying cause of the disturbance, because adequate treatment requires correction of the underlying disorder, if possible.

Transitional Physiology After Birth

As part of a discussion of normal physiology, it is important to understand the in-utero environment just before delivery of the newborn and its effect on neonatal acid-base analysis shortly after birth. Hyperventilation of pregnancy is a known phenomenon with corresponding maternal $PaCO_2$ levels of approximately 31 to 34 mm Hg.[141] This relative respiratory alkalosis in the mother is compensated for by a corresponding metabolic acidosis in the mother and therefore in the fetus. As a result, umbilical arterial blood gases have a normal pH range of 7.20 to 7.28, with a corresponding base deficit ranging from 2.7 ± 2.8 mEq/L to 8.3 ± 4.0 mEq/L.[142,143] In other words, a mild metabolic acidosis in the newborn shortly after birth can be expected and explained by normal physiology. In particular, factors such as gestational age,[144] mode of delivery, type of anesthesia, the use of phenylephrine instead of ephedrine to maintain normal blood pressure during cesarean delivery, and maneuvers to decrease vena caval compression all can have impact on fetal acid production. The



• **Fig. 20.6** Acid-base nomogram illustrating the 95% confidence limits for compensatory responses to primary acid-base disorder. (From Cogan MG, Rector Jr FC. Acid–base disorders. In: Brenner BM, Rector Jr FC, eds. The Kidney. Philadelphia, PA: WB Saunders; 1986.)

TABLE 20.5 Expected Compensatory Mechanisms Operating in Primary Acid-Base Disorders

Acid-Base Disorder	Primary Event	Compensation	Rate of Compensation
Metabolic Acidosis			
Normal anion gap	Decreased HCO_3^- concentration	Decreased PCO_2	For 1 mEq/L decrease in HCO_3^- concentration, PCO_2 decreases by 1–1.5 mmHg
Increased anion gap	Increased acid production Increased acid intake	Decreased PCO_2	For 1 mEq/L decrease in HCO_3^- concentration, PCO_2 decreases by 1–1.5 mmHg
Metabolic Alkalosis			
	Increased HCO_3^- concentration	Increased PCO_2	For 1 mEq/L increase in HCO_3^- concentration, PCO_2 increases by 0.5–1 mmHg
Respiratory Acidosis			
Acute (<12–24 h)	Increased PCO_2	Increased HCO_3^- concentration	For 10 mmHg increase in PCO_2, HCO_3^- concentration increases by 1 mEq/L
Chronic (3–5 days)	Increased PCO_2	Increased HCO_3^- concentration	For 10 mmHg increase in PCO_2, HCO_3^- concentration increases by 4 mEq/L
Respiratory Alkalosis			
Acute (<12 h)	Decreased PCO_2	Decreased HCO_3^- concentration	For 10 mmHg increase in PCO_2, HCO_3^- concentration increases by 1–3 mEq/L
Chronic (1–2 days)	Decreased PCO_2	Decreased HCO_3^- concentration	For 10 mmHg decrease in PCO_2, HCO_3^- concentration decreases by 2–5 mEq/L

HCO_3^-, Bicarbonate
Modified from Brewer ED. Disorders of acid–base balance. *Pediatr Clin North Am.* 1990;37:429–447.

final common pathway for these factors is in optimizing uteroplacental blood flow.[145] The practice of delayed cord clamping appears to have minimal to no impact on umbilical cord gases in term newborns.[146]

Metabolic Acidosis

Metabolic acidosis is a common problem, particularly in the critically ill newborn. Metabolic acidosis occurs when the drop in pH is caused by the accumulation of acid other than H_2CO_3 in the ECF, resulting in loss of available HCO_3^-, or by the direct loss of HCO_3^- from body fluids. Patients who have metabolic acidosis are divided into those with an elevated anion gap and those with a normal anion gap.

The anion gap reflects the unaccounted acidic anions and certain cations in the ECF. The unmeasured anions normally include the serum proteins, phosphates, sulfates, and organic acids, whereas the unaccounted cations are the serum potassium, calcium, and magnesium ions. Thus, in clinical practice, the anion gap is estimated with the formula:

$$\text{Anion Gap} = [NH_4^+]_{serum} - ([Cl^-]_{serum} + [HCO_3^-]_{serum}).$$

The normal range of the serum anion gap in newborns is 8 to 16 mEq/L, with slightly higher values in very premature newborns. Accumulation of strong acids because of increased intake, increased production, or decreased excretion results in an increased anion gap acidosis, whereas loss of HCO_3^- or accumulation of H^+ results in a normal anion gap acidosis. A decrease in serum potassium, calcium, and magnesium concentrations, an increase in serum protein concentration, or a falsely elevated serum sodium concentration can also result in an increased anion gap in the absence of metabolic acidosis. In clinical practice, although a serum anion gap value greater than 16 mEq/L is highly predictive of the presence of lactic acidosis and a value less than 8 mEq/L is highly predictive of the absence of lactic acidosis, an anion gap value between 8 and 16 mEq/L cannot be used to differentiate between lactic and non-lactic acidosis in the critically ill newborn.[147] Therefore, if the anion gap is within this high normal range and lactic acidosis is suggested, measurement of serum lactate is indicated. At present, with the routine availability of measurement of lactic acid from low-volume blood samples by blood gas machines, the determination of the origin of acidosis ("anion gap," i.e., net strong acid gain, or "non-anion gap," i.e., buffer loss–induced acidosis) has become simpler in most clinical presentations of metabolic acidosis.

An increased anion gap metabolic acidosis in the newborn is most commonly caused by lactic acidosis secondary to tissue hypoxia, as seen in hypoxic-ischemic encephalopathy, hypothermia, hypovolemia, severe respiratory distress, sepsis, necrotizing enterocolitis, and other severe neonatal illnesses. Other important but much less common causes of an increased anion gap metabolic acidosis in the neonatal period are inborn errors of metabolism, renal failure, and intake of toxins (Box 20.3). Box 20.4 lists inborn errors of metabolism that can manifest themselves as increased anion gap metabolic acidosis in the newborn period.

In the syndrome of late metabolic acidosis of prematurity, first described in the 1960s, otherwise healthy premature infants at several weeks of age demonstrated mild to moderate increased anion gap acidosis and decreased growth. All the infants were receiving high-protein cow's milk formula and presented with higher net acid excretion compared with controls. However, this type of late metabolic acidosis is rarely seen today, likely because of the use of special premature infant formulas and changes in regular formulas with decreased casein-to-whey ratios and lower fixed acid loads.

A normal anion gap metabolic acidosis most commonly occurs in the newborn as a result of HCO_3^- loss from the extracellular

• BOX 20.3 Common Causes of Metabolic Acidosis

Increased Anion Gap

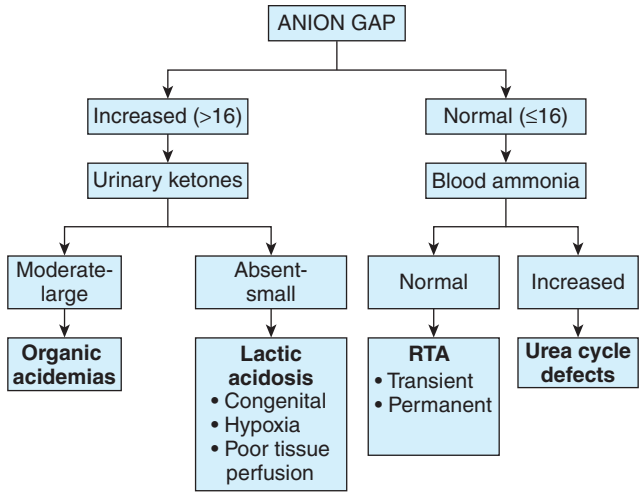
- Lactic acidosis caused by tissue hypoxia
  - Asphyxia, hypothermia, shock
  - Sepsis, respiratory distress syndrome
- Inborn errors of metabolism
  - Congenital lactic acidosis
  - Organic acidosis
- Renal failure
- Late metabolic acidosis
- Toxins (e.g., benzyl alcohol)

Normal Anion Gap

- Renal bicarbonate loss
  - Bicarbonate wasting caused by immaturity
  - Renal tubular acidosis
- Carbonic anhydrase inhibitors
  - Gastrointestinal bicarbonate loss
  - Small bowel drainage: ileostomy, fistula
  - Diarrhea
- Extracellular volume expansion with bicarbonate dilution
- Aldosterone deficiency
- Excessive chloride in intravenous fluids

• BOX 20.4 Inborn Errors of Metabolism Associated With Metabolic Acidosis

- Primary lactic acidosis
- Organic acidemias
- Pyruvate carboxylase deficiency
- Pyruvate hydroxylase deficiency
- Galactosemia
- Hereditary fructose intolerance
- Type I glycogen storage disease



• **Fig. 20.7** Diagnostic approach of increased anion gap and normal anion gap metabolic acidosis in the newborn. Newborns with lactic acidosis may not have an increased anion gap, and lactate concentration should be directly measured if this is suspected from the history and physical examination. *RTA*, Renal tubular acidosis. (Modified from Lorenz JM, Kleinman LI, Markarian K, et al. Serum anion gap in the differential diagnosis of metabolic acidosis in critically ill newborns. *J Pediatr*. 1999;135:751–755.)

space through the kidneys or the GI tract. Hyperchloremia develops with the HCO_3^- loss because a proportionate rise in serum chloride concentration must occur to maintain the ionic balance or to correct the volume depletion in the extracellular compartment. The most common cause of normal anion gap metabolic acidosis in the preterm newborn is a mild, developmentally regulated, proximal renal tubular acidosis with renal HCO_3^- wasting. In newborns with this disorder, the serum HCO_3^- concentration usually stabilizes at 14 to 18 mEq/L in the early postnatal period. The urinary pH is normal once the serum HCO_3^- has stabilized at this level because the impairment in proximal tubular HCO_3^- reabsorption is not associated with an impaired distal tubular acidification of similar magnitude.[139] The diagnosis of this temporary cause of acidosis can be established by the recurrence of a urinary alkaline pH when serum HCO_3^- concentration is raised above the threshold after HCO_3^- or acetate supplementation. Even term newborns have a lower renal threshold for HCO_3^- than adults with normal plasma HCO_3^- levels in the range of 17 to 21 mEq/L. In most infants, plasma HCO_3^- concentration increases to adult levels over the first year as the proximal tubule matures. Other common causes of normal anion gap metabolic acidosis seen in neonatal intensive care units are GI HCO_3^- losses, often caused by increased ileostomy drainage, diuretic treatment with carbonic anhydrase inhibitors, and dilutional acidosis, with rapid expansion of the extracellular space through the use of non-HCO_3^- solutions such as 0.9% sodium chloride in the hypovolemic newborn.

The presence of metabolic acidosis in the newborn may be suspected by the clinical presentation and the history of predisposing conditions, including perinatal depression, respiratory distress, blood or volume loss, sepsis, and congenital heart disease associated with poor systemic perfusion or cyanosis. Metabolic acidosis is confirmed by blood gas measurements. Specific laboratory evaluation of electrolytes, renal function, lactate, and serum and urine amino acids may be undertaken, depending on the diagnosis that is suggested clinically. Fig. 20.7 shows a simple flow diagram outlining an approach to diagnosis of metabolic acidosis in the newborn. It is important to emphasize that newborns might not manifest an increased anion gap in the setting of lactic acidosis.[147] However, the availability of lactic acid measurement along with the blood gas measurements in today's intensive care units aids in the rapid determination of the type of the lactic acidosis.

The morbidity and mortality associated with metabolic acidosis depend on the underlying pathologic process, the severity of the acidosis, and the responsiveness of the process to clinical management. By far, the most important intervention for a newborn with a metabolic acidosis is identification of the pathologic process responsible for the development of acidosis and the taking of measures to correct it. It is important to emphasize that the indiscriminate administration of a base, such as sodium bicarbonate, as supportive therapy for all types of metabolic acidosis is unproven in its efficacy.[148]

The question facing neonatal providers is: how low does the pH need to fall before base administration might be associated with improvement in clinical condition for newborns with metabolic or severe mixed acidosis? An early study suggested that $NaHCO_3$ administration to mechanically ventilated preterm and term neonates receiving neuromuscular blockade with an arterial pH of less than 7.25 *transiently* improved systemic and organ blood flow.[149] However, in a more recent study using more sophisticated

hemodynamic evaluation in hemodynamically stable preterm neonates born at 30 weeks' gestation or earlier, myocardial contractility was found to be unaffected by acidosis even at pH values close to 7.00 during the first 2 weeks postnatally. In the same study, similar to what is seen in adults, worsening acidosis in more than 3-day-old preterm neonates was associated with decreased systemic vascular resistance and increased left ventricular output. This effect was not seen on preterm neonates 3 days old or less.[150] It is clear that more data are needed to adequately address this question, especially because of the concern for harm associated with the administration of base. Indeed, a number of historical studies have shown an association between the use of base and increased mortality and incidence of IVH,[151–153] increased cerebral blood volume regardless of the rate of administration,[154] and decreased intracellular pH with cellular injury.[155] To assess the frequency of prevention or treatment of metabolic acidosis, a survey of 120 Italian NICUs with a 97.5% response rate revealed that 55% of NICUs routinely correct metabolic acidosis with sodium bicarbonate and 37.6% include buffer salts in parenteral nutrition in an attempt to prevent the occurrence of metabolic acidosis.[156]

If a decision has been made to administer base, the clinician has traditionally had three options: sodium bicarbonate, sodium (or potassium) acetate, and tromethamine. $NaHCO_3$ is the most widely used buffer in the treatment of metabolic acidosis in the neonatal period. Bicarbonate should not be given if ventilation is inadequate because its administration results in an increase in $PaCO_2$ with little to no increase in pH and an increase in intracellular acidosis. Therefore, $NaHCO_3$ should be administered slowly and in diluted form only to newborns with documented metabolic acidosis and adequate alveolar ventilation. Once a blood gas measurement has been obtained, the dose of $NaHCO_3$ required to fully correct the pH can be estimated with the use of the following formula:

$$\text{Dose of } NaHCO_3\,(\text{mEq}) = \text{Base Deficit}\left(\frac{\text{mEq}}{\text{L}}\right) \times \text{Body Weight}\,(\text{kg}) \times 0.3.$$

$NaHCO_3$ is confined mostly to the ECF compartment. Although there are controversies regarding the actual bicarbonate space in humans, body weight (kg) × 0.3 (or 30% of the infant's total body weight) represents its estimated volume of distribution in the neonate. Most clinicians would use half of the calculated total correction dose for initial therapy to avoid overcorrection of metabolic acidosis. Subsequent doses of $NaHCO_3$ are then based on the results of additional blood gas measurements.

When clinicians are faced with a chronic non–anion gap metabolic acidosis caused by a prematurity-related proximal renal tubular acidosis with bicarbonate wasting, many choose to replace these losses over time. In this instance, either sodium acetate or potassium acetate can be used as an alternative to $NaHCO_3$. Sodium acetate is a conjugate base of a weak acid (acetic acid) with a pK_b of 9.25; pK_b is a measure of the strength of a base, which depends on its base dissociation constant. Sodium acetate has been shown in one study to be an effective alternative to $NaHCO_3$ in correcting this type of acid-base abnormality when added to parenteral nutrition.[157] The median dosages of acetate used in this randomized controlled trial were 2.6 mmol/kg/day on postnatal day 4 of life and 4.1 mmol/kg/day on postnatal day 8. Neonates randomized to receive acetate had an increased base excess, pH, and $PaCO_2$, and they received less bicarbonate boluses compared with control neonates.

Historically, tromethamine has been used as an alternative buffer to sodium bicarbonate, but in recent times its commercial availability has been limited. In the United States, it appears to have been removed from the market; however, it continues to be available elsewhere.[158] The theoretical advantages of tromethamine over $NaHCO_3$ in the treatment of metabolic acidosis include its more rapid intracellular buffering capability, its ability to lower $Paco_2$ levels directly, and the lack of an increase in the sodium load.[159] Tromethamine lowers $Paco_2$ by covalently binding H^+ and thus shifting the equilibrium of the reaction

$$H^+ + HCO_3^- \leftrightarrow H_2CO_3 \leftrightarrow H_2O + CO_2$$

to the left, resulting in a decrease in the amount of CO_2 and an increase in the amount of HCO_3^-. Tromethamine administration has been associated with the development of acute respiratory depression, most likely secondary to an abrupt decrease in $PaCO_2$ levels, as well as from rapid intracellular correction of acidosis in the cells of the respiratory center.[160] In addition, when large doses of tromethamine are administered, hyponatremia,[161] hypoglycemia, hyperkalemia, an increase in hemoglobin oxygen affinity, and diuresis followed by oliguria can occur. As tromethamine is hyperosmolar, a slow infusion rate is recommended at a dose calculated from the formula:

$$\text{Dose of Tromethamine}\,(\text{mL}) = \text{Base Deficit}\left(\frac{\text{mEq}}{\text{L}}\right) \times \text{Body Weight}\,(\text{kg}).$$

Finally, during the correction of metabolic acidosis, regardless of the method chosen, particular attention should be paid to ensuring an appropriate potassium balance. Because potassium moves from the intracellular to the extracellular space in exchange for H^+ when acidosis occurs, the presence of a total body potassium deficit might not be appreciated during metabolic acidosis. Hypokalemia may become evident only as the pH increases and potassium returns to the intracellular space. Furthermore, intracellular acidosis cannot be completely corrected until the potassium stores are restored. Therefore, close monitoring of serum electrolyte levels and careful potassium supplementation are important during the correction of metabolic acidosis in the sick newborn.

Respiratory Acidosis

Respiratory acidosis occurs when a primary increase in $PaCO_2$ develops secondary to impairments in alveolar ventilation that result in an arterial pH of less than 7.35. Primary respiratory acidosis is a common problem in newborns and causes include hyaline membrane disease, pneumonia owing to infection or aspiration, PDA with pulmonary edema, chronic lung disease, pleural effusion, pneumothorax, and pulmonary hypoplasia. The initial increase in $PaCO_2$ is buffered by the non-HCO_3^- intracellular buffers without noticeable renal compensation for at least 12 to 24 hours (see Table 20.5). Renal metabolic compensation reaches its maximum levels within 3 to 5 days, and its effectiveness in the newborn is influenced mainly by the functional maturity of proximal tubular HCO_3^- transport. Management of respiratory acidosis is directed toward improving alveolar ventilation and treating the underlying disorder. This may include escalation of noninvasive respiratory support, intubation and mechanical ventilation, alternative modes of mechanical ventilation, and/or administration of surfactant.

Metabolic Alkalosis

Metabolic alkalosis is characterized by a primary increase in the extracellular HCO_3^- concentration sufficient to raise the arterial pH above 7.45. In the newborn, metabolic alkalosis occurs when there is a loss of H^+, a gain of HCO_3^-, or a depletion of the extracellular volume with the loss of more chloride than HCO_3^-. It is important to understand that metabolic alkalosis generated by any of these mechanisms can be maintained only when factors limiting the renal excretion of HCO_3^- are also present.

Metabolic alkalosis can result from a loss of H^+ from the body, from either the GI tract or the kidneys, that induces an equivalent rise in the extracellular HCO_3^- concentration. The most common causes of this type of metabolic alkalosis in the newborn period are continuous nasogastric aspiration, persistent vomiting, and diuretic treatment. Less common causes of H^+ losses are congenital chloride-wasting diarrhea, certain forms of congenital adrenal hyperplasia, hyperaldosteronism, post-hypercapnia, and Bartter syndrome.

Metabolic alkalosis can also result from a gain of HCO_3^-, such as occurs during the administration of buffer solutions to the newborn. In the past, a metabolic alkalosis was intentionally created when $NaHCO_3$ or tromethamine was used to maintain an alkaline pH to decrease pulmonary vasoreactivity in infants with persistent pulmonary hypertension, a practice not recommended anymore. Currently, iatrogenic metabolic alkalosis is primarily due to long-term excessive administration of HCO_3^-, lactate, citrate, or acetate in IV fluids and blood products. Because excretion of HCO_3^- is normally not limited in the newborn, metabolic alkalosis resulting from HCO_3^- gain alone should rapidly resolve after administration of HCO_3^- is discontinued. However, if the alkalosis is severe and urine output is limited, inhibition of the enzyme carbonic anhydrase by the administration of acetazolamide may enhance elimination of HCO_3^-.

Metabolic alkalosis can also result from a loss of ECF containing disproportionately more chloride than HCO_3^-, the so-called contraction alkalosis. During the diuretic phase of normal postnatal adaptation, preterm and term newborns retain relatively more HCO_3^- than chloride.[119] The obvious clinical benefits of allowing this physiologic extracellular volume contraction to occur, especially in the critically ill newborn, clearly outweigh the clinical importance of a mild contraction alkalosis that develops after recovery. No specific treatment is needed in such cases, because with the stabilization of the extracellular volume and renal function after recovery, acid-base balance rapidly returns to normal. Contraction alkalosis due to other causes, however, may require treatment.

As mentioned previously, for metabolic alkalosis to persist, factors limiting the renal excretion of HCO_3^- must be present. The kidneys are usually effective in excreting excess HCO_3^-, but this ability can be limited under certain conditions, such as decreased glomerular filtration rate, increased aldosterone production, and the more common clinical situation of volume contraction–triggered metabolic alkalosis with potassium deficiency. In the last condition, there is a direct stimulation of Na^+ reabsorption coupled with H^+ loss in the proximal tubule and an indirect stimulation of H^+ loss in the distal nephron by the increased activity of the RAAS. Contraction alkalosis responds to administration of saline to replace the intravascular volume in conjunction with additional potassium supplementation to account for renal potassium wasting. In the other disorders, however, the primary problem of reduced glomerular filtration rate or elevated aldosterone concentration must be treated for the alkalosis to resolve.

One of the most commonly encountered clinical scenarios of chronic metabolic alkalosis actually occurs in the form of a mixed acid-base disorder in a preterm neonate with chronic lung disease receiving long-term diuretic treatment. Such a newborn initially has a chronic respiratory acidosis that is partially compensated for by renal HCO_3^- retention. Prolonged or aggressive use of diuretics can lead to total body chloride and potassium depletion and contraction of the extracellular volume, thus exacerbating the metabolic alkalosis. By stimulating proximal tubular Na^+ reabsorption and thus H^+ loss, distal tubular H^+ secretion, and renal ammonium production, the diuretic-induced hypokalemia contributes to the severity and maintenance of the metabolic alkalosis. Furthermore, metabolic alkalosis per se worsens hypokalemia, because potassium moves intracellularly to replace hydrogen as the latter shifts into the extracellular space. Although the serum potassium concentration may be decreased, the serum levels in the newborn do not accurately reflect the extent of total body potassium deficit because potassium is primarily an intracellular ion, with approximately 98% of the total body potassium being in the intracellular compartment. In addition, the condition is accompanied by marked hypochloremia and hyponatremia. Hyponatremia occurs in part because sodium shifts into the intracellular space to compensate for the depleted intracellular potassium. If the alkalosis is severe, alkalemia (pH >7.45) can supervene and result in hypoventilation. In this situation, potassium chloride supplementation, and not sodium chloride supplementation, reverses hyponatremia and hypochloremia, corrects hypokalemia and metabolic alkalosis, and increases the effectiveness of diuretic therapy. Because chloride deficiency is the predominant cause of the increased pH, ammonium chloride or arginine chloride also corrects the alkalosis. These agents do not affect the other electrolyte imbalances such as the hypokalemia, so they should not be the only therapy given.

It is important to be proactive and stay ahead of the potassium losses in infants receiving long-term diuretic therapy rather than to attempt to replace potassium after intracellular depletion has occurred. Because the rate of potassium repletion is limited by the rate at which potassium moves intracellularly, correction of total body potassium deficits can require days to weeks. In addition, there is also a risk of acute hyperkalemia if serum potassium levels are driven too high during repletion, particularly in newborns in whom an acute respiratory deterioration may occur. Indeed, with worsened respiratory acidosis, potassium will move from the intracellular to the extracellular space. The routine use of potassium chloride supplementation and close monitoring of serum sodium, chloride, and potassium levels are therefore recommended during long-term diuretic therapy to prevent these common iatrogenic problems.

Respiratory Alkalosis

When a primary decrease in $PaCO_2$ results in an increase in the arterial pH beyond 7.45, respiratory alkalosis develops. The initial hypocapnia is acutely titrated by the intracellular buffers, and metabolic compensation by the kidneys returns the pH toward normal within 1 to 2 days (see Table 20.5). Respiratory alkalosis is the only simple acid-base disorder in which, at least in adults, the pH can be completely normalized by the compensatory mechanisms.[140] The cause of respiratory alkalosis is hyperventilation, which in the spontaneously breathing newborn is most often

caused by fever, sepsis, retained fetal lung fluid, mild aspiration pneumonia, CNS disorders, or urea cycle defects.

In the NICU, the most common cause of respiratory alkalosis is iatrogenic secondary to hyperventilation of the intubated newborn. Because findings suggest an association between hypocapnia and the development of periventricular leukomalacia (PVL)[162,163] and chronic lung disease[164] in ventilated preterm neonates, avoidance of hyperventilation during resuscitation and mechanical ventilation is of utmost importance in the management of sick preterm newborns. It appears that later onset of hypocarbia is more deleterious in terms of the development of PVL (at 26 hours of age vs. 15 hours of age), with 88% of these patients having poor neurodevelopmental outcome at a median age of 46 months.[165] Managing preterm neonates with permissive hypercapnia has made the incidence of hypocapnia and respiratory alkalosis much less common than it was in previous decades. However, it is of note that *hypercapnia in the transitional period* is associated with increased incidence of periventricular hemorrhage/IVH in very preterm neonates.[166,167] In one study of ventilated preterm neonates, the incidence of hypocapnia was 4%, and hypocapnia was more common in the first 3 days of age.[168] The treatment of neonatal respiratory alkalosis consists in the specific management of the underlying process causing hyperventilation.

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21

Neonatal Pharmacology

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KEY POINTS

- The key feature of neonatal physiology is rapid maturation, resulting in extensive variability in pharmacokinetics and pharmacodynamics, further aggravated by other covariates, like pharmacogenetics or unique disease characteristics. Variability is the essence of neonatal care and neonatal pharmacology.
- Thorough understanding of these factors, especially developmental changes, that affect pharmacokinetics (absorption, distribution, metabolism, elimination) in neonates helps to provide accurate dose adjustments to assure effective drug therapy.
- Adverse drug reactions are common and important aspects of neonatal drug therapy. Pharmacovigilance is based on prevention, detection, and assessment, but all these issues need to be adapted to the specific characteristics of this population. Minimization or prevention of such events can usually be achieved.
- To stimulate neonatal drug development, product development should be based on neonatal physiology and pharmacology, while still incorporating already available knowledge from non-neonatal settings to facilitate neonatal drug development. Furthermore, the central role of nurses and families in research and the value of the entire neonatal team in the design, implementation, and interpretation of clinical therapeutic studies is crucial.

Dynamic changes related to growth and maturation in newborns create unique complexities in drug therapy that affect the variability in pharmacokinetics (PK) (absorption, distribution, metabolism, elimination) and pharmacodynamics (PD) (both desired efficacy and unwanted side effects). Pharmacologic studies during this period of rapid growth and physiologic maturation reveal diverse patterns of changes. These patterns also reflect the maturation of different drug-metabolizing enzymes or pathways of elimination throughout infancy. Therapeutic drug monitoring (TDM) is applied to only a limited number of drugs in neonatal care (e.g., phenobarbital, gentamicin), because the serum or plasma concentrations need to correlate with both effects and possible side effects. Dose adjustments to reach the desired concentrations can be estimated at the bedside with use of simple calculations, although we expect that such observations will be incorporated in model informed precision dosing (MIPD) concepts, tailored to neonates.[1] Pharmacogenetics (PGx) or pharmacogenomics of drug-metabolizing enzymes, transporters, or receptors explain a large part of the observed variations among individuals in their responses to drugs. Developmental expression of enzymes, receptors, and organ function add additional levels of complexity in drug disposition and effects in the immature, preterm, or critically ill newborn.

Principles of Neonatal Therapeutics

In neonates, thorough understanding of factors ("covariates") that influence drug concentrations enhance accurate and effective drug therapy and may help to identify causes of treatment failure or therapy-related toxicity. Many relevant covariates are not incorporated in a therapeutic plan, although the impact on effectiveness and safety of a given drug added to current drug therapy may be tremendous. Since PK (concentration–time profiles) and PD (concentration–effect profiles) in newborns follow the same general principles that govern drug actions in patients of all ages, the diagnosis, drug selection, and route of administration needed to achieve a therapeutic goal must consider the impact of absorption, distribution, metabolism, and elimination (ADME) on the dose–exposure relationship. In newborns these principles should include special consideration of the unique physiologic and pharmacologic features of these developmentally immature patients (Box 21.1).

Diagnosis

Effective treatment begins with an accurate diagnosis and assessment of symptoms. Although this applies to all areas of therapeutics, treatment in newborns presents specific challenges. Their small size and fragility may preclude useful, but inordinately invasive, diagnostic procedures. For example, newborns with chronic lung disease are treated for "bronchospasm" on the basis of the findings of decreased air entry, desaturation, and abnormal breath sounds. Relief of these symptoms with aerosolized bronchodilators may be interpreted as confirming this diagnosis. Although this may be correct, increased humidity or movement of the endotracheal tube bevel away from a pliable tracheal wall during aerosol treatment may also result in improvement. A similar argument can be made for suspected neonatal infection, the clinical diagnosis of a patent ductus arteriosus, neonatal pain management, drug-related renal or hepatic toxicity, or the claimed link between gastroesophageal reflux and apnea. Therefore, evaluation of an ineffective therapy should include reconsideration of the diagnosis, in the same way as conclusions about why a therapy succeeded should be made cautiously.

Absorption

Absorption is the movement of a drug into the systemic circulation. It generally requires the crossing of membranes or membrane barriers and is characterized by rate (time to peak) and

• BOX 21.1 Pharmacologic Principles and Pitfalls in the Management of Very Low Birth Weight Infants

Diagnosis
Limited diagnostic procedures

Absorption
Intravenous
- Drug injection away from patient
- Uneven mixing of drugs and intravenous fluids
- Delayed administration due to very low flow
- Part of the dose discarded with tubing changes

Intramuscular
- Poor perfusion limits absorption
- Danger of sclerosis or abscess formation
- Depot effect

Oral
- Poorly studied
- Affected by delayed gastric emptying
- Potentially affected by reflux
- Passive venous congestion may occur with chronic lung disease, decreasing absorption

Distribution (Affected by)
Higher (85%) total body water (vs. 65% in adults)
Lower body fat, i.e., about 1% body weight (vs. 15% in term newborns)
Low protein concentration
Decreased protein affinity for drugs

Metabolism
a. Half-life prolonged and unpredictable
b. Total body clearance decreased
c. Affected by nutrition, illness, and drug interaction
d. Affected by maturational changes

Elimination
Decreased renal function, both glomerular filtration rate and tubular secretion/absorption

extent (percentage of dose). The most commonly used route in neonates is *intravenous administration*. However, even this route of administration has challenges related to neonatal pathophysiology and limitations of intravenous infusion systems (e.g., low flow, small volume, dead space volume, limited flush volume).[2–4] Consequently, drugs should be infused into the patient as close as possible to the site of the venous access. If a drug is injected farther away from the infant and at a low rate, the drug may reach the patient far too slowly or incompletely to achieve effective concentrations. Infusion solution filters may further hamper drug delivery by blocking large molecules, by adsorption of the drug to the filter, or by allowing a heavier drug to settle in the filtration chamber and mix slowly.[2–4] For drugs in which the peak concentration matters (e.g., aminoglycosides) or when the driving force for tissue penetration is a concentration gradient between circulation and tissue (e.g., meningitis), these limitations may result in suboptimal therapy.

Intramuscular administration of drugs is used for slow release (e.g., vitamin A or K, palivizumab) but is a poor substitute for intravenous access and should be avoided for multiple doses. Absorption following intramuscular injection relates to muscle blood flow and depends on maturation and disease characteristics (e.g., hypothermia, shock). Furthermore, intramuscular administration may result in tissue sclerosis, causing sterile abscesses, or create large intramuscular collections, which are subsequently absorbed slowly, producing a "depot effect" in which serum concentrations evolve slowly (both rise and fall) over time.

Oral administration of drugs is preferred for treatment of chronic illnesses in newborns, but this route is not very well studied in acutely ill preterm neonates. Maturation affects gastric and intestinal pH and motility, pancreatic activity and bile acid secretion, or pre-systemic drug metabolism and transport (first pass) in the intestinal wall. The gastric fluid composition (bile salts, osmolarity, pH) displays age-dependent changes, further affected by type and frequency of feeding.[5] Many newborns experience gastroesophageal reflux and delayed gastric emptying. This prolongs and delays absorption, which reduces the peak concentration and may also reduce the total dose absorbed. Passive intestinal venous congestion caused by elevated right atrial pressure decreases drug absorption in adults and may do so in premature infants with severe bronchopulmonary dysplasia.[6] Co-administration of medications to newborns with small volumes of milk or during continuous gastric or duodenal feedings may also alter absorption. Volumes can affect drug solubility in the intestinal lumen while milk can affect bioavailability (e.g., iron or quinolone absorption when combined with milk).

Buccal, lingual, or rectal administrations are additional enteral routes that are all associated with variability. For clinicians, this means that if enteral drug therapy fails, the impact of the route or feeding patterns on drug absorption must be considered. Finally, unanticipated absorption of drugs or excipients intended for topical effects (e.g., cutaneous, inhalation, perineural) should be considered, as these can also be associated with relevant side effects.[7,8]

Distribution

Distribution is the partitioning of drugs among various body fluids, organs, and tissues. The distribution of a drug within the body is determined by several factors, including organ blood flow, pH and composition of body fluids and tissues, physical and chemical properties of the drug (e.g., lipid solubility, molecular weight, and ionization constant), and drug transporter activity but also by the extent of drug binding to plasma proteins (albumin, α_1-acid glycoprotein) and other macromolecules.[7,9]

Physiologic differences between fetuses, preterm neonates, children, and adults affect drug distribution. Total body water content ranges from 85% in premature newborns to 75% in term newborns to 65% in adults.[10–13] Conversely, body fat content ranges from 0.7% or less in extremely premature newborns to 12% in term newborns. These differences affect distribution of water-soluble (e.g., aminoglycosides, acetaminophen), lipophilic (e.g., propofol) or nonpolar drugs (e.g., fentanyl). The protein-binding capacity of drugs in the circulation is lower in early infancy because of lower circulating plasma protein concentrations (e.g., albumin, α_1-acid glycoprotein) and less binding affinity. Competitive binding with bilirubin is another specific issue to be considered in neonates.[7] With rare exceptions, only the free (unbound) drug crosses membranes, exerts pharmacologic actions, and undergoes metabolism and elimination. However, measurements of drug concentrations usually reflect total circulating drug concentrations, which consist of both free and protein-bound drug concentrations. Thus even when total circulating drug concentrations in

the newborn may be low by adult standards, the free drug concentrations may still be equivalent or even higher than those in the adult because of decreased protein binding. Disease (sepsis) or therapeutic interventions (extracorporeal circulation) can further affect distribution.

Metabolism

Drug-metabolizing enzymes are crucial in the extent of drug biotransformation. Although the liver is considered the major organ responsible for drug biotransformation, other organs such as the intestines, lungs, and kidneys also contribute to drug metabolism. These metabolizing enzymes can be classified as participating in non-synthetic phase I reactions (e.g., oxidation, reduction, hydrolysis) or synthetic phase II reactions (e.g., glucuronidation, sulfation, acetylation). Their metabolites can subsequently be eliminated by renal, biliary, or other elimination routes and may also be therapeutically active or contribute to adverse effects.

The primary type of enzymes involved in phase I reactions are cytochromes P450 (CYPs). CYPs mature at different rates and in different patterns with the highest impact in early infancy. In addition, single nucleotide polymorphisms (SNPs) or substitutions in the DNA sequence for a CYP may reduce its metabolizing activity or completely eliminate it if the polypeptide cannot be formed (cf. PGx). Conversely, some individuals inherit multiple copies of a CYP, producing "supermetabolizers" (CYP2D6). The phase II conjugation enzymes, such as the uridine 5-diphosphoglucuronosyltransferases (UGTs) have several forms with different substrate specificity (e.g., UGT1A1 for bilirubin, UGT2B7 for morphine) although they are not as absolutely selective as the CYPs.

All enzymes display isoenzyme specific maturation, and this affects drug metabolism throughout infancy. Consequently, the clearance of almost all drugs is decreased in neonates compared with older children and adults, but important variations in rates of maturation occur among drug classes and among individuals, which prevents simple generalization. Three patterns can be identified with (a) most CYPs being low at birth and increasing to adult levels in the first months or years (e.g., CYP2D6, UGT1A1), (b) high at birth and decreasing thereafter (e.g., CYP3A7), and (c) stable expression (e.g., plasma esterase, sulfation).[14] Although this classification is very helpful to explain and even predict maturational drug disposition, it should be used cautiously. We cannot simply miniaturize "major" and "minor" routes of elimination as documented in adults to (pre)term neonates. In the absence of an adult major route, a minor route, either metabolic or primary elimination, may be a more relevant route of clearance in neonates (e.g., caffeine elimination is renal in neonates and metabolic [CYP1A2] in adults). A similar argument can be made for acetaminophen. Glucuronide conjugation is usually low at birth. In contrast, conjugation through sulfation is usually active at birth. These different patterns are reflected in the developmental changes in acetaminophen metabolism in early life.[15]

Besides age-driven maturation, other factors such as nutrition, disease characteristics, PGx, or drug–drug interactions further affect the phenotypic activity of enzymes and organs responsible for drug metabolism in the newborn. Maturational changes in hepatic blood flow, drug transport into hepatocytes, synthesis of serum proteins, protein binding of drugs, and biliary secretion further confound accurate predictions about drug metabolism after birth.

Elimination

Drug elimination can occur through several mechanisms. Elimination occasionally occurs by the hepatobiliary route, transcutaneously, or by the lungs, but is most commonly by the kidneys. Renal elimination is the most important pathway for unchanged drugs or metabolites, either by glomerular filtration or by renal tubular transport (reabsorption, secretion).

Glomerular function increases steadily after birth, whereas tubular function matures more slowly, causing a glomerular-to-tubular imbalance.[16,17] Based on pooling of GFR estimates (polyfructose, Cr-EDTA, mannitol or iohexol), a quantitative description using weight and postmenstrual age (PMA) showed that half of the adult value is reached at 48 weeks PMA.[18] In a very recent analysis on differences between preterm and term neonates, Salem et al. documented that both postnatal and gestational age (GA) are relevant for GFR until 1.25 years.[19] This postnatal increase in glomerular function relates to higher cardiac output, reduced renal vascular resistance, redistribution of intrarenal blood flow, and changes in intrinsic glomerular basement membrane permeability. These age-dependent dynamics of neonatal renal function markedly influence drug elimination. Similar to drug metabolism, however, the variability in renal elimination capacity and drug clearance is further affected by other covariates, such as hypoxemia, nephrotoxic drugs, hypoperfusion, hypothermia, and intercurrent renal diseases.[20]

The maturation of biliary elimination capacity appears to be fast, attaining adult activity within the first months of life.[20,21] Drugs that are conjugated within the liver may also be eliminated, excreted through bile only to enter the intestinal tract, where they may be deconjugated and undergo enterohepatic recirculation, similarly to bilirubin. Although biliary elimination is not well studied in newborns, clinical conditions such as parenteral nutrition-associated cholestasis suggest that it may be quite variable among specific patients and conditions.

Transporters play important roles in the uptake or removal of drugs. Compared with enzymes, the ontogeny is still not well documented, although patterns have been described and incorporated in physiologically based PK models. A recent review suggests that different developmental patterns for individual transporters are emerging.[9] Organic anion transporter polypeptides provide facilitated transport of anions in many tissues, including the kidney and liver. P-glycoprotein (PGP), the permeability glycoprotein, is an efflux transporter that belongs to the adenosine triphosphate-binding cassette/multiple drug resistance family of transporters and prevents absorption of many compounds across the intestinal wall or into the brain. PGP expression has been described as limited at birth, reaching adult levels at 3 to 6 months of age.[22] To put these findings into perspective, the limited PGP efflux activity increases opioid concentrations in the central nervous system of newborns and likely explains the higher incidence of apnea in neonates following opioid exposure.

Pharmacogenetics and Pharmacogenomics

The Human Genome Project has described the structures of many proteins (enzymes, transporters, receptors, postreceptor signaling pathways) that have a role in the PK and PD of many drugs currently used in neonatal, pediatric, and adult pharmacotherapy. Genetic variants that alter activity have been identified, many based on SNPs in these proteins. As mentioned earlier, genetic

variation in drug-metabolizing enzymes can have a significant influence on the relative activity within a particular individual. Large interindividual variation occurs for several isoenzymes and is often explained by inherited differences in activity. Changes in a single nucleotide in the DNA for one of these enzymes is designated with an asterisk, such as CYP2C9*1, and these changes can alter the protein structure enough to decrease, completely inactivate, or increase its enzymatic activity. SNPs have been identified for many CYPs, and ethnic variations in these SNPs help predict when their activity is likely to be reduced or increased. A similar case can be built for drug transporters and/or receptors.

Knowledge of pharmacogenetic and pharmacogenomic factors that affect PK (metabolism, transport) or PD (receptor) has been important for understanding ways to avoid unanticipated drug effects in adults and to some extent in children. The aim to individualize pharmacotherapy with the use of PGx reflects the fact that specific effects/side effects are not just randomly distributed but relate to genetic variation in the level of activity of transporters, drug-metabolizing enzymes, and/or receptors.[23] Tailoring neonatal pharmacotherapy to individual patients with use of this knowledge holds great promise and serves as a model of precision medicine in newborns.[23,24]

The most commonly applied approach to evaluate PGx in neonates is to search for similar signals initially reported in adults or children (*from adult to newborn* approach). For drug-metabolizing enzymes such as CYP2C19 (pantoprazole dealkylation), CYP2D6 (tramadol O-demethylation, codeine), *N*-acetyl transferase 2 (isoniazid acetylation), or UGT2B7 (morphine glucuronidation), the impact of polymorphisms on neonatal drug metabolism has been documented.[23,25] However, drug-metabolizing enzymes also mature during the first months or years of life. Consequently, it can be difficult to determine to what extent the variability in drug metabolism is explained by genetic expression, maturation, or other covariates. Drug metabolism is often reduced in neonates, and scaling of drug dose by simple body weight or allometrically with an exponential function will not fully compensate for differences in clearance that exist in this newborn population. Clearance often differs several-fold among adults, and the same degree of variation is emerging among neonates, whether due to maturation of the expression of these enzymes by GA or due to induction of protein synthesis after birth.

In addition to this approach, PGx should also be tailored to neonates and not only mirror observations initially described in adults. Pharmacogenetic studies may also provide information on the ontogeny of processes that have not yet been well described, such as transporter or receptor ontogeny (pharmacogenetic concordance). A recent illustration of such an exploration is the impact of polymorphisms on neonatal abstinence syndrome following maternal opioid intake. Specific catechol-*O*-methyltransferase (*COMT*, 158 adenine [A] > guanine [G]) and μ-opioid receptor (*OPRM1*, 118 A > G) polymorphisms influenced the extent and the duration of neonatal abstinence syndrome.[26] Pharmacogenetic studies may focus on the concordance between genotype and phenotype within cohorts of newborns and young infants. A structured approach to assess the contribution of genetic variation in addition to maturation to pharmacokinetic/pharmacodynamic variability has been suggested and is based on five questions:[23]

1. What gene products (if known) are relevant for the disposition of a given compound?
2. What is the developmental trajectory (if known) of functional (e.g., transporter, enzyme, or receptor) activity?
3. Does allelic variation affect the function(s) of a given compound?
4. Does allelic variation affect the developmental drug disposition phenotype?
5. What is the developmental context of the relevant genes?

Pharmacokinetic Principles

Pharmacokinetics describes the time course of changes in drug concentrations within the body. Although rates of change are often described with differential equations, concepts useful at the bedside are emphasized here.[27]

Compartment

In PK, *compartment* refers to fluid and tissue spaces into which drugs penetrate. These compartments may or may not be equivalent to anatomic or physiologic volumes. In the simplest case the compartment may correspond to the vascular space and equal the volume of a real body fluid, blood. Large or quite polar molecules may be confined to this central compartment until they are eliminated by elimination or metabolism. Many drugs, however, diffuse reversibly out of the central compartment into tissues or other fluid spaces, referred to generically as *peripheral or tissue compartments*. Diffusion can be driven, in part, by differences in concentration and protein binding between compartments or can be affected by active transport (influx/efflux) processes. These kinds of compartments are seldom sampled directly, but their involvement in kinetic processes may be recognized from the graphical or mathematical description of the kinetics of a drug. The number of exponential terms necessary to adequately describe the kinetic profile of a drug designates the number of compartments involved, recognizing that many more compartments may exist. For clinical application, rarely more than three compartments are required to describe the PK of any drug.[27–29]

The apparent volume of distribution might be better termed the *volume of dilution* because it is a mathematical description of the volume (L or L/kg) that dilutes a dose (mg or mg/kg) to produce the observed circulating drug concentration (mg/L or μg/mL). To simplify cancellation of units, concentrations are expressed here as milligrams per liter (mg/L), which is the same as micrograms per milliliter (μg/mL), the more conventional unit for drug concentrations in the US:

$$\text{concentration (mg/L)} = \frac{\text{Dose (mg/kg)}}{\text{Apparent Volume of Distribution (L/kg)}}$$

For many drugs the volume of distribution does not correspond to a specific physiologic body fluid or tissue, hence the term *apparent*. In fact, the volume of distribution for drugs that are bound extensively in tissues may exceed 1.0 L/kg, a physiologic impossibility that emphasizes the arithmetic, nonphysiologic nature of the apparent volume of distribution. The determination of the volume of distribution is described later.

First-Order Kinetics

Removal of most drugs from the body can be described by first-order (exponential or proportional) kinetics, in which a constant

proportion or percentage of a drug is removed per unit of time (e.g., 50% in one half-life interval), rather than a constant amount per unit of time (zero-order kinetics, see later in this chapter). Consequently, for drugs exhibiting first-order kinetics, the higher the concentration, the greater the amount removed during a time interval. The following equations describe the concentration (C) of a drug whose first-order kinetics has a rate constant, k (/hour), at time t and an initial concentration of C_0 achieved after administration of a dose.

In differential equation form, the change in concentration with time is:

$$\frac{dC}{dt} = -KC$$

The solution to this differential equation gives the exponential form, describing C at time t:

$$C_t = C_0 e^{-kt}$$

If this equation is transformed with use of the natural logarithm (ln), it becomes:

$$\ln C_t = \ln C_0 - kt$$

The last equation is the equation of a straight line, so a graph that plots ln C_t versus t has an intercept of ln C_0, the concentration at $t = 0$, and a slope of $-k$, the rate constant for the change in concentration. This can be used to calculate the half-life (see next section) and to estimate appropriate dosages. Multiple rate constants in more complex equations are distinguished with the letter k and numbered subscripts or with Greek letters.

Half-Life

The elimination half-life ($t_{1/2}$) of a drug is the time required for its concentration to decrease by 50%. Consequently, half-life is linked to a first-order kinetic process because the same proportion, 50%, of the drug is removed during equal periods. Half-life can be determined mathematically from the elimination rate constant, k, as:

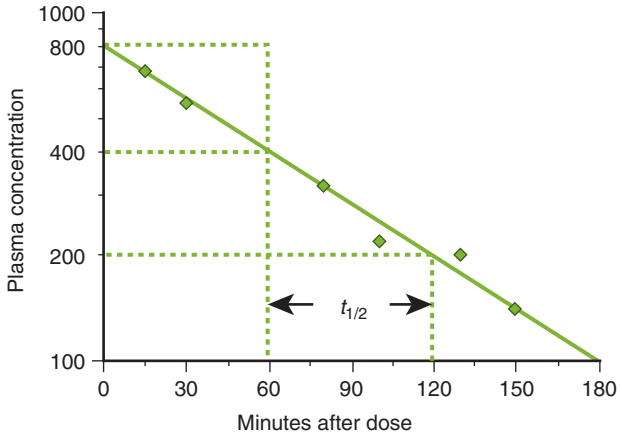
$$t_{1/2} = \frac{\text{Natural Logarithm 2}}{k} = \frac{0.693}{k}$$

Fig. 21.1 illustrates a graphical method for determination of half-life. Drug concentrations measured serially are graphed on semi-logarithmic axes, and the best-fit line is determined either visually or by linear regression analysis. In this illustration of first-order kinetics, the concentration decreases by 50% (from 800 to 400 mg/L) during the first hour and decreases by another 50% (from 400 to 200 mg/L) during the second hour. Thus the half-life is 1 hour. More drug is removed during one half-life at higher concentrations, although the proportion removed remains constant. The exponential equation for this graph is:

$$C = 800e^{-0.693t}$$

where $k = 0.693$/hour and $C_0 = 800$, allowing a mathematical calculation of half-life with use of the equation previously described:

$$t_{1/2} = \frac{0.693}{k\,(/\text{hour})} = \frac{0.693}{0.693/\text{hour}} = 1\,\text{hour}.$$



• **Fig. 21.1** Apparent single-compartment first-order plasma drug disappearance curve illustrating graphic determination of half-life from the best-fit line of serial plasma concentrations.

Multicompartment First-Order Kinetics

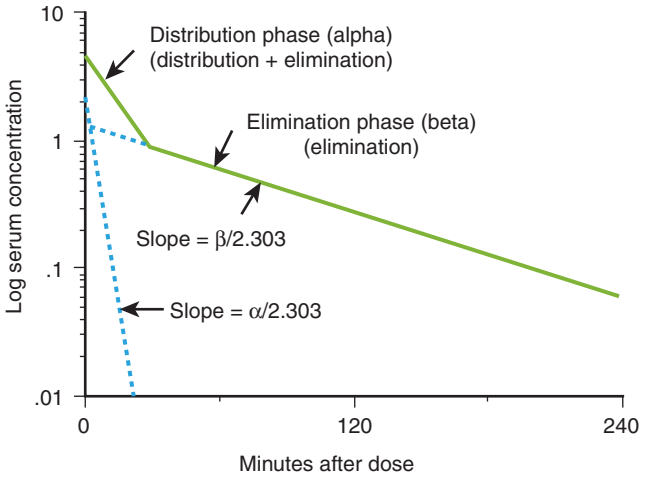
The rate of removal of many drugs from the circulation is often biphasic. An initial rapid decrease in concentration is referred to as the distribution (α) phase, often lasting 15 to 45 minutes, which is followed by a sustained slower rate of removal, the elimination (β) phase. Such biphasic processes are best visualized from semi-logarithmic graphs of drug concentration versus time. When such semi-logarithmic graphs show kinetics that best fit two straight lines, the kinetics are described as *biexponential*, or reflective of a drug that shows *two-compartment first-order pharmacokinetics* (Fig. 21.2). Two exponential terms are needed to describe the change in concentration over time, as:

$$C = Ae^{-\alpha t} + Be^{\beta t}$$

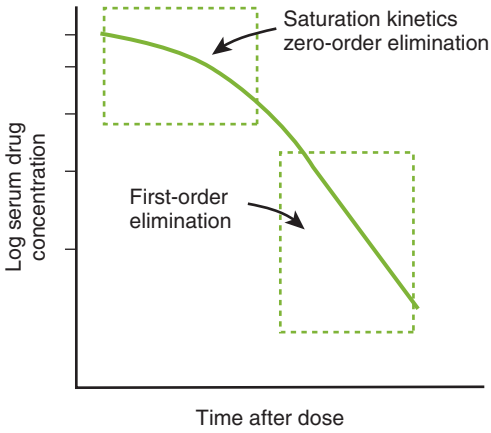
In this equation the rate constant for distribution is designated α to discriminate it from the rate constant for terminal elimination (β), where A and B are the $t = 0$ intercepts for the lines describing distribution and elimination, respectively. Division by 2.303 converts logarithms to natural logarithms.

After an intravenous dose, drug loss from the vascular space during the distribution phase occurs through both distribution and elimination (see Fig. 21.2). The rate constant of distribution (α) can be determined by a plot of the difference between the total amount of drug lost initially and the amount of drug lost through elimination.[27] This produces the line with the steeper slope (equal to $\alpha/2.303$) below the serum concentration graph in Fig. 21.2. The single slope of the distribution phase and of the terminal elimination phase does not imply that distribution or elimination occurs through a single process. The observed rates usually represent the sum of several simultaneous processes, each with differing rates, occurring in various tissues.

When the time course of drug elimination is observed for prolonged periods, a third rate of elimination, or γ phase, may also be observed and is usually attributed to the elimination of a drug that has re-equilibrated from deep tissue compartments back into the plasma. Such kinetics are designated *three-compartment first-order pharmacokinetics*. The kinetics of a drug are expressed with the smallest number of compartments that accurately describes its concentration changes over time.



• **Fig. 21.2** Multi-compartment serum drug disappearance curve. α, Rate constant for distribution; β, rate constant for terminal elimination.



• **Fig. 21.3** Saturation, or zero-order (serum concentration–dependent), and first-order (serum concentration–independent) pharmacokinetics.

Apparent Single-Compartment First-Order Kinetics

When a semi-logarithmic graph of concentration versus time reveals a single slope with no distribution phase, the kinetics are characterized as *apparent single-compartment first-order kinetics* (see Fig. 21.1). This kind of kinetics can occur when a drug remains entirely within the vascular space or central compartment or when a drug passes very rapidly back and forth between the blood and peripheral sites until it is metabolized or eliminated by first-order kinetics. The adjective *apparent* is used because careful study (e.g., very early sampling) often shows that distribution occurs even though the kinetic curve has only a single slope. Single-compartment kinetics implies that the drug rapidly and completely distributes homogeneously throughout the body, which rarely occurs clinically.

In many pharmacokinetic studies in newborns, blood samples are not obtained early enough to allow calculation of the distribution phase, and the kinetics are described as single-compartment kinetics. If sampling begins after the distribution phase, the concentration–time points may fit a single-compartment first-order model, which determines the elimination rate constant (β).

Zero-Order Kinetics

Some drugs demonstrate *zero-order kinetics*, in which a constant *amount* of drug, rather than a constant proportion or percentage, is removed per unit of time. This relationship can be expressed as:

$$\frac{dC}{dt} = -k$$

It is important to understand when zero-order kinetics occurs, how to recognize it, and how it affects drug concentrations. Zero-order kinetics is sometimes referred to as *saturation kinetics* because it may occur when excess amounts of drug completely saturate enzymes or transport systems such that they metabolize or transport only a *constant amount* of drug over time. Zero-order processes produce a curvilinear shape in a semi-logarithmic graph of concentrations versus time (Fig. 21.3). When drug concentrations are high from a drug overdose or the pathway for elimination is impaired as in renal dysfunction, the kinetics may become zero order initially and be followed by first-order kinetics at lower concentrations. For drugs exhibiting zero-order kinetics, small increments in dose may cause disproportionately large increments in serum concentration. Certain drugs or excipients (inactive substances that serve as vehicle or medium for a drug or other active substance, like filler, preservative or coloring agent) administered to newborns exhibit zero-order kinetics at concentrations observed in the clinical setting and must be recognized for potential accumulation (Box 21.2).

• BOX 21.2 Drugs That Demonstrate Saturation Kinetics With Therapeutic Doses in Newborns

Caffeine
Furosemide
Indomethacin
Phenytoin
Ethanol and other alcohols (excipients)

Noncompartmental Analysis

Noncompartmental analysis is based on describing drug exposure measured by the area under the concentration–time curve (AUC) without any assumptions about the pattern of elimination or the number of compartments.[28] Central to this analysis is the determination of drug clearance (CL) from the dose and the AUC:

$$CL = Dose/AUC$$

If the dose is administered intravenously, then noncompartmental analysis allows the direct determination of drug clearance with use of this relationship. Estimation of the elimination half-life is generally done with the slope of the log-transformed concentration measurements made during the end of a pharmacokinetic study. With an estimation of clearance and elimination rate, the apparent distribution volume (V) can also be estimated from:

$$CL = V \times k$$

Thus, noncompartmental PK provides a simple means to assess fundamental pharmacokinetic parameters that may be useful for dosing patients when detailed knowledge of the complete pharmacokinetic profile is not available or not needed.[27]

Advanced Mathematical Approaches: Population- and Physiology-Based Pharmacokinetic Models

Pharmacokinetic studies in newborns remain challenging due to sample size, heterogeneity of study population, and patient burden (blood sampling). The optimal PK sampling strategy must consider both the timing and number of samples per patient, and minimize sample volume using innovative bioanalytical methods.[30]

The population approach describes the concentration versus time profile for samples in all patients simultaneously, estimating population parameters that describe the average pharmacokinetic profile of the complete study group and patient-specific parameters that define the individual patients in the study. It can use fewer samples taken from each patient, if the samples are taken at different specified times, time windows or even opportunistic times when other blood samples are taken over the complete time profile of interest for the study analysis.[31] For example, one group of 28 to 32 weeks gestation neonates might have samples drawn at 1, 4, and 12 hours, while another group of 28 to 32 weeks gestation neonates might have blood sampled at 0.5, 2, and 8 hours. The concentrations from these two groups of similar patients are then analyzed in aggregate so as to provide information during both the distribution phase and the elimination phase, thus describing the kinetics with a limited volume of blood sampled from each patient.

Furthermore, the population approach allows the investigation of patient covariates of interest that might explain differences within the population of patients enrolled in the trial. Typical covariates such as postnatal age, GA at birth, weight, sex, organ dysfunction, and disease conditions can also be assessed for their contribution to differences seen between participants in a clinical study. These covariates can be very helpful for gaining a better understanding of factors that may alter the PK of infants that might otherwise be considered similar. Because of both conditions (fewer samples, exploration of covariates), the use of population pharmacokinetic studies in clinical studies is also strongly supported by the relevant regulatory agencies as an important contribution to drug research in this population.[28,29,32]

The PBPK approach is gaining interest as the preferred methodology to reliably predict PK in the neonatal population, especially during drug development studies. In a PBPK approach, drug-specific information (solubility, pH, molecular weight) is combined with anatomical and physiological knowledge—in this case specific to (pre)term neonates, including diseases—into a predictive model. To achieve this, the anatomical structure of the human body is represented by physiologically relevant compartments that are connected via the blood circulation.[33–35]

Target Drug Concentration Strategy

Drug treatment of newborns commonly uses the *target drug concentration strategy* (Table 21.1), in which drug therapy corrects a specific problem by producing an effective concentration of free drug at a specific site of action.[28] The target site of drug action (e.g., central nervous system, subcutaneous tissue) is usually inaccessible for monitoring of concentrations. A specific concentration or range of circulating concentrations is correlated with the effective concentration at the site of action, which provides a "therapeutic" concentration range. However, caregivers should be aware that such target drug concentrations are commonly extrapolated from other patient populations and are only rarely validated in newborns.[24]

The requirements for effective and accurate application of the target drug concentration treatment in adults have been discussed by Spector et al.[36] When applied to newborns, these requirements highlight the special problems of drug therapy in these patients and the special circumstances in which clinical drug concentration monitoring is appropriate. Some of these requirements are as follows:

- Availability of a reliable, valid analytic procedure for accurate measurement of drug concentrations in small blood volumes;
- A wide variation in PK among individuals with the knowledge that population-based kinetics do not accurately predict individual kinetics;
- Drug effects proportional to plasma drug concentrations;
- A narrow concentration range between efficacy and toxicity (narrow therapeutic index);
- Constant pharmacologic effect over time, in which tolerance does not develop;
- Clinical studies that have determined the target and toxic drug concentration ranges.

Therapeutic Drug Monitoring (TDM)

Table 21.1 describes the basic assumptions of TDM: the total plasma drug concentrations correlate with dose but also with the circulating unbound drug concentrations and the unbound drug concentration at the site of action. Measurements of drug concentrations are usually total drug concentrations, while the active portion is the unbound portion (see discussion in the Distribution section). Two broad indications for TDM are to (1) attain effective concentrations and (2) avoid toxic concentrations. However, drug concentration ranges are not absolute reflections but only indirect markers of effective therapy. Patient response, not a specific drug concentration range, is the end point of therapy.[37]

Although the concentrations of aminoglycoside or glycopeptide antibiotics are monitored frequently in newborns, the current perception is that toxicity is much less common in newborns than in adults.[38,39] Because of the limited evidence of toxicity in newborns, it is more important to measure these concentrations to achieve effective concentrations for treatment of culture-proven infections than to avoid toxicity. When the desired concentration

TABLE 21.1 Target Drug Concentration Strategy

Drug dose	↔	Plasma total drug concentration	↔	Plasma unbound drug concentration	↔	Target site unbound drug concentration	↔	Desired pharmacologic effect

range and kinetic parameters are known, dosages may be estimated to reach that concentration with single bolus doses or bolus doses followed by continuous infusions. TDM hereby typically covers a PK assessment based on a drug concentration, with subsequent dose adaptation, or not. We anticipate that in the near future, such dose adaptation will be guided by MIPD tools. MIPD is a new dosing paradigm, combining mathematical models for drugs and diseases with individual patient characteristics (e.g., TDM, weight, age) and disease characteristics (e.g., pathogen susceptibility) to calculate the optimal dose, preferably at the bedside. As computer technology becomes ever more readily available bedside, this is a very realistic, feasible aim with the potential to significantly improve the interpretation of TDM results as integrated with other relevant information of the individual patient.[1]

Pharmacokinetic-Based Dosing

The following equations can be used both to guide dosing and to derive kinetic parameters for individual patients:

$$\text{Dose} = \Delta C \cdot \text{Vd} = \left[C_{\text{desired}} - C_{\text{initial}}\right] \cdot \text{Vd}$$
$$(\text{mg/kg}) = (\text{mg/L})(\text{L/kg}) = (\text{mg/L})(\text{L/kg})$$

where C is the concentration and Vd is the apparent volume of distribution.

This equation may be used to estimate dosage changes needed to increase or decrease concentration. For the first dose, the starting concentration is zero; for doses after the first, the calculation of the volume of distribution should use the change (Δ) in concentration from the preceding trough to the peak associated with that dose. To reach a desired concentration rapidly, a loading dose can be administered, followed by a sustaining infusion. The equation for calculation of infusion doses to maintain a constant concentration is as follows:

$$\text{infusion rate} = k \cdot \text{Vd} \cdot \text{C}$$
$$(\text{mg/kg})\left(\text{min}^{-1}\right) = \left(\text{min}^{-1}\right)(\text{L/kg})\,(\text{mg/L})$$

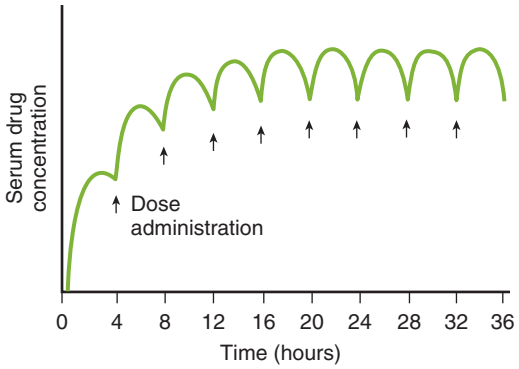
where C is the concentration, Vd is the volume of distribution, and k is the rate constant of elimination.

A steady state is reached when tissue concentrations are in equilibrium and the amount of drug removed equals the amount of drug infused. The time needed to reach a steady state depends on the elimination half-life. While the time needed is *not* shortened by the administration of a loading dose, a loading dose can allow the patient to achieve therapeutic concentrations rapidly, and these can be maintained with an infusion during the steady state.

It is important to consider that if drug clearance decreases, the steady-state concentration during an infusion will increase proportionally. Additionally, the half-life increases and the rate constant decreases. Since concentrations are not measured for most of the drugs administered by continuous infusion in the neonatal intensive care unit (NICU), it is important to consider to adjust dosages for factors that reduce clearance, such as kidney or liver dysfunction or reduced kidney or liver blood flow, to avoid high and potentially toxic concentrations.

Repetitive Dosing and the "Plateau Principle"

During a typical course, drug doses are administered before complete elimination of previous doses, and the drug accumulates in the body. Consequently, during repeated administration, the peak and trough concentrations after each dose will increase until a steady-state situation has been reached. *Steady-state*, or *plateau*, concentrations are reached when the amount of drug eliminated equals the amount of drug administered during each dosing interval. During repetitive dosing, the steady-state concentrations achieved are related to the half-life, dose, and dosing interval relative to the elimination half-life.



• **Fig. 21.4** Multiple dosing with accumulation of serum drug concentrations to the steady-state concentration.

Fig. 21.4 illustrates a hypothetical concentration–time curve for a drug with a half-life of 4 hours administered orally every 4 hours, so the dosing interval corresponds to one half-life. Several important principles of PK are illustrated in Fig. 21.4. Drug concentrations increase and decrease with drug administration (absorption) and elimination. For dosing intervals of one half-life, accumulation is 88% complete after the third dose, 94% complete after the fourth dose, and 97% complete after the fifth dose. At steady state, the peak and trough concentrations between doses are the same after each dose. If a drug is administered with a dosing interval equal to one half-life, the steady-state peak and trough concentrations are two times those reached after the first dose. If the dosing interval is shortened to half of a half-life, the concentration decreases less before the next dose, more total drug is administered per day, and the steady-state peak and trough concentrations are considerably higher (3.4 times the peak and trough concentrations after the first dose). Therefore, the shorter the dosing interval to half-life ratio, the higher the drug accumulation. As noted during infusions, the *length of time* required to reach steady-state concentrations depends primarily on the elimination half-life, not the dosing interval.

Clearance

Clearance of drugs, as for creatinine, describes the *volume of blood* from which all the drug is removed per unit of time. Clearance is proportional to organ blood flow and the intrinsic capacity of organs to metabolize or remove a drug from the circulation. In its simplest form, clearance is proportional to the flow to a single organ (Q) and to the arterial–venous difference in drug concentrations compared with the amount of drug in the circulation, expressed as follows:

$$\text{CL} = \text{Q} \cdot \frac{C_{\text{arterial}} - C_{\text{venous}}}{C_{\text{arterial}}}$$

Total body clearance usually reflects the combined clearance of multiple organs with different enzyme activities and different

rates of blood flow. Clearance can be measured by the rate of appearance of a drug outside the body (such as urinary creatinine clearance) or by the rate of disappearance of a drug from the blood compared with the blood concentration. For calculations, *clearance* is defined as the dose divided by the AUC and by the rate of drug input per steady-state concentration (Css) average, where the rate of input is the dose/dosing interval (τ). For a drug administered by continuous infusion, this is simply the infusion rate (milligrams per kilogram per hour) divided by Css as follows:

$$\mathrm{CL} = \frac{\mathrm{Dose}}{\mathrm{AUC}} = \frac{\mathrm{Dose}/\tau}{\mathrm{Css}} = \frac{\mathrm{Infusion\ Rate}}{\mathrm{Css}}$$

Once clearance is known, this equation can be rearranged to calculate the dose necessary to achieve any desired steady-state concentration:

$$\mathrm{Infusion\ Rate} = \mathrm{Css} \cdot \mathrm{CL}$$
$$(\mathrm{mg/kg})\,\mathrm{hr}^{-1} = (\mathrm{mg/L})(\mathrm{mL/kg})\,\mathrm{hr}^{-1}$$

Total body clearance changes significantly for several drugs during the period of fetal and infant development because renal clearance and/or the activity of drug-metabolizing enzymes increase with advancing gestational and/or postnatal age. If available, values for clearance and the volume of distribution at different stages of development can be used to estimate the dosages needed to achieve and maintain "therapeutic" concentrations associated with desired clinical responses. To illustrate this for renal clearance, a higher dose (mg/kg) and a further extended time interval are applied for aminoglycosides or vancomycin to compensate for the higher distribution volume (peak concentration) and the lower renal clearance (trough concentration).[40,41]

Studies of the analgesic fentanyl illustrate the developmental changes in metabolic clearance and how they may be used to calculate dosages to reach and maintain concentrations associated with effective analgesia. Analgesia has been associated with a serum fentanyl concentration of 1 to 2 ng/mL.[42] If analgesic treatment is initiated with a continuous infusion of fentanyl, five half-lives are needed to reach a steady state. The fentanyl half-life ranges from 3 hours in term newborns to 12.7 hours in preterm newborns.[42–44] Because of this prolonged half-life, the patient may be inadequately treated for a long time unless a loading dose is administered to reach an effective concentration more rapidly. In general, initiation of analgesic treatment and increases in infusion dosages of analgesics should begin with a loading dose based on the estimated volume of distribution in the central compartment (circulation) and the desired concentration. Use of a loading dose shortens the time to reach higher effective analgesic concentrations but also increases the likelihood of toxicity, as has been reported with opioids or milrinone.

The available data on GA-related changes in fentanyl clearance show that there is an increase with advancing GA[42–44] and postnatal age.[42,44,45] The linear graph of clearance versus GA from 38 neonates in whom treatment began within 47 hours after their birth was used to derive mean rates of clearance at different GAs as shown in Table 21.2.[42,45]

Other investigators studied single-dose fentanyl kinetics during anesthesia and found an apparent central volume of distribution of fentanyl in neonates of 1.45 L/kg.[43] This distribution volume is smaller than the steady-state volume of distribution of 5.1 L/kg also calculated after a single dose of fentanyl.[43] In turn, the apparent steady-state volume of distribution after a single bolus dose of a lipophilic drug is usually smaller than that associated with continuous drug infusions, during which tissues throughout the body become saturated with drug. The steady-state distribution volume for fentanyl during continuous infusions was calculated as 17 L/kg.[42] Because fentanyl is a highly lipid-soluble drug, it distributes rapidly from the central compartment into the peripheral tissue compartment. This large distribution volume likely reflects the period during the infusion when drug is leaving the circulation to penetrate peripheral tissues, such as fat and brain. Because it may take 15 to 60 hours to achieve a steady-state concentration (five half-lives) after a fentanyl infusion is begun or the infusion rate is increased, a patient may need repeated bolus doses to maintain effective plasma concentrations in the central compartment. The best approach is to repeat the calculated loading dose until the desired clinical effect is achieved. This also illustrates why, for sedation specifically, dosing should be adjusted to achieve the desired clinical effect. Clearance calculations, however, can guide the starting dosages to achieve effective sedation, as illustrated later.

TABLE 21.2 Developmental Pharmacokinetics of Fentanyl

Gestational Age (Wk)	Clearance at 0–47 h After Birth (mL/min/kg)
29	9.6
33	11.4
37	13.2
41	15.0

Data from Saarenmaa E, Neuvonen PJ, Fellman V. Gestational age and birth weight effects on plasma clearance of fentanyl in newborn infants. *J Pediatr*. 2000;136:767–770.

The kinetic parameters for fentanyl in premature newborns reported by Koehntop et al.[43] can be used to calculate a loading and infusion dose to reach a fentanyl concentration of 2 ng/mL, which is considered an analgesic concentration.[46] This is estimated for a premature newborn at 33 weeks GA (note that nanograms per milliliter is equivalent to micrograms per liter) as:

$$\mathrm{C}(\mu\mathrm{g/L}) = \frac{\mathrm{Loading\ Dose}\ (\mu\mathrm{g/kg})}{\mathrm{Vd}_{\mathrm{central}}\ (\mathrm{L/kg})}$$

$$\mathrm{Loading\ Dose}\ (\mu\mathrm{g/kg}) = 2\left(\frac{\mu\mathrm{g}}{\mathrm{L}}\right) \times 1.45(\mathrm{L/kg}) = 2.9\ \mu\mathrm{g/kg}$$

$$\mathrm{Infusion\ Rate}\left(\frac{\mu\mathrm{g}}{\mathrm{kg}}\cdot\mathrm{h}\right) = \mathrm{C}\left(\frac{\mu}{\mathrm{L}}\right) \times (\mathrm{ml/kg}\times\mathrm{h}) = 2\ \frac{\mu\mathrm{g}}{\mathrm{L}} \times 11.4\ \mathrm{mL/kg/min} \times 1\frac{\mathrm{L}}{1000}\ \mathrm{mL} \times 60\,\mathrm{min/h} = 1.4\ \mu\mathrm{g/kg}\cdot\mathrm{h}$$

Studies have also reported on increased fentanyl clearance with advancing postnatal age.[42,44,45] This postnatal increase in clearance of fentanyl likely relates either to maturation of CYP3A4

(the enzyme responsible for fentanyl metabolism) activity or to increased hepatic blood flow after birth, because fentanyl has a high hepatic extraction rate. For drugs such as fentanyl with a high hepatic extraction rate, the rate-limiting factor in clearance is the flow of blood to the liver.[46] Some researchers have observed that increased intra-abdominal pressure reduces fentanyl clearance, which is likely caused by reduced hepatic blood flow.[43,45] Clinical covariates known to alter fentanyl clearance should be used to adjust starting dosages, but dosing should be adjusted primarily for the desired clinical effect. Such an approach should also consider subsequent development of drug tolerance (i.e., a higher concentration is needed to attain a similar effect).

Modeling and Simulations

Pharmacokinetic modeling and simulations can be used to estimate the impact of important developmental changes and disease processes on pharmacokinetic parameters for distribution volume, elimination rate, and total body clearance. These can identify clinical situations and conditions when dosages are likely to require modification (e.g., drug–drug interactions, genetic polymorphisms, advancing age, renal impairment). Mathematical simulations create theoretical pharmacokinetic profiles for patients after a dose using the range of pharmacokinetic parameters determined from a patient population. These can then be calculated for 100 to 1000 hypothetical patients to define the expected range of concentrations that are likely after a dose. For drugs such as antiinfectives of which serum concentrations have been correlated with effectiveness, this provides estimates of how large a dose is needed to reach effective concentrations. Modeling and simulation methods can also be used for clinical study design to support decisions about the number of participants, optimal times of sampling, covariates, phenotypic analyses, and population analyses.

Clinical Applications of Pharmacokinetics

How to Estimate Dose Adjustments

Gentamicin and phenobarbital will be used to illustrate the clinical application of the principles of PK and TDM discussed earlier. The calculations can be performed with standard arithmetic calculators and provide close enough estimates of the kinetics for drugs with a long half-life to adjust dosages at the bedside.

Gentamicin

Assume that the optimal gentamicin target concentrations are:

$$\text{Peak} = 6-10\,\mu\text{g/mL}$$
$$\text{Trough} = 0.5-2\,\mu\text{/mL}$$

Following the fourth conventional dose (4 mg/kg) of gentamicin to a hydropic premature newborn, the peak concentration was 4.5 µg/mL; 18 hours after the peak was obtained, the trough was 2.25 µg/mL. It appears that the distribution volume is greater than anticipated, because the peak concentration is lower than expected, and the half-life is longer than anticipated, because the trough is higher than expected. The time of drug administration and that of blood sampling was confirmed (an important step), so the half-life is 18 hours, because the concentration decreases by 50% from 4.5 to 2.25 µg/mL in 18 hours (assuming that the kinetics are exponential and first order):

$$\begin{aligned}\text{Vd (mL/kg)} &= \text{Dose (mg/kg)} \times (1000\,\mu\text{g/mg})\ \Delta\text{C}\ (\mu\text{g/mL})\\ &= 4.0\ (\text{mg/kg}) \times 1000/4.5 - 2.25\ (\mu\text{g/mL})\\ &= 4000\ \text{mL}/2.25\ \text{kg} = 1.777\ \text{mL/kg}.\end{aligned}$$

To ensure a trough concentration of 2.0 µg/mL or less, doses are administered every two half-lives, or every 36 hours. When two half-lives have passed after the fourth dose, the gentamicin concentration should be about 1.1 µg/mL (50% of 2.25 µg/mL). Increasing the concentration from the 1.1 µg/mL trough to more than 6 µg/mL requires a concentration difference of 4.9 µg/mL or more. With a distribution volume of 1777 mL/kg, a dose of 8.7 mg/kg should raise the concentration from a trough of 1.1 µg/mL to a peak of 6.00 µg/mL:

$$\text{Vd (mL/kg)} = \text{Dose (mg/kg)} \times (1000\ \mu\text{g/mg})/\Delta\text{C}\ (\mu\text{g/mL})$$

$$1777\ \text{mL/kg} = \text{Dose (mg/kg)} \times (1000\ \mu\text{g/mg})/4.9\ (\mu\text{g/mL})$$

$$\text{Dose (mg/kg)} = (1777/1000)/\text{kg} \times 4.9\ \text{mg} = 8.7\ \text{mg/kg}.$$

In one half-life, this concentration will decrease to 3.0 µg/mL, and in two half-lives, or 36 hours, it will decrease to 1.5 µg/mL. Another 8.7 mg/kg dose will raise the peak concentration by 4.9 µg/mL to 6.4 µg/mL, which will fall to 3.2 µg/mL in one half-life and to 1.6 µg/mL in two half-lives. The variation between the peak and trough concentrations after the last dose is within the desired range for the optimal gentamicin concentrations defined previously.

Phenobarbital

Seizures that were hard to control developed in a 3.6-kg asphyxiated newborn. Seizures continued after two 20 mg/kg phenobarbital doses until an additional 10 mg/kg dose was administered. A maintenance dose of 7 mg/kg/day was started 24 hours after the loading doses were administered. At 10 days, the infant was increasingly somnolent. The phenobarbital level measured in a blood specimen drawn 2 hours after administration of the oral maintenance dose was 50 µg/mL. Additional doses were withheld, and the phenobarbital concentration was checked daily; the results were as follows:

- 24 hours: 40 µg/mL
- 48 hours: 31 µg/mL
- 72 hours: 25 µg/mL
- 96 hours: 21 µg/mL

The maintenance dose (7 mg/kg) was resumed immediately after the 21 µg/mL concentration was measured and produced a peak concentration of 30 µg/mL after administration of the dose. These concentrations and dosages can be used to calculate the volume of distribution and a dose to maintain the phenobarbital concentration between 20 and 30 µg/mL as:

$$\begin{aligned}\text{Vd}\left(\frac{\text{L}}{\text{kg}}\right) &= \frac{\text{Dose}\,(\text{mg/kg})}{\text{C}\,(\mu\text{g/mL} = \text{mg/L})}\\ &= \frac{7.0\,(\text{mg/kg})}{(30-21)(\text{mg/L})}\\ &= \frac{7\,(\text{mg/kg})}{9\,(\text{mg/L})}\\ &= 0.78\,\text{L/kg}\end{aligned}$$

Half-life can be estimated from inspection, because the concentration decreased from 50 to 25 μg/mL in 72 hours. Thus it should take 72 hours for the concentration to decrease by one half-life from 30 to 15 μg/mL. Assuming that the elimination rate does not change, the concentration will decrease approximately 5 μg/mL every 24 hours, or one-third of a half-life. Dividing the half-life into fractions is an approximation because it estimates the change in concentration as linear rather than exponential. To be more accurate, the concentration decreases by 59% in half of one half-life. Although this approximation violates certain principles of PK, it allows estimation of the change in concentration for each one-third of a half-life as one-third of the change during one half-life. Thus the concentration decreases by about 5 μg/mL in 24 hours. The following approach can be used to estimate the daily phenobarbital dose needed to return the concentration to 30 μg/mL, a change in concentration of 5 μg/mL:

$$\Delta C\ (\text{mg/L}) = \frac{\text{Dose (mg/kg)}}{\text{Vd (L/kg)}}$$

$$5\ (\text{mg/L}) = \frac{\text{Dose (mg/kg)}}{(0.78)\ (\text{L/kg})}$$

$$3.6\,\text{mg/kg} = \text{Dose (mg/kg)}$$

Adverse Drug Reactions

Adverse event assessment includes signal *detection*, *causality* assessment, and *severity* assessment. For signal detection and incidence, as well as for causality and severity assessment, disentangling confounders from adverse drug events remains a major challenge. In a prospective study on the epidemiology of adverse drug reactions (ADRs) in 200 consecutively admitted neonates, 136 ADRs occurred in 60 neonates (30%), 20 were life threatening, and 24 were of moderate intensity (prolonged hospital stay); about 50% of the neonates had multiple ADRs. In a more recent study of a mixed population of 313 neonates admitted to the NICU or the intermediate care unit, 116 ADRs occurred in the neonates (17%), and 44% of these ADRs needed specific treatment.[47] The reasons behind drug-related morbidity and mortality are diverse and complex. In addition to a lack of specific labeling for this special patient population, absence of age-appropriate neonate-friendly formulations and a high frequency of (poly)pharmacy, there is also the immature organ function and substantial comorbidities that will further increase the risks of ADRs in neonates. To improve *causality* detection, Du et al. suggested and validated an algorithm to detect ADR in the NICU.[48] However, intra- and interrater assessment of causality of ADRs remained only fair (kappa scores ≤0.3) in a recently reported prospective observational study on neonatal events.[49]

Related to *severity assessment*, the International Neonatal Consortium (INC) Adverse Event Severity Scale has recently been reported. Immediate functional consequences (on age-appropriate behavior and basal physiological functions), together with resulting care changes, were established as the parameters of the generic severity scale. This generic severity scale was subsequently tailored to 35 event-specific severity criteria related to neurological, cardiovascular, respiratory, infectious, gastrointestinal, infectious, or general neonatal care events.[50]

Illustrations of Adverse Drug Reactions in Neonates

Chloramphenicol was released in the 1940s, and the recommended dosages were 50 to 100 mg/kg/day for patients weighing 15 kg or less. Before 1959, the year that Sutherland reported three cases of sudden death in newborns treated with high dosages of chloramphenicol (up to 230 mg/kg/day), the drug was considered "well tolerated and nontoxic."[51] Burns et al. reported the disturbing results of a controlled trial of the following four prophylactic treatment regimens for newborn sepsis: (1) no treatment, (2) chloramphenicol alone, (3) penicillin and streptomycin, and (4) penicillin, streptomycin, and chloramphenicol. The groups that received chloramphenicol (100 to 165 mg/kg/day) had higher mortality rates (60% and 68%), and the deaths of these newborns demonstrated the stereotyped sequence of symptoms and signs caused by chloramphenicol accumulation, coined *grey baby syndrome*.[52] This syndrome consisted of abdominal distention, poor peripheral perfusion and cyanosis, vasomotor collapse, irregular respirations, and death within hours of onset of these symptoms.

The discovery of the mechanism (glucuronidation deficiency) of chloramphenicol toxicity in newborns illustrates several important aspects of neonatal pharmacology. Because chloramphenicol was considered well-tolerated in older children and adults, it was regarded as nontoxic to newborns. Higher doses were administered to newborns despite recognition that its clearance required glucuronide conjugation, which was known to be immature in newborns. The unexpected finding that chloramphenicol in dosages of 100 to 165 mg/kg/day could be lethal to newborns was demonstrated because the study conducted by Burns et al. included appropriate control groups.[52] Similar case observations with the mechanism involved are summarized in Table 21.3 to illustrate that these observations can still be seen.

Reduction and Prevention of Medication Errors in Newborn Care

Medication errors represent a significant and often preventable cause of morbidity and mortality in neonates. While many errors are without consequences, others result in serious adverse effects. Medication errors incur significant costs, ranging from obvious ones such as direct patient injury, prolonged hospital stays, and additional corrective treatments to more subtle ones such as the costs associated with monitoring and regulation of medication use.[53,54] In two studies performed in NICUs, about one-third of the "iatrogenic" events were preventable, and one-quarter were related to drugs.[53,54] For drug errors, ordering and prescription as well as administration-related errors were observed. The top five preventable errors relate to incorrect doses, medications that are inappropriate for the medical condition, failure to monitor newborns for drug-related side effects, failure of communication, and failure to monitor drug concentrations.

The process for ordering, preparing, dispensing, and administering medications is often complicated and may contribute directly to errors. The frequency of those errors, however, may be reduced in almost every unit. A barcode medication administration system (verification of drug and patient), in combination with a double-check when further manipulation (such as dilution) is needed, is required to effectively reduce this burden of

TABLE 21.3 Illustrations of Formulation (Active Compound, Excipient)-Specific Drug-Induced Illnesses in Neonates and the Mechanism Involved

Compound/ Formulation	Clinical Syndrome	Mechanism Involved
Sulfonamides (1956)	Kernicterus	Highly albumin bound antibiotic, competitive with endogenous compounds, including bilirubin. This results in higher free bilirubin concentration and subsequent kernicterus.
Benzyl alcohol (1982)	Gasping syndrome	Benzyl alcohol, coadministered as preservative in parenteral formulations results in accumulation in preterm neonates because of their limited metabolic (alcohol dehydrogenase) clearance capacity. Accumulation results in metabolic acidosis followed by seizures, bradycardia, gasping respirations, hypotension preceding cardiovascular collapse, and ultimately death.
Dexamethasone (2000)	Cerebral palsy	High-dose dexamethasone exposure in neonatal life results in an increased risk for cerebral palsy in later life, likely due to increased neuroapoptosis.
Lopinavir/ ritonavir (2005)	Alcohol accumulation	Lopinavir/ritonavir (Kaletra) is an effective treatment of human immunodeficiency virus (HIV). Its syrup contains both ethanol and propylene glycol. Impaired metabolism results in accumulation and subsequent hyperosmolality, lactic acidosis, renal toxicity, central nervous system impairment, cardiac arrhythmia, hemolysis, and collapse (FDA black box warning).
Ceftriaxone plus calcium (2009)	Cardiovascular collapse	Simultaneous administration of calcium-containing infusions and ceftriaxone results in intravascular precipitation, as observed during autopsy (FDA black box warning).
Caffeine (2015)	Apnea of prematurity	In a pilot study, high versus regular dosing was associated with an increased incidence of cerebellar bleeding in the high-dose group.

targeted, preventable, medication errors in the NICU setting.[55] Although these kinds of complex and expensive computerized systems may help reduce medication errors, caregivers can take steps that are completely within their control to reduce medication errors without waiting for changes in the entire pharmacy process within the hospital. Prescriptions and drug orders are a means of communicating, yet clinicians often devote too little attention to make these documents legible, clear, and unambiguous. Prescribers should keep the following recommendations in mind to ensure that they communicate their medication orders more effectively:

- Write out instructions rather than use abbreviations.
- Avoid vague instructions (e.g., "take as directed").
- Specify exact dosage strengths.
- Avoid abbreviations of drug names (e.g., MS could mean morphine sulfate or magnesium sulfate).
- Avoid "U" as an abbreviation for units as the "U" may be mistaken for a "0."
- Avoid trailing zeroes (e.g., 5.0 mg).
- Use leading zeroes (e.g., 0.5 mg).
- Minimize the use of verbal orders.
- Ensure that prescriptions and signatures are legible, even if it means printing the prescriber's name that corresponds to the signature.

Changes in the pharmacy process can also be effective, as highlighted by Campino et al., who evaluated the impact of a care bundle intervention (protocol standardization, education) on the number of errors and documented significant reductions in both calculation errors (1.35% to 0%) and accuracy error rates (from 54.7% to 23% at the bedside and from 38.3% to 14.6% in the hospital pharmacy).[56] In a systematic review on interventions to reduce medication errors in neonatal care, several effective interventions were identified related to technology (like electronic prescribing), organization (guidelines, procedures), personnel (staff education), pharmacy (clinical pharmacy services), hazard and risk analysis (error detection tools), or a combination of such interventions (care bundle concept).[55]

Drug Elimination in Breast Milk

Breast milk-related drug exposure remains a source of confusion and concern for physicians and families.[57,58] However, knowledge is increasing, and access to information through, for example, LactMed (http://toxnet.nlm.nih.gov/newtoxnet/lactmed.htm) has improved. The Pregnancy and Lactation Labeling Final Rule provides a tool to generate and collect more reliable information on maternal drug use during breastfeeding. A common misconception about safety is "*when in doubt, do not provide breastfeeding*," since breastfeeding itself provides benefits to the infant and the mother. On average, the nursing infant receives around 2% to 3% of a maternal dose through milk, but drugs that are organic bases or are lipid soluble may reach higher concentrations in milk than in maternal serum. However, this should not by definition affect the infant. To illustrate this more balanced approach, breastfeeding in infants of women taking antiepileptic drugs was associated with improved neurodevelopmental outcome compared with those who either did not breastfeed or breastfed for less than 6 months.[59] Similarly, breastfeeding by opioid-taking women may help to control neonatal abstinence syndrome, and may even increase signs of withdrawal when reduced or stopped.[60] Likewise, it may also be associated with neonatal oversedation.[61] Overall, the available data regarding drug exposure of the newborn through human milk have been organized, in decreasing levels of concern, from drugs that are associated with adverse effects on the infant during nursing to those that are of concern pharmacologically to those that have not been associated with problems during nursing. The list of drugs that clearly cause problems during nursing is surprisingly short.[62,63]

Neonatal Drug Development Remains an Obvious and Shared Need

Recent legislative initiatives have resulted in a substantial increase in pharmacologic studies in children, and a significant increase in label changes. Unfortunately, only a few have included drug label changes for neonates.[64,65] Neonatologists should realize that almost all compounds currently used in neonates were initially developed for other populations, with subsequent tailoring to neonates. Key to stimulating and facilitating neonatal drug development is basing product development on neonatal physiology and pharmacology, while incorporating already available knowledge from non-neonatal settings. Furthermore, the central role of families in research and the value of the entire neonatal team in the design, implementation and interpretation of studies are crucial.[66] Surfactant hereby serves as a very obvious illustration of the potential impact of neonatal drug discovery.

During drug development, dosing in neonates should be based on integrated knowledge of disease, neonatal physiology, and PK and PD of a given drug.[64] Subsequent PD assessment, covering both efficacy as well as safety, necessitates valid population-tailored tools and methods. Recently, the INC has been developed as part of the Critical Path Initiative to serve as a forum for the neonatal community to develop consensus statements (e.g., standardization of methods, standard-of-care consensus statements, population-specific biomarkers, modeling approaches, trial designs, clinical outcome assessment tools, formulation issues) with the ultimate goal to improve, to accelerate neonatal drug development.[66,67] It is crucial to realize that the INC is an international multi-stakeholder construct, facilitating interaction between regulators, academia, clinical researchers, industry, parents and NICU staff from the United States, Europe, Japan, and Canada. With its focus on neonatal drug development, the INC construct itself is embedded in the Critical Path Initiative, a broader FDA national strategy to drive innovation in the scientific processes through which medical products are developed, evaluated, and manufactured.

Summary

Drug therapy for newborns requires application of the basic principles of PK (drug absorption, distribution, metabolism, and elimination) and PD (both desired efficacy and unwanted side effects) to estimate and individualize dosages. However, owing to a lack of gestational age-appropriate kinetic data in the rapidly maturing fetus and newborn, drug therapy is still commonly based on empiric, off-label prescriptions. Combined with the extensive drug exposure of neonates in the NICU, this is hazardous and likely explains the high frequency of adverse, sometimes fatal, drug reactions. Methods appropriate for the study of therapeutics in newborns present unique difficulties, but recent legislative and methodological progress provides additional assistance for investigators and helps advance this area of pharmacology.[68–72] Recent collaborative initiatives should further stimulate development of both new and already existing drugs for use in neonates.[66,67]

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22

Neonatal Pain and Stress

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KEY POINTS

- Pain and discomfort in infants are common occurrences in intensive care.
- Identification and management of neonatal pain remain challenging.
- Many neonatal pain scoring tools assist in classifying pain, but many were not validated in clinical practice.
- Neonatal pain recognition with near-infrared spectroscopy and amplitude-integrated electroencephalography does not always align with clinical manifestations of pain, raising questions about how best to define, diagnose, and treat pain.
- Untreated neonatal pain can result in long-term adverse outcomes due to alterations in brain structure and hormone imbalance, resulting in abnormal childhood pain responses.
- Pharmacologic treatment of pain is widely used, but nonpharmacologic interventions may be just as effective.

Background

In the 1980s, a public outcry regarding the recognition and management of pain in hospitalized patients prompted the US Department of Health and Human Services, The Joint Commission, and other professional organizations to mandate pain management practices for all patients, including neonates. An initial statement endorsing pain management for neonates undergoing surgical interventions, regardless of age and cortical immaturity, was jointly issued by the American Academy of Pediatrics Committees on Fetus and Newborn, the Committee on Drugs, the Section on Anesthesiology, the Section on Surgery as well as the American Society of Anesthesiologists.[1] The Acute Pain Management Guideline Panel of the US Agency for Health Care Policy and Research[2] also endorsed the need for pain management in neonates.

The subsequent conundrum was that neonatal caregivers were expected to assess and treat perceived neonatal pain and discomfort in the absence of evidence-based methods with which to base their assessment and therapy. The lack of verbal skills, immature behaviors in response to pain, and the nonspecific nature of physiologic indicators of pain in a critically ill patient all combine to make accurately assessing pain in term and preterm neonates challenging. The challenge of pain assessment is further compounded by typical NICU interventions such as mechanical ventilation, physical restraints, and pharmacologic sedation, all of which mask distress behaviors. Because the gold standard of pain assessment (self-reporting using validated scales) is not applicable to neonatal patients, providers must reply on physiologic, behavioral, and biobehavioral indicators as surrogates for self-reporting pain.

Taxonomy

Defining neonatal pain and its myriad presentations may help clinicians to recognize and therefore treat it. The proposed taxonomy includes distinctions between acute episodic pain, acute recurrent pain, prolonged pain, persistent pain, and chronic pain (Table 22.1).[3] The origin and resultant physiologic status for various painful stimuli can be quite different. Tools for assessment of neonatal pain and stress must be based on an understanding of the normal development of behavioral responses to pain and stress—an infant is not a small adult.

An international survey of experts in neonatal pain, including physicians, nurses, researchers, and parents from around the world, used the Delphi method to propose a definition of chronic pain in neonates. They put forth the following:

"Chronic pain can often not be associated with a specific cause. It has no obvious endpoint in sight and is no longer proximate to an event or procedure. Chronic pain may alter perception causing non-noxious events to be perceived as painful, leading to a chronic pain response. It depletes stress hormones, increases energy consumption, and therefore interferes with growth. As a consequence, chronic pain may likely prolong hospitalization and add to existing neonatal morbidities."[4]

The optimal approach to neonatal pain management relies on a concerted, mindful practice that involves:

- Reducing the frequency of painful procedures
- Decreasing environmental stressors
- Facilitating neurologic development
- Determining the best technique to minimize the pain and stress associated with procedures
- Empowering nurses to manage pain assessment and treatment
- Using a balanced multimodal approach to pain control

Parents remain an underutilized resource in managing neonatal pain,[5] in part because of medical paternalism.[6] Parent awareness of their own role, as well as how NICU staff engage with and educate them, impacts how they advocate for pharmacologic and nonpharmacologic analgesia for their children.[7] When providers perceive maternal information regarding premature infant pain as sufficient and necessary, maternal involvement during painful procedures increases.[8] Parents randomized to receive a pain information booklet were better prepared to understand infant pain cues and comforting techniques and took more active roles in their hospitalized infant's pain care.[9,10]

TABLE 22.1 Suggested Starting Point for Defining the Pain Terms Used for Neonatal Pain

Pain Term	Onset	Duration	Character*	Primary Hyperalgesia
Acute episodic	Immediate	0–120† min	Sharp, well-localized	Present, mild, short-lasting
Acute recurrent	Immediate	Variable	Sharp, well-localized	Present, moderate or severe
Prolonged‡	Rapid, may be gradual	1 h–24† h	Sharp, diffusely localized	Present, moderate or severe
Persistent‡	Rapid or gradual, cumulative	1–7 days	Dull/sharp, diffusely localized	Present, moderate or severe
Chronic	Usually gradual	8 days or longer	Dull, diffusely localized	May be present or absent, mild if present

Pain Term	Secondary Hyperalgesia	Allodynia	Behavioral Phenotype	Physiological Phenotype
Acute episodic	Probably absent	Probably absent	Strongly reactive and reflexive	High peak, sympathetic activation
Acute recurrent	Present, mild or moderate	Probably absent	Weakly reactive or reflexive	Prolonged peak, sympathetic activation
Prolonged‡	Mild or absent	Probably absent	Strongly reactive on stimulation	High plateau, sympathetic activation
Persistent‡	Present, mild or moderate	May be present, mild/moderate	Hyperreactive initially, later hyporeactive	Normal or low sympathetic activation
Chronic	Present, moderate, or severe	May be present, moderate/severe	Hyporeactive more often, could also be hyperreactive	Normal or suppressed sympathetic drive

*Based on descriptions in adult patients but may be discerned by a careful physical examination.
†Some infants with increased sensitivity to pain may have a slower decay of the acute pain following an invasive procedure, thus justifying some overlap in the durations of acute episodic pain and prolonged pain.
‡Continuous pain may be characterized as either "prolonged" or "persistent."
From Anand KJS. Defining pain in newborns: need for a uniform taxonomy? *Acta Paediatr.* 2017;106(9):1438–1444.

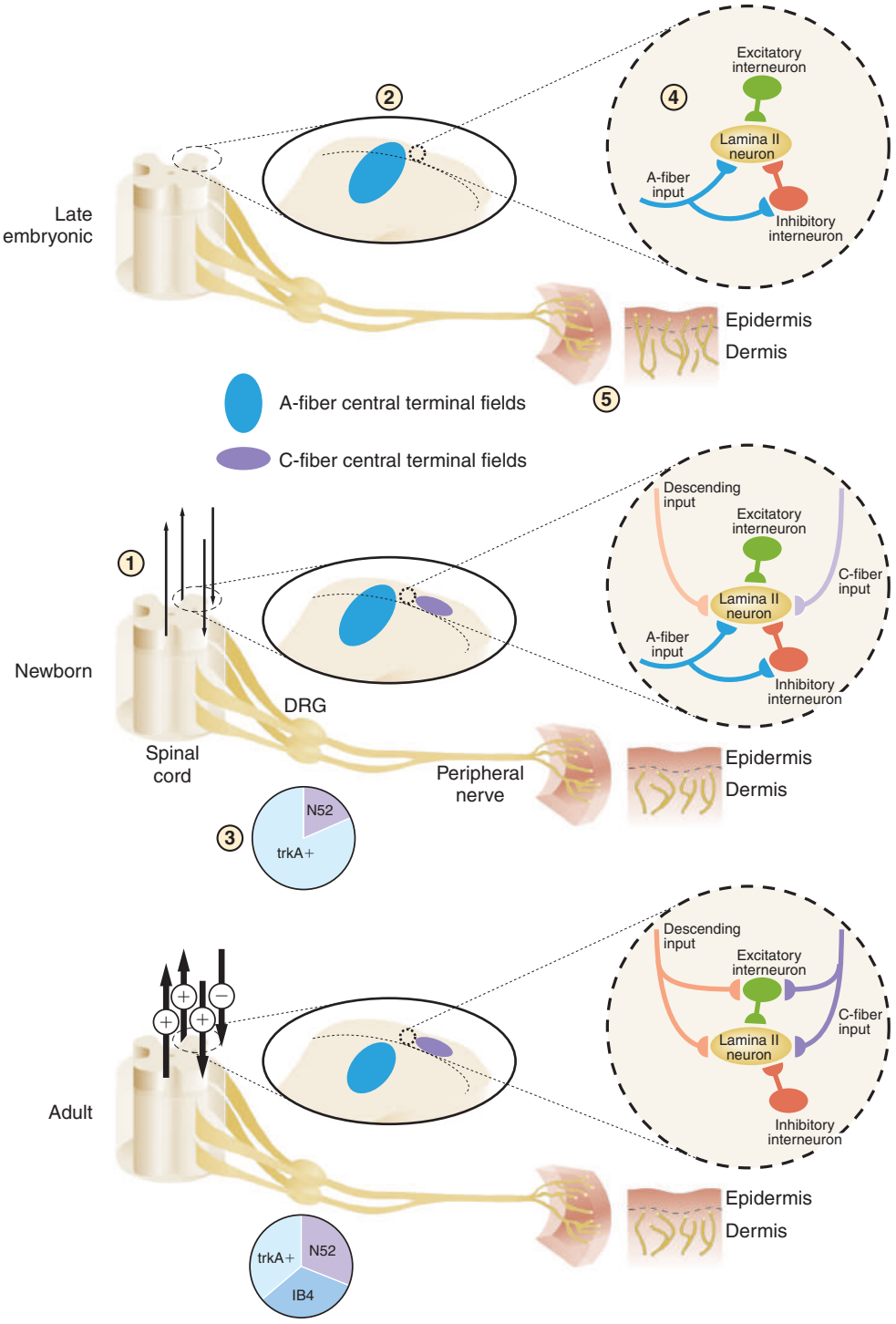
Ontogeny and Development of Pain and Stress Responses

Pain is a progressive, adaptive process that develops gradually throughout fetal life. The human fetus must develop both neurons to sense pain and the cortex to process painful sensations to experience pain.[11] Direct thalamocortical fibers not specific to pain emerge between 23 and 30 weeks' gestational age.[12] These thalamic afferent neurons reach the cortical plate as early as 24 weeks' gestation.[13] Neuronal connections within the cortex appear to form at approximately 22 weeks' gestation.[14] Cortical activity can be captured by electroencephalogram (EEG) as early as 24 weeks as well. However, thalamocortical pathways may not begin functioning until 29 to 30 weeks' gestational age,[12] suggesting that higher cortical level pain processing may be limited despite the presence of a behavioral response.[15] Facial movements consistent with pain response are noted as early as 28 weeks. The possibility of interpreting these facial movements with 4-dimensional ultrasound to assess fetal pain responses is an area of intrigue for those performing fetal surgery.[16]

When infants are born preterm, the sensory system is immature (Fig. 22.1). Afferent input from both noxious and nonnoxious stimuli terminate in the dorsal horn of the spinal cord in a diffuse manner on multiple cells, resulting in the infants' inability to distinguish between noxious and nonnoxious stimuli and limits the care provider's ability to correctly interpret the infants' behavioral response. In the neonatal rat, separation of sensory input is not complete until 3 to 4 weeks after birth (approximately 1 to 2 years in humans);[17] this prevents the newborn from consistently differentiating touch from painful sensory input. The responses of the infant are therefore nonspecific. Local tissue injury resulting from repeated heel sticks and invasive procedures trigger increased proliferation of nerve endings in surrounding tissues, particularly when this damage occurs early in gestation. As a result, scars (e.g., from heel sticks, old intravenous sites) and surrounding tissues can remain hypersensitive well beyond the neonatal period.[18]

Infants may lose any discriminatory ability with repeated painful exposures and develop hypersensitive states for long periods. This hypersensitivity persists even if nonnoxious stimuli are introduced.[18] The responses are less synchronized in the immature central nervous system because of underdeveloped myelination and slower synaptic transmission as manifested in longer and more variable latencies.[18] In addition, the neonate lacks sufficient descending modulatory control, thereby limiting their ability to benefit from endogenous control over noxious stimuli compared with adults.[19,20]

Neurophysiologic investigations have dramatically changed our understanding of nociception in newborns. Using research methods such as near-infrared spectroscopy and EEG techniques such as amplitude-integrated EEG, it is clear that cerebral cortical activation occurs with noxious stimulation, such as needle pokes, as early as 24 weeks' gestation. Moreover, cortical activation has been noted even in infants who manifest no behavioral response to a stimulus.[21] The converse has also been found in studies reporting that oral sucrose administration decreases clinical observational scores with no changes noted in cortical activation from noxious stimulation.[22,23] Such findings raise the question that our patients may be perceiving pain at a cortical level despite our pain-relieving



• **Fig. 22.1** *1*, In early postnatal life, descending fibers are present, but inhibitory and excitatory influences are weak or absent. The connections gradually strengthen, becoming fully functional at the end of the third postnatal week. *2*, A-fibers are the first primary afferents to enter the dorsal horn gray matter and are present during the last few embryonic days. Their distribution is diffuse with exuberant, more superficial projections gradually retracting over the first 3 postnatal weeks. C-fibers are present in the dorsal horn during late embryonic stages but only enter the gray matter 2 to 3 days before birth. Unlike A-fibers, they project to topographically appropriate regions in lamina II of the spinal cord as soon as they enter. C-fiber synaptic connectivity is present, although weak at the time of birth, with connections strengthening over the first 2 postnatal weeks. *3*, At birth, the majority (approximately 80%) of dorsal root ganglion (DRG) neurons express the nerve growth factor (NGF) receptor trkA. Over the first postnatal week, this population reduces, with approximately half of these neurons losing their trkA expression and beginning to express receptors for glial cell line-derived neurotrophic factor (GDNF) (identifiable as the IB4-binding population). *4*, The balance of excitation and inhibition in the superficial dorsal horn develops postnatally through changes in both local interneuron circuitry and descending fibers. A-fiber input is stronger in the neonate and weakens as the influence of C-fiber input increases. *5*, Primary afferent innervations of the skin occur earlier than central projections. By late embryonic stages, primary afferents of all classes have reached the skin and innervate through the dermis into the epidermis. These projections die back during the immediate perinatal period to leave the full adult situation of dermal innervations present soon after birth. (From Beggs S, Fitzgerald M. Development of peripheral and spinal nociceptive systems. In: Anand KJ, Stevens BJ, McGrath PJ, eds. Pain in Neonates and Infants. 3rd ed. Philadelphia: Elsevier; 2007:1124.)

interventions (pharmacologic and nonpharmacologic) and that pain scoring tools may only provide limited information, possibly underestimating pain in neonates.[24]

Noxious stimuli in adults result in the release of inflammatory and trophic factors that activate and sensitize nociceptors in the injured tissue. Such noxious stimuli lead to nociceptive afferent input to the central nervous system, exciting nociceptive circuits in the spinal cord, brainstem, thalamus, somatosensory cortex, cingulate cortex, and amygdala.[25] However, noxious stimuli in infants do not evoke similar patterns of central nervous system activity. The response to noxious stimuli is more diffuse and less spatially focused in infants. Studies in rats demonstrate significant alterations in neuronal circuitry with maturation. These animal data may parallel developmental changes that have been noted in humans.

Behavioral responses to noxious stimuli in infants are not always predictable because of the immaturity of the central nervous system. Typical changes in facial expression related to noxious stimulation are inconsistent before 34 weeks' gestational age.[26] This makes an assessment of pain and response to therapeutic intervention unreliable. Significant structural and functional changes occur in pain pathways during development, and these continue after birth.

In summary, the immature infant's nervous system

- Lacks the ability to discriminate consistently between noxious and nonnoxious stimuli, often reacting with similar behavior to a variety of stimuli
- Lacks the ability to modulate pain responses
- Does not consistently manifest signs or symptoms that allow care providers to accurately assess the infant's level of pain and discomfort

Recognizing and Treating Pain

Neonatal care providers and parents all play front-line roles in recognizing and ameliorating neonatal pain. The factors at play in assessing the depth and breadth of pain and determining its treatment include nationality, level of education of bedside providers, use of clinical pain scales, time of day, and unit acuity. Surveys of neonatal providers from around the world note deficits in recognizing and treating pain. Despite efforts to garner awareness and provide systematic approaches toward ameliorating pain, the provision of analgesia is not consistent.

A prospective cohort of 6680 neonates enrolled in 243 NICUs in 18 European countries, dubbed the EUROPAIN (EUROpean-Pain-Audit-In-Neonates) cohort,[27] cataloged demographics, methods of respiration, use of continuous or intermittent sedation, analgesia, or neuromuscular blockers, pain assessments, and drug withdrawal symptoms over the first 28 days of admission. Wide variations in practice were documented regarding the recording of pain scores, genres of respiratory support and their association with analgesia, and use of analgesia for any neonates receiving any modality of respiratory support. Despite the results of trials such as NEOPAIN,[28] the median use of sedation or analgesia for intubated neonates was 89%, with 74% of these neonates receiving opioids, 25% receiving midazolam, and 25% receiving neuromuscular blockers. The large, prospective nature of this study provides a compelling snapshot of how pain is assessed and how pharmacologic analgesia is prescribed for many neonates in the NICU. Continuous pain assessments occurred in fewer than one third of NICU admissions and daily in only 10% of neonates.[29]

Infant Pain Scores

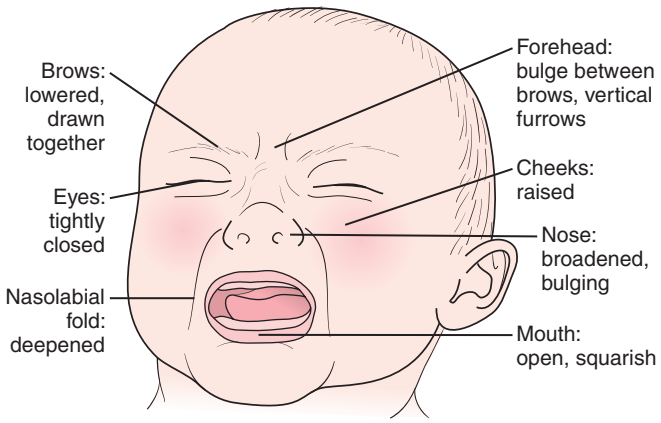
Multiple neonatal pain scoring tools integrate physiologic, behavioral, and biobehavioral indicators into a total score; these tools assess the response to noxious stimuli by categorizing the infant's behavioral or physiologic reactions or a combination of both. The behavioral responses include limb movements, muscle tone, crying, and characteristic facial expressions; crying is the least reliable indicator of neonatal pain. The physiologic measures include heart rate, oxygen saturation, and respiratory rate. Specific facial expression changes are believed to be the most reliable indicators of pain (brow bulge, eye squeeze, nasolabial furrow, taut lips, and open mouth). See Fig. 22.2 for examples of these characteristic facial changes. The use of facial expression changes can be challenging when the infant's face is partially covered with adhesives used to secure tubes and lines, respiratory equipment, or with a phototherapy mask in place.

Many discrete scoring tools exist to assess pain in neonates, but the majority have been validated only for research purposes.[30] Scoring tools that provide a multidimensional assessment of pain are preferred; however, they are still subjective. Some tools lack sensitivity and specificity by relying, for example, only on changes in vital signs.[31] Recent publications list currently available neonatal pain assessment tools.[24,32] Only five of these neonatal pain scales have undergone rigorous psychometric testing with patients serving as their own controls:

- Neonatal Facial Coding System
- Premature Infant Pain Profile (PIPP)
- Neonatal Pain and Sedation Scale (NPASS)
- Behavioral Infant Pain Profile (BIPP)
- Douleur Aiguë du Nouveau-né (DAN).

The PIPP scale is one of the most commonly used and well-validated scales. A review of 62 studies of the PIPP scores over 13 years reinforced this scoring measure as valid, reliable, clinically feasible, and useful at measuring acute, procedural, or postoperative pain[33] with strength lying in a composite (measuring behavioral and physiological indicators) approach. In 2014, researchers refined the scoring tool further, termed the PIPP-Revised score, to better encompass markers of pain and distress across the spectrum of gestational age.[64]

Ideally, a neonatal pain-scoring tool would be matched with the anticipated types of pain that an infant may suffer (such as



• **Fig. 22.2** Facial expressions of physical distress and pain in the infant. (From Hockenberry MJ, Wilson D: *Wong's Clinical Manual of Pediatric Nursing*, ed 8, St Louis: Elsevier; 2011.)

acute, procedural, postoperative, or chronic pain). A common conundrum is that providers find themselves treating chronic pain related to complications of intensive care using tools designed and validated for acute, procedural, and postoperative pain.[4] Several studies have tried to match specific pain scales to specific clinical populations. For example, a prospective comparison of multiple pain scores in neonates after cardiac surgery demonstrated that the COMFORT score was most closely correlated with the infants' pain and analgesic response.[34] COMFORT and N-PASS scales are most effective for pain assessment in mechanically ventilated infants while PIPP and CRIES are recommended for assessment of acute and postoperative pain.[35] Another example is a validated scoring tool called the Echelle Douleur Inconfort Nouveau-Né (EDIN), which was developed specifically to assess prolonged pain in preterm infants.[36]

Practically speaking, it is difficult to train and maintain provider skills in the use of multiple scoring tools in a single NICU. Determining which scoring tool best matches local patient composition allows care providers to become experts in using a single tool or two. The current reliance on routine assessment and documentation of pain scores as a proxy for routine pain assessment for preterm and term neonates throughout their hospital stay may not adequately capture what patients experience, both because of the manner with which they are assigned[37] and the lens through which any given tool was developed.[3]

Bedside Noninvasive Neurophysiologic Measures to Evaluate Pain and Stress

Changes in infant electrophysiological and hemodynamic brain activity occur in response to noxious stimuli.[38] Brain-derived approaches such as near-infrared spectroscopy[39] and amplitude-integrated electroencephalography to capture neonatal and infant pain are promising, as they can assess cortical activation in neonates in response to tactile and painful stimuli.[40] A proposed template of nociceptive brain activity can guide researchers in measuring and interpreting neonatal and infant pain with more precision.[38] The evoked brain activity described by this template is meant to aid researchers but is not yet validated or generalized for direct pain measurement in individual patients.[38]

Clinical pain scores, based on pain-related behavioral changes, do not consistently overlap with noxious-evoked brain activity.[38] Simultaneously measuring physiology, behavior, and cortical response to painful stimuli will help elucidate how best to assess, and then treat, neonatal pain.[41] The *Procedural Pain in Premature Infants (Poppi)* study[42] was one such trial. Because NIRS and EEG do not consistently reflect noxious-evoked brain activity when pain scores and biomarkers such as cortisol suggest a painful response, these tools raise questions about how to define a pain response and what composite measures should be used in research and clinical settings.[43]

Long-Term Consequences of Neonatal Pain and Stress

Untreated pain and stress experienced during the neonatal period are linked to adverse long-term outcomes. The acute physiologic responses to pain—elevations of cortisol, catecholamines, and lactate; hypertension, tachycardia, respiratory instability, glucose instability, and changes in cerebral blood flow—affect developing organs, especially the brain. Exposure to pain and painful procedures is related to the acuity of the hospitalization; studies evaluating a direct relationship between pain and outcomes must account for multiple clinical confounders.

Data suggest that structural changes on MRI at both terms corrected[44] and 7 years of age[45] may be related to increased exposure to pain. Untreated pain in the NICU is also associated with altered pain perception to subsequent immunizations after circumcision without anesthesia, abnormal cortisol responses to stress in later infancy[46] and at school age,[47] and altered pain responses in childhood.[48] Cortisol levels and overall hypothalamic pituitary adrenal axis function at school age for children born preterm are influenced by pain and stress in the neonatal period.[47] Higher cortisol levels at 4 years of age are associated with sensory processing and cortisol reactivity to stress.[49]

Greater neonatal pain exposure is associated with lower body weight percentiles at 32 weeks' corrected gestation.[50] A prospective daily assessment of acute pain and chronic pain/stress concluded that increased pain and stress exposures in the first 4 weeks of life result in altered habituation and stress responses for former preterm infants at 36 weeks' corrected gestational age.[51] There is concern that such hormonal changes might lead to the development of cardiovascular disease and type 2 diabetes in adulthood.[52,53]

Higher numbers of skin-breaking procedures from birth to term-corrected gestation predict lower cognitive and motor development indices of Bayley Scales of Infant Development at 8 and 18 months, after controlling for early illness severity, overall morphine exposure, and days of postnatal dexamethasone.[54] Prolonged placement of indwelling arterial lines to decrease the frequency of skin-breaking painful procedures in the preterm neonates improved thalamic growth and school-age neurodevelopment.[55]

Chronic pain can affect growth, immune function, recovery, and length of hospitalization. In addition, a growing body of evidence has drawn attention to the potentially deleterious effects of repeated handling, stress, and pain on long-term memory, social and cognitive development, and neural plasticity. Greater neonatal invasive procedures are associated with lower thalamic and amygdala volumes, leading to poorer cognitive, visual-motor, and behavioral outcomes.[56]

Clinical Pain and Stress Management Strategies

Fetal Interventions

The fetus has increasingly become a candidate for interventions to repair fetal anomalies and to treat fetal disorders such as severe anemia secondary to hemolytic diseases. The type of anesthesia prescribed to pregnant mothers during fetal surgery falls into one of three categories: anesthesia for minimally invasive fetal surgery, anesthesia for open fetal surgery, and anesthesia for an Ex Utero Intrapartum Treatment (EXIT) procedure.[57] Prescription of maternal anesthesia requires an understanding of placental pharmacology to ensure the fetus is appropriately anesthetized for each procedure. Fetal pain management should aim to attenuate fetal physiologic and hormonal stress responses.[58,59]

Fetal anesthesia is not without risk.[60] Fetal exposure to opioids may result in smaller brain volumes in neonates after birth.[61] There are no human studies examining the effect of anesthesia on the developing fetal brain.[57] Despite this, the US Food and Drug Administration issued a warning in 2016[62] regarding impaired brain development in children, including fetuses during the

third trimester of pregnancy, following exposure to the inhalational anesthetics isoflurane, sevoflurane, and desflurane, and the intravenous agents propofol and midazolam. The impact of these recommendations places the burden of weighing the risks and benefits of anesthesia for fetal surgery on the parents and providers for each fetal and neonatal surgical procedure.[63]

Postoperative Pain Management Strategies

Pain management after surgical intervention, like acute pain management, requires knowledge of the developmental status and function of the end-organ system and the potential adverse and toxic effects of specific analgesic agents. Unfortunately, the current body of neonatal pain literature focuses heavily on procedural pain management and lacks systematic data on acute perioperative pain management in neonates.[64] In the developing world, if infants survive the challenges of diagnosis and surgery for their congenital anomalies, postoperative pain is often not considered or under-managed.[65]

Postoperative pain trajectory in neonates is variable. Ilhan et al. described typical and atypical pain trajectories in infants under 6 months undergoing major thoracic or abdominal surgery, with those in the atypical group having more days and instances of pain. These infants had higher rates of iatrogenic neonatal abstinence syndrome.[66] Taylor et al., surveyed 10 NICUs regarding their postoperative pain assessment and management practices; they found that pain assessment documentation was inconsistent. Nursing documentation was done for most infants, whereas few physicians documented any assessment. Most infants were treated with opioids, benzodiazepines, or both, and some infants (7%) received no analgesia despite recent major surgery.[67] Van der Marel et al.[68] evaluated the use of rectal acetaminophens as an adjuvant treatment to continuous morphine infusion in postoperative neonates and could not demonstrate any additional analgesia effect. However, a quality improvement initiative by Grabski et al. demonstrated that postoperative IV acetaminophen for 48 hours and provider education reduced opioid use, postoperative ventilation time, and use of parenteral nutrition without worsening pain scores.[69] Neonates likely require less morphine to control pain and discomfort than older infants do, based on monitoring of pain scale data. Bouwmeester et al.[70,71] determined that neonates required less morphine for postoperative pain control and that the dose requirement increased with age. Both studies found that morphine was equally effective whether given by bolus or continuous infusion.

Epidurals are effective in treating postoperative pain and alleviating unwanted side effects of systemic opioids in adults and older children. While the literature does support benefits of epidurals for perioperative pain control for term and preterm neonates,[72] there is concern that the risk of placing thoracic epidurals by physicians without sufficient experience exposes neonates to the possibility of catastrophic neurologic outcomes.[73] In a study of infants <6 months of age who required thoracotomy for congenital pulmonary malformations, those randomized to epidural anesthesia had faster time to full feedings and reduced intensity of postoperative care when compared to those randomized to systemic analgesia.[74] Regional anesthesia, when used alone, may also reduce the incidence of postoperative apnea in preterm infants. Studies of parent- and nurse-controlled analgesia (PNCA) as an alternative to continuous opioid infusion for pain management in postsurgical infants in the NICU provides promising data that PNCA may provide more individualized care and potentially reduce opioid consumption.[75,76]

Mechanical Ventilation

Despite an ongoing shift in neonatology toward noninvasive respiratory support for neonates with immature lungs or infants with lung injury, invasive mechanical ventilation is still frequently utilized to save lives. Adults who require mechanical ventilation are routinely sedated. This finding prompted research surrounding opiate sedation in ventilated neonates,[77] despite limited information regarding safety and efficacy in neonates. Preemptive use of pharmacologic sedation during mechanical ventilation in newborns, especially preterm infants, remains controversial.[78]

Mechanical ventilation in neonates is associated with an increase in hormonal stress responses, including increased cortisol and catecholamine levels.[79,80] In the past, infants who appeared uncomfortable while ventilated demonstrated asynchronous respiratory effort (i.e., "fighting" the ventilator), compromised gas exchange, and altered stress responses. Pain and stress in newborns receiving mechanical ventilation was associated with decreased pulmonary compliance, atelectasis, and intrapulmonary shunting. However, with the introduction and use of surfactant replacement therapy, noninvasive ventilatory support, and synchronized ventilation, many of these problems have been minimized or eliminated.[81–83]

A randomized, double-blind, placebo-controlled clinical trial of nearly 900 neonates (NEOPAIN) reported no beneficial effect of preemptive morphine infusions in ventilated preterm infants and an increased incidence of severe intraventricular hemorrhage in preterm infants born at 27 to 29 weeks' gestation receiving preemptive morphine.[28] A parallel study conducted by Simmons in 2003 randomized 150 ventilated neonates to lower dose preemptive analgesia with morphine. This study failed to find any benefit of empiric morphine analgesia and did not recommend this practice.[84] Secondary analysis of the NEOPAIN trial found that morphine infusions were independently associated with increased risk for air leak, a longer requirement for ventilatory support[85], and a longer time to reach full-volume enteral feedings.[86] Neither this trial nor the smaller pilot trials that preceded it[87,88] provided evidence that routine narcotic sedation during mechanical ventilatory support in neonates is beneficial.

A subsequent trial conducted in 2013 randomized premature neonates to continuous infusion of fentanyl or placebo. Similar to prior trials of morphine results demonstrated increased duration of mechanical ventilation and no decrease in prolonged pain in the treatment group. Continuous fentanyl infusion reduced acute pain scores and had fewer side effects than open-label fentanyl boluses. The authors concluded that, just like morphine, "there is no place for the routine use of continuous fentanyl infusion in ventilated preterm newborns because of a lack of continued pain score reduction and increased side effects of continuous infusion compared with the bolus administration of fentanyl."[89]

A Cochrane Review combining outcomes of these and other, smaller trials evaluated the effects of opioid analgesics on pain, duration of mechanical ventilation, mortality, growth, and development in neonates requiring mechanical ventilation.[90] The authors found no differences in mortality, duration of mechanical ventilation, and short- and long-term neurodevelopmental outcomes. As morphine sedation prolongs ventilatory support and time to full enteral feedings, an increase in complications related

to the use of intravenous lines (bloodstream infections) and parenteral nutrition (cholestasis) can occur.[91] Two independent reviews concluded "there is insufficient evidence to recommend routine use of opioids in mechanically ventilated newborns."[91,92]

One comprehensive review of analgesia and sedation for ventilated neonates produced five standard-of-care recommendations[93]:

- Reduce neonatal stress and use nonpharmacological analgesia during invasive ventilation.
- Favor intermittent boluses of opioids, administered after pain scores and before invasive procedures, during short, expected periods of mechanical ventilation.
- Do not use morphine infusion in preterm infants under 27 gestational weeks.
- Always use algometric (pain sensitivity) scores to titrate analgesic drug doses.
- Use premedication before endotracheal intubation.

Additionally, studies of midazolam infusions as an alternative to either morphine or fentanyl infusions for intubated and ventilated infants have raised safety concerns.[94] Midazolam infusions are thus not recommended for neonates.

Procedures

Infants undergoing intensive care endure many painful procedures, often several times each day, with the most immature infants receiving the most significant number of painful stimuli. Pain relief strategies are underused for routine minor yet painful neonatal procedures.[95] Minimizing negative impacts of untreated pain is best achieved with a balance of medications, including opioid-sparing medications and nonpharmacologic agents and behaviors.[96]

Positive correlations between clinical pain scores and malondialdehyde, a marker for oxidative stress, suggest a significant relationship between procedural pain and oxidative stress in preterm neonates.[97] Mode of delivery may alter acute pain response in full-term neonates, with those born vaginally having higher saliva cortisol levels and clinical pain response than those delivered via C-section.[98] Healthy term neonates who are sleeping or drowsy have decreased pain scores and shorter duration of crying compared to awake term neonates when exposed to a painful stimulus.[99]

Both nonpharmacologic and pharmacologic treatment strategies can decrease or completely ameliorate procedural pain and stress.[55] Nonpharmacologic strategies including, but not limited to, sucrose, breastfeeding, nonnutritive sucking, sensorial saturation, and skin-to-skin care can be adapted for many procedures. These strategies are further discussed in "Nonpharmacologic Analgesia."

Blood Sampling and Monitoring

Heel sticks are routinely performed to obtain blood samples from neonates. The heel should be warmed to aid in blood sampling. The most appropriate method for relieving pain from a heel stick has yet to be determined; however, nonpharmacologic therapies are efficacious and without detrimental effects making these the first line of analgesia for heel sticks.[100] A prospective study comparing the ability of fentanyl, facilitated tucking, and sensorial saturation at decreasing both clinical pain scores and cytokine markers of stress concluded that both fentanyl and sensorial saturation were efficacious at decreasing pain and stress from heel lance.[101] Several meta-analyses found skin-to-skin care, termed *kangaroo care*, to be effective at decreasing composite pain indicators for single painful procedures such as heel lance or venipuncture.[102,103] Swaddling,[104] nesting,[105] and sucrose[106] are effective at diminishing acute pain from heel lance. Kangaroo care is efficacious over time at relieving pain from repeated blood draws.[107] Neonates undergoing venipuncture had lower pain scores than those who underwent heel stick for blood sampling. In selected neonates, venipuncture should be used preferentially over heel stick.

A eutectic mixture of local anesthetics (EMLA) cream does not relieve the pain of a heel lance.[108,109] EMLA does not diminish the nociceptive pain response captured from cortical monitoring.[38] Multiple studies have also concluded that acetaminophen alone does not reduce the pain from heel lance.[110] Further, morphine was ineffective at mitigating nociceptive brain activity or diminishing pain scores for heel lance in a prospective, blinded, randomized controlled trial.[42]

Tracheal Intubation

Premedication prior to tracheal intubation facilitates the procedure, controls pain and agitation, and minimizes physiologic disturbances such as bradycardia, tachycardia, and increased intracranial pressure in the newborn. Premedication optimizes conditions for the procedure, increasing success for proceduralists at every skill level. In 2010, the American Academy of Pediatrics issued a policy statement with grade A evidence-based recommendations recommending that all newborn infants receive analgesic premedication for endotracheal intubation except for emergency intubations during resuscitation or infants in whom instrumentation of the airway is likely to be extremely difficult.[111,112] A key component of premedication is analgesia.

Premedication for intubation is inconsistent across NICUs. Concerns about rapid medication availability, the ability to maintain the airway, and the ability to provide ongoing ventilatory support continue to cause controversy. The debate about the best medication regimen is ongoing in the literature, particularly surrounding the INtubate, SURfactant, and Extubate (INSURE) procedure which involves intubation to administer surfactant with subsequent rapid extubation to positive pressure,[113] the concern being that sedation for intubation may make it more challenging to extubate rapidly. One center reported using naloxone to facilitate extubation after INSURE for infants with a poor respiratory drive from sedation for intubation.[114] As the Less Invasive Surfactant Administration (LISA) procedure becomes more common, sedation to facilitate this procedure, with associated analgesia, has been encouraged.[115]

Once the decision has been made to premedicate, the next question is which medications to use. Medication combinations typically include a vagolytic to stabilize patient vital signs, an analgesic to provide pain control, and a muscle relaxant.

Atropine is the preferred vagolytic for intubation. A dose of atropine at 0.02 mg/kg IV or IM, with no minimum dosage, has a grade A evidence-base for its efficacy as a vagolytic.[111,112]

Options for pain control are more varied with studies of opioids such as morphine, fentanyl, and remifentanil. The current best choice is fentanyl, a fast-acting opioid, as morphine has been shown to be no better than a placebo in randomized trials due to its slow onset of action.[111,112] Remifentanil, a short-acting opioid with extremely rapid onset and half-life, is also an acceptable option for analgesia,[116,117] although when given for INSURE in

one study, it predisposed to the rigid chest and provided suboptimal analgesia.[118] Careful administration of fentanyl and remifentanil can prevent rigid chest.[119]

Rapid-onset muscle relaxants also carry grade A evidence-based recommendations for the ability to facilitate the procedure; however, many providers have concerns about depressing spontaneous respiratory drive. Options include depolarizing and nondepolarizing neuromuscular blockers. Nondepolarizing neuromuscular blockers, such as vecuronium or rocuronium, are more common. Vecuronium is given at a dose of 0.1 mg/kg, whereas rocuronium, which has a shorter onset of action and half-life, is given at a dose of 0.5 mg/kg.[119]

Propofol, an anesthetic that provides no analgesia, was thought to be a possible substitute for the combination of atropine and a muscle relaxant because it allows continued spontaneous breathing. Pharmacologic studies failed to identify an optimal propofol dose and patients experienced unpredictable hypotension without achieving adequate sedation.[120] In addition, there is the risk of pain when injected into small veins and extreme pain with extravasation.[111]

Midazolam, a sedative, is contraindicated when intubating preterm neonates, as it has been associated with adverse events in a small prospective, randomized controlled trial.[112]

Circumcision

Neonatal male circumcision is the most common pediatric surgical procedure, performed in 38% to 39% of male infants worldwide.[121] In 2012, the AAP Circumcision Policy Statement[122] not only affirmed the benefits of circumcision but unequivocally stated that analgesia must be provided during neonatal circumcision. Effective, evidence-based options for analgesia include EMLA cream, a topical anesthetic; dorsal penile nerve block, which provides local analgesia; subcutaneous ring block or caudal block, which also provides local analgesia; topical lidocaine; or acetaminophen 10 mg/kg orally.[121] However, anesthesiologists argue that topical EMLA may be insufficient.[123] Subcutaneous ring block has been found to be more effective than EMLA or dorsal penile nerve block. Nonpharmacologic techniques such as breastfeeding, positioning, nonnutritive sucking, sucrose solution, or dextrose solution, when used alone, are not acceptable alternatives for pain control but provide added benefit when used in concert with pharmacologic analgesia.

A randomized clinical trial assessing neonatal circumcision pain with a Gomco versus Mogen clamp, performed in 251 infants, concluded that the Mogen clamp is associated with less physiologic signs of pain but with no difference in CRIES pain scores.[124]

Other Invasive Procedures

Placement of a central venous catheter is facilitated by topical anesthesia with EMLA or infiltration of the skin with lidocaine. In addition, a parenteral opioid, such as morphine or fentanyl, is typically required. Ultrasound-guided central line placement is less painful than standard peripherally inserted catheter (PICC) placement.[125] Intravenous paracetamol is no better than sucrose at mitigating pain with PICC placement.[126]

The pain of lumbar puncture is compounded by both the needle puncture and the distress caused by the body position required for the procedure. EMLA has been shown to decrease the pain of lumbar puncture.[127] Fentanyl does decrease the pain of lumbar puncture with the caveat that it can cause respiratory depression.[128]

Chest tube insertion requires an intravenous opioid, adequate local analgesia (lidocaine), or both.

Laser surgery for retinopathy of prematurity requires pain control. A prospective study concluded that fentanyl infusion was more efficacious than sucrose alone.[129] Fentanyl combined with propofol helped prevent the need for intubation for this procedure while providing adequate analgesia.[130]

Pharmacologic Analgesia

The severity of the pain, its etiology, available administration routes, and potential side effects should all be considered during selection of an analgesic. Once medication administration has begun, careful monitoring for efficacy and side effects can decrease potential adverse events. A key component of effective pain management is continued reassessment after each intervention is introduced, although this is difficult to do with limited pain assessment tools.

Nonopioid Analgesics

Nonsteroidal Antiinflammatory Drugs (Indomethacin, Ibuprofen, Ketorolac)

Prostaglandins are part of the mechanism of local inflammatory response that results in pain. Inhibiting prostaglandin synthesis allows nonsteroidal anti-inflammatory drugs (NSAIDs) to inhibit pain. NSAIDs have many adverse physiologic and developmental effects, including sleep cycle disruption, increased risk of pulmonary hypertension, cerebral blood flow alterations, decreased glomerular filtration rate, alteration in thermoregulatory control, and changes in platelet function. The development of the central nervous, cardiovascular, and renal systems depends on prostaglandins, so these potential adverse effects are particularly worrisome for neonates and infants.

A retrospective analysis by Walsh et al. showed that ibuprofen use was not associated with more side effects in children younger than 6 months compared to those older than 6 months. However, adverse events were increased in the less than 6-month cohort who were prescribed ibuprofen compared to acetaminophen,[131] mainly gastrointestinal bleeding and renal injury. Infants less than 21 days of age and less than 37 weeks' corrected gestational age were more likely to have bleeding events when given ketorolac for postoperative pain than older, more mature infants less than 3 months of age in a retrospective review.[132]

Acetaminophen

Acetaminophen is the most widely administered analgesic in patients of all ages. It inhibits the activity of cyclooxygenase in the central nervous system, decreasing the production of prostaglandins, and peripherally blocking pain impulse generation. Neonates are able to form the metabolite that results in hepatocellular damage; however, it is not necessary to withhold acetaminophen in newborns because of concerns of liver toxicity. In fact, the immaturity of the newborn's cytochrome P-450 system may actually decrease the potential for toxicity by reducing production of toxic metabolites.[133]

Pharmacodynamic and pharmacokinetic studies of acetaminophen in infants recommend less frequent enteral dosing intervals

of acetaminophen (every 8 to 12 hours in preterm and term neonates) because of slow clearance and higher rectal dosing due to decreased absorption. Rectally administered acetaminophen has a longer half-life, but absorption is highly variable because it depends on the individual infant and the placement of the suppository. It should also be noted that the suppository may contain the entire drug in its tip and should be divided lengthwise if a partial dose is desired. Intravenous acetaminophen has become available in recent years with dosing recommendations that lack validated pharmacokinetic or pharmacodynamic correlates.[134] While rectal acetaminophen does not appear to decrease narcotic needs,[68] IV acetaminophen can decrease the need for morphine analgesia in preterm infants[135] and those undergoing major noncardiac surgery.[69,136]

A 2020 Cochrane review emphasized that data are insufficient to establish acetaminophen as an effective analgesic for procedural pain in neonates.[137] Acetaminophen may increase the response to later painful exposures after assisted vaginal birth and may decrease overall opiate needs after major surgery.[137]

Epidemiologic data suggest a link between perinatal acetaminophen exposure and an increased risk of developing asthma,[138] autism spectrum disorder (ASD),[139–141] and attention-deficit hyperactivity disorder.[142] While the association between acetaminophen and autism correlates with maternal use during pregnancy, its risk is mainly seen when ASD is comorbid with a hyperkinetic condition. Meanwhile, the association with asthma is strongest with acetaminophen use in infancy. As with all epidemiologic studies, causation is difficult to ascertain.

Opioid Analgesics

Opioids are believed to provide the most effective treatment for moderate to severe pain in patients of all ages, but there is a wide range of inter-patient pharmacokinetic variability. Opioid dosing depends on the severity of pain as well as the age and clinical condition of the infant.

Morphine

Morphine remains the gold standard opioid for pharmacologic pain treatment in neonates, although not necessarily because it has been shown to be the most effective. It is derived from the opium poppy and metabolized in the liver by uridine diphosphate glucuronyltransferase into two active metabolites: (1) morphine-6-glucuronide (M6G), a potent opiate receptor agonist, and (2) morphine-3-glucuronide (M3G), a potent opiate receptor antagonist. Both metabolites and some unchanged morphine are excreted in the urine. The predominant metabolite in preterm and full-term neonates is M3G. Because of slow renal excretion, both metabolites can accumulate substantially over time, especially M3G.[143] There is thus the potential for late respiratory depression because of a delayed release of morphine from less well-perfused tissues and the sedating properties of the active M6G metabolite.

Using the lowest dose possible to achieve the needed analgesia is advised. Escalating morphine doses will also increase the levels of M3G in the infant, interfering with the goal of adequate analgesia. Doses as low as 1 to 5 µg/kg/h can provide adequate analgesia, minimizing the risk of accumulation of high M3G levels, given that metabolite's prolonged half-life.[70,71]

Clearance or elimination of morphine and other opioids is prolonged in infants because of the immaturity of the cytochrome P-450 system; it approaches that of adults after 6 months of age. Chronologic age is a better indicator than gestational age of infant opioid metabolism.[144]

Infants are at greater risk for opioid-associated respiratory depression because of their immature respiratory control mechanisms. There is an increase in unbound or free morphine and M6G available to reach the brain as a result of the reduced concentration of albumin and α_1 acid glycoproteins.

Hypotension, bradycardia, and flushing constitute the response to the histamine release associated with rapid intravenous administration of morphine. Histamine release may occasionally cause bronchospasm in infants with chronic lung disease. Morphine sedation may result in the extended need for ventilatory support in neonates.[85] The duration of morphine is also a predictor of severe necrotizing enterocolitis.[145]

Dosing recommendations currently reflect the wide range of inter-patient pharmacokinetic variability. In the past, 0.03 mg/kg of morphine IV was suggested as a starting dose in infants not receiving mechanical ventilation, whereas 0.05 to 0.1 mg/kg of morphine IV was recommended as an appropriate starting dose in infants on ventilators. Significantly lower doses are now recommended.[146] Titration to the desired clinical effect is required by adjusting the dose and the frequency of administration while continually assessing needs and responses. As the use of morphine for analgesia and sedation in neonates is explored further, it is becoming clear that some of the risks (as discussed in the Long-Term Effects of Neonatal Opioid Exposure section) without clear improvement in measures of pain should encourage conscientious morphine prescription.[30] With this in mind, efforts to reduce opioid exposure in the hospitalized neonatal and pediatric population are being undertaken with strategies aimed at standardizing analgesia management pathways, including weaning as well as the use of pain and withdrawal scores.[147,148]

Fentanyl

Fentanyl is 80- to 100-fold more potent than morphine and causes less histamine release, making it potentially a better choice for infants with hypovolemia, hemodynamic instability, chronic lung disease, or congenital heart disease. Another clinical advantage of fentanyl is its ability to reduce pulmonary vascular resistance, which can benefit infants who have undergone cardiac surgery, have persistent pulmonary hypertension, or need extracorporeal life support. Bolus doses of fentanyl must be administered over a minimum of 3 to 5 minutes to avoid chest wall rigidity, a serious side effect observed after rapid infusion. This adverse effect is treatable with naloxone or a muscle relaxant. Intranasal fentanyl has been shown to reduce pain scores during retinopathy of prematurity screening examinations without increasing the risk of respiratory depression.[149]

Fentanyl has a quick onset and relatively short duration of action. In infants, 3 to 12 months of age, total body clearance of fentanyl is greater than that of older children, and the elimination half-life is longer because of its increased volume of distribution.

Fentanyl is highly lipophilic. A rebound transient increase in plasma fentanyl levels is a phenomenon known to occur after discontinuation of therapy in neonates. It is a result of the accumulation of fentanyl in fatty tissues, which can prolong its effects after continued use; therefore, caution must be exercised in the use of repeated doses or continuous infusions. Because of tachyphylaxis, continuous infusion rates of fentanyl are often increased to maintain constant levels of sedation and pain control. Infusion dosing can reach substantial levels, which then require a prolonged withdrawal period.

Enterally Dosed Opioids

Methadone is used in infants, primarily in the context of withdrawal from either maternal narcotic use or chronic narcotic use in the NICU. The limited data regarding its efficacy and pharmacokinetics in this population suggest no dosing modifications are needed from those recommended for adults.[150] The respiratory depressant effect of methadone is longer than its analgesic effect; it is metabolized slowly and has a long half-life.

Codeine use in any pediatric patient is not recommended due to safety concerns.[151] No data are available regarding its effectiveness in neonates, and its use in this population is discouraged. Acetaminophen and codeine can be given in a set formula, consisting of acetaminophen (120 mg) and codeine phosphate (12 mg/5 mL) with alcohol (7%). The dose prescribed is limited by the appropriate dose of codeine and the safe dose of acetaminophen. This combination is not recommended in neonates.

Oxycodone dosing at 0.05 to 0.15 mg/kg orally every 4 to 6 hours has been used in neonates. However, no data are available to recommend oxycodone in neonates.

Nalbuphine is a mixed agonist-antagonist opioid receptor drug; therefore its administration in infants of opioid-addicted mothers may precipitate withdrawal. This agent is equianalgesic with morphine and has an analgesic ceiling effect. It is not recommended for neonates, although it may be useful during opioid drug withdrawal treatment.[152]

Long-Term Effects of Neonatal Opioid Exposure

Experimental Animal Studies

Perinatal and neonatal opioid exposure in experimental animals is associated with both short- and long-term adverse neurologic effects that should prompt clinicians to wonder whether use of such medications is warranted. Opioid receptor-mediated signaling likely has a role in several aspects of early brain development.[153] The developing cerebral circulation is extremely vulnerable to physiologic perturbations and the effects of drugs. Cerebrovascular effects of drug exposure early in development can have lifelong consequences, including increased risk for stroke. Perinatal narcotic exposure restricts brain growth, induces neuronal apoptosis, and alters behavioral pain responses later in life.[154]

The acute effects of exogenous narcotics, including morphine, on the developing cerebral circulation, have been described in piglets and include modulation of prostaglandin-induced pial artery dilation during hypoxia, alteration in endothelin production, and increases in endothelin A receptor mRNA expression.[155] Morphine causes dose-mediated responses to mRNA expression in mice that suggest long-term alterations in hippocampal functioning.[156] Endogenous opioids are important regulators of cerebrovascular tone and angiogenesis. Permanent neurobehavioral and neuropathologic changes are demonstrable in a rodent model of neonatal stress and morphine exposure.[157–159] Morphine causes rodent thalamic cell death.[160] Certain alleles of morphine biotransformation gene variants in combination with increased morphine exposure in the neonatal period are linked to anxiety, depression, and externalizing behaviors at 18 months corrected age.[161]

Understanding the clinical relevance of these animal studies regarding the long-term effects of neonatal opioids is difficult because of species differences in the timing of brain development, the development of opiate receptors and major neurotransmitter systems, and the pharmacokinetics of administered opioids.

Clinical Studies

Clinical studies addressing the short- and long-term effects of prolonged opiate use in neonates are limited. The few that exist are contradictory and confounded by illness severity. Possible confounders including illness acuity, concurrent medications such as steroids and benzodiazepines, and morphine given preemptively make these results difficulty to interpret.[162] Cohort studies using regression analysis to control for drug exposure, gestational age, acuity, and other variables, are inconsistent but concerning in their reports.

The EPIPAGE cohort in 2008 noted that preterm infants exposed to more than 7 days of sedation or analgesia were at increased risk of severe or moderate disability at 5 years. However, this association did not remain significant when adjusting for gestational age and infant acuity.[163] Conversely, the EPIPAGE 2 cohort[164] published data in 2014 noting that ventilated preterm infants treated with continuous opioid and/or midazolam infusion in the first 7 days of age were more likely to survive without measurable differences in moderate or severe sensorimotor impairment at 2 years of age.[165]

A 2015 cohort study of preterm infants noted that low-dose morphine analgesia in the NICU was associated with altered cerebral structure and behavioral problems that resolved in childhood.[166] A cohort study published in 2016 noted that premature infants correlated worse cognitive scores on the Bayley III test administered at 20 months of age with high levels of opioid exposure in the neonatal period after controlling for neonatal and social confounders.[167]

Preterm infants evaluated at 36 weeks' post-conceptual age in the prospective, randomized 2004 NEOPAIN study demonstrated neurobehavioral abnormalities if exposed to morphine.[168] A small pilot follow-up of the NEOPAIN trial in 2012 included only 19 patients at age 5 to 7 years and showed an association between those given preemptive morphine infusions and smaller head circumference, lower body weight, and increased social problems, and altered response latencies.[169] Two-year follow-up results of the study randomizing ventilated infants to fentanyl versus placebo for analgesia concluded in 2017 that infants randomized to fentanyl had impaired hand-eye coordination not explained by acuity alone.[170]

A cohort of preterm infants admitted to a single NICU was tracked prospectively for acuity, painful procedures, and morphine exposure. Results published in 2016 noted that MRIs performed at term equivalent gestation demonstrated a strong statistical association of morphine exposure with smaller cerebellar volumes and poor motor and cognitive outcomes despite rigorous control for confounders.[171]

Several follow-up studies tracked some of the 150 neonates included in a 2013 randomized controlled trial of preemptive analgesia for ventilated, preterm neonates.[84] Five-year follow-up controlling for open-label morphine and clinically relevant variables implied a trend toward more negative outcomes in subjects who received morphine concerning intelligence, visual-motor integration, behavior, chronic pain, and health-related quality of life. Morphine use, both preemptive and open-label, was also associated with worse performance on the visual analysis subset of the intelligence quotient testing.[172] When the same investigators conducted follow-ups at 8 to 9 years of age, they obtained different results. IQ testing did not differ between the two groups, and children who were exposed to morphine had fewer problems on the subscales of inhibition, organization of materials, and monitoring as observed by parents and on the subscale

of planning and organization as observed by their teachers.[173] No differences were found in thermal detection and pain thresholds, the incidence of chronic pain, or overall neurologic functioning between those who received continuous morphine infusion in the NICU for preemptive analgesia and those who did not arrive at 8 to 9 years of age.[174]

The Preterm Epo Neuroprotection Trial (PENUT Trial), published in 2020, enrolled 941 extremely low gestational age infants and evaluated survivors at 2 years of corrected age. Prolonged combined use of opioids and benzodiazepines increased the risk of poorer neurodevelopmental outcomes.[175]

The conflicting nature of these studies, multiple clinical confounders, and the fact that few of these studies are adequately powered to address the issue of long-term effects of neonatal morphine exposure make the question of the long-term effects of morphine on the developing brain an area in need of robust research.

Long-term use of narcotics and other drugs leads to drug tolerance and the need to wean slowly to avoid drug withdrawal. The drugs themselves, as well as drug tolerance and drug withdrawal, may all contribute to adverse effects on brain development and neurodevelopmental outcomes.

Topical and Local Anesthetics

Lidocaine reduces the pain and stress of venipuncture and IV catheter placement. ELMA is a cream containing the active ingredients lidocaine and prilocaine. The cream can be placed on the area where anesthesia is desired and then covered with an occlusive dressing for 1 hour before a procedure. Longer application times provide deeper local anesthetic penetration but can lead to toxicity. There is a slight risk of methemoglobinemia with the use of EMLA cream in patients who are G6PD deficient and in infants younger than 12 months who are also receiving methemoglobinemia-inducing drugs such as acetaminophen, sulfonamides, nitrates, phenytoin, and class I antiarrhythmics. A rare occurrence, methemoglobinemia, can happen when hemoglobin is oxidized by exposure to prilocaine. EMLA should not be used in patients with methemoglobinemia. A study of 30 preterm infants found that a single 0.5-g dose of EMLA applied for an hour did not lead to a measurable change in methemoglobin levels. A systematic review concluded that EMLA diminishes the pain during circumcision. Limited efficacy was noted with pain from venipuncture, arterial puncture, and percutaneous venous line placement.[176] EMLA was not found to diminish pain from heel lancing.[109] A Cochrane review concluded that there is insufficient evidence of the effectiveness or safety of EMLA in term or preterm infants to support clinical recommendations.[177] Oral sucrose or glucose may be as effective as EMLA for venipuncture.

Sedatives

Benzodiazepines

Benzodiazepines such as lorazepam and midazolam are sedatives that activate gamma-aminobutyric acid receptors and should not be used in place of appropriate pain medication, as this class of medication has no analgesic effect. For painful procedures, an analgesic must be used in conjunction with the benzodiazepine. Benzodiazepines are administered to decrease irritability and agitation in infants, providing sedation.

In ventilated infants, benzodiazepines can help to avoid hypoxia and hypercarbia from breathing out of synchrony with the ventilator, although the common use of synchronized infant ventilators makes this clinical problem much less likely. When given continuous infusions, dosing often escalates rapidly to maintain apparent sedation creating a need for prolonged weaning. In the 1999 NOPAIN trial, midazolam was associated with a significantly higher incidence of adverse neurologic events such as death, severe intraventricular hemorrhage, and periventricular leukomalacia.[88] Meta-analysis concluded that there are "insufficient data to promote the use of intravenous midazolam infusion as a sedative for neonates undergoing intensive care…[and there are] concerns about the safety of midazolam in neonates."[94]

Use of such benzodiazepines is associated with abnormal neurologic movements in both preterm[178] and term infants.[179] Prolonged midazolam dosing in preterm infants has been correlated with abnormal hippocampal growth and poorer neurodevelopmental outcomes.[180] Despite this, midazolam is commonly prescribed for infants receiving mechanical ventilation.[27] Clinicians are concerned and should use these drugs with caution in the NICU.

Dexmedetomidine and Clonidine

Dexmedetomidine is a potent and relatively selective α_2-adrenergic receptor agonist indicated for the short-term sedation of patients in intensive care settings, especially those receiving mechanical ventilatory support. The drug is given by either IV bolus doses for short procedural sedation (1 to 3 μg/kg) or continuous intravenous infusion (0.25 to 0.6 μg/kg/h). Because dexmedetomidine does not produce significant respiratory depression, it has been used for procedural interventions in spontaneously breathing infants.[181–183] Animal studies have been reassuring, and some have demonstrated that dexmedetomidine may be neuroprotective.[184–186]

As neonatologists become more familiar with dexmedetomidine, its use has increased;[187–191] however, short- and long-term safety and effectiveness information in infants is limited. A trial prescribing dexmedetomidine for infants undergoing therapeutic hypothermia reported no adverse events with decreased opioid usage for those in the treatment arm.[192] Dexmedetomidine successfully reduces benzodiazepine exposure when given as an intranasal sedative for preterm infants undergoing MRI.[193] In contrast, the use of dexmedetomidine in a retrospective study of surgical neonates did not show reduced opioid exposure highlighting the need for effectiveness data.[194]

Clonidine, another α_2-agonist similar to dexmedetomidine, is not deemed efficacious or safe based on available data to recommend its use as a sedative for infants receiving mechanical ventilation, according to a recent Cochrane review.[195]

Gabapentin

Gabapentin is a gamma-aminobutyric acid (GABA) analog that acts by binding the α_2-δ subunit on voltage-gated calcium channels, thereby blocking the release of excitatory neurotransmitters that cause pain.[196] It is predominantly renally excreted, and children under 5 years require approximately 30% higher doses, presumably due to age-related changes in renal function.[197] Gabapentin utilization in NICUs is highly variable, with patients ranging from term infants, ≤28 weeks' gestation preterm infants, and infants with chronic genetic, neurological, and gastrointestinal diagnoses.[198] Its use in neonates has been limited to those with refractory pain or agitation that fails to respond to standard therapy with opiates and benzodiazepines. In a retrospective observational study of 22 infants in a level IV NICU, gabapentin

successfully reduced N-PASS scores and the need for analgesic and sedative medications.[199] Gabapentin has also been used for visceral hyperalgesia due to neurologic or gastrointestinal morbidities. A study by Edwards et al. in this population noted improvement in chronic irritability and feeding intolerance as well as decreased usage of opioids and benzodiazepines.[200] Caution is advised as adverse events occurred with abrupt discontinuation.[200] This limited but growing literature suggests there may be a subset of neonates with refractory pain, agitation, or neurodevelopmentally impaired infants with feeding intolerance, for whom gabapentin may be a useful adjunct.

Nonpharmacologic Analgesia

Nonpharmacologic interventions for prevention or relief of neonatal pain and stress are numerous, can be effective, and have minimal risks or disadvantages for patients, yet are frequently underused.[201] This may be in part to inconsistent findings on effectiveness, small pain relief provided and/or the lack of a clear superior method.[202] Nonetheless, nonpharmacologic strategies to minimize pain should always be undertaken, especially with commonly performed procedures in the NICU.[203] The 2016 American Academy of Pediatrics policy update on prevention and management of pain endorses nonpharmacologic management as a key component to infant analgesia.[32]

Oral sucrose is the most widely studied nonpharmacologic "analgesic"; it safely and effectively reduces the acute behavioral response to procedural pain from one-time painful events.[106] Clinical equipoise suggests further studies of sucrose must enroll controls who have efficacious analgesia.[204] Sucrose does not, however, reduce spinal reflex responses, cortical activity,[22,23] or hyperalgesia, raising philosophical and ethical questions about the use of sucrose for procedural analgesia. "Sugar may be better understood not as an analgesic, removing or relieving pain, but as a compensating pleasure."[205] When combined with other nonpharmacologic analgesic interventions, sucrose continues to effectively decrease neonatal behavioral responses to pain. Sucrose plus nonnutritive sucking, for example, provides improved pain relief (as assessed by behavioral scores) from heel lance compared to placebo or either method alone.[206] However, despite immediate pain relief, glucose used for analgesia does not appear to mitigate the adverse effects of pain on brain development at 18-month neurodevelopmental follow-up.[207] Sucrose use in highly preterm, unstable, and ventilated neonates is not adequately addressed in the literature, nor is the effect of repeated sucrose administration.[23]

Several other nonpharmacologic therapies provide efficacious analgesia, alone and in concert with each other. Nonnutritive sucking with pacifiers reduces pain responses to heel prick, injections, venipuncture, and circumcision procedures.[208–210] Nonnutritive sucking is also efficacious in combination with breast milk and/or facilitated tucking (flexed position) to mitigate acute procedural pain.[211] Infant massage has been demonstrated to decrease plasma cortisol and catecholamine levels in preterm infants.[212,213] Preterm neonates benefit from skin-to-skin contact[214] or facilitated tucking[215] to ameliorate acute procedural pain responses. Among positioning strategies, facilitated tucking by parents for at least 30 minutes duration, starting 15 minutes before, during, and 15 minutes after a painful procedure, was recommended in a systematic review as the first position of choice during painful procedures in the NICU.[216]

Skin-to-skin contact (also termed *kangaroo care*) is associated with greater physiologic stability and reduced responses to and recovery from acute pain.[103] Kangaroo care is also efficacious for repetitive painful procedures in the NICU.[107] Kangaroo care can decrease Neonatal Infant Pain Scale scores after vitamin K injections.[217] When compared to oral sucrose, kangaroo care during heel lancing is more effective for pain relief in preterm infants.[218]

Breastfeeding in combination with kangaroo care reduces the physiologic and behavioral responses to acute pain and stress and is the first line of treatment.[219–221] Breastfeeding[222] and feeding expressed breast milk[223] can decrease acute procedural pain, as can the odor of maternal breast milk.[224] In full-term infants, direct breastfeeding is more effective in reducing pain responses than maternal holding, skin-to-skin, topical anesthetics, and music therapy, and more effective than sucrose solutions.[225]

Another approach is multisensory stimulation (sensorial saturation) of preterm infants undergoing painful procedures. This approach entails simultaneous gentle massage, soothing vocalizations, eye contact, smelling perfume, and sucking on a pacifier. This technique was associated with analgesia and calming the infants in several reports from one unit.[226–229]

Music therapy may reduce the behavioral and physiologic responses to acute procedural pain.[230] Maternal rocking, distinct from kangaroo care, diminishes neonatal distress.[231] Warmth is also an analgesic for acute, procedural pain in healthy term infants.[232,233] Other interventions such as white noise[234] and flaxseed pillows[235] are under investigation for their effects as either an acute analgesic or modulator of chronic pain.

Summary

Recognition and treatment of pain and discomfort in neonates remain challenging. Despite significant progress in the understanding of human neurodevelopment, pharmacology, and more careful attention to the care of sick infants, there is still much to learn. Potentially best practices include avoiding aggressive interventions, prioritizing nonpharmacologic pain management, and grouping interventions and exams with nursing care times. Providers can limit skin-breaking procedures by batching or decreasing blood draws and by maximizing the use of indwelling central lines in lieu of heel lancing for lab draws as able. When pharmacologic analgesia is prescribed, providers should utilize the least amount of drug that controls the pain and discontinue the medication as soon as possible; escalation of drug doses may exacerbate the problem. Bedside providers should feel empowered to prevent pain by making time for appropriate and effective pharmacologic and nonpharmacologic analgesia.[236] Mindful practice to minimize pain and distress mitigates adverse effects on neurodevelopment.

Suggested Readings

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23

Palliative Care

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KEY POINTS

- Palliative care is the total care of a patient with a life-limiting illness regardless of the disease trajectory or treatment options chosen.
- There is a special focus on pain/symptom management, communication, quality of life, family support, and grief support.
- Up to 3% of pregnancies are complicated by a life-limiting diagnosis. Roughly one-third of deaths in children's hospitals in the United States occur in neonatal intensive care units (NICUs). Many babies and families in the NICU benefit from palliative care.
- There is a special role for palliative care when the likelihood of long-term survival is uncertain or minimal or when there is a low likelihood of survival without severe morbidity. Decisions around the most appropriate treatment plan are often based around views of acceptable quality of life.
- While palliative care is essential to individuals in the NICU, it is often overlooked, and there are many barriers to it being considered or implemented in suitable cases.
- Palliative care can be readily integrated in to the management of neonates with life-limiting illnesses and can be provided concurrently with cure-oriented or life-extending care.

What Is Palliative Care?

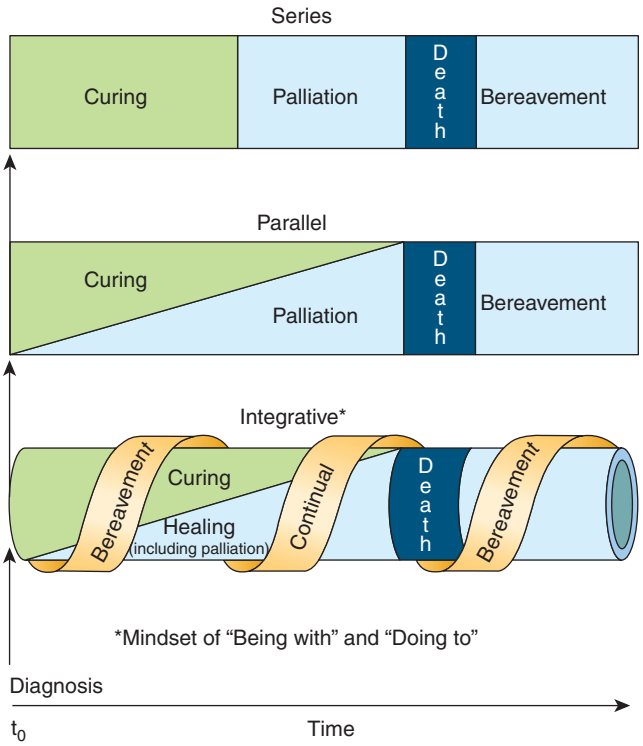
The concept of palliative care has been acknowledged in medicine for centuries. The name is derived from the Latin word *palliare*, meaning "to cloak," and has been more broadly interpreted as "to alleviate without the intent of curing." Over the years the definition and scope of palliative care have evolved.[1] The field gained great momentum with adults during the 1960s with the hospice movement and the realization that modern medicine alone does not address all of the issues patients with serious and life-limiting illnesses experience. More recently, the incorporation of palliative care in pediatrics has become a critical focus for the field.[2–11] The World Health Organization defines pediatric palliative care as the total care of a child with a life-limiting illness. It involves caring for the mind, body, and spirit of the child and supporting the family through the process. It begins with diagnosis and continues regardless of the disease trajectory or treatment options chosen. There is a special focus on pain and symptom management and alleviating distress through a multidisciplinary approach.[12] Palliative care is not limited to end-of-life (EOL) care. It can occur concurrently with other treatments in an attempt to cure a patient or prolong his or her life independently of if or when the transition to EOL care occurs. Hospice care is a small and important part of palliative care. Once it has been determined that there is no chance of meaningful survival, the focus of care shifts to pain and symptom management, grief support, preparing for the dying process, and bereavement support.[13]

In recent years, the American Academy of Pediatrics has acknowledged the importance of this growing field and recognizes that all large healthcare organizations providing care to children with life-limiting illnesses should have a dedicated pediatric palliative care team. They have discussed in detail the principles for palliative care and the importance of educating trainees.[14,15] Governing academies and families alike have come to see the importance of the provision of palliative care in pediatrics and more specifically in taking care of the sickest neonates. US News and World Report's Best Children's Hospitals Rankings in Neonatology now include a section for neonatal intensive care unit (NICU)-specific palliative care programs.

Despite the increasing focus on neonatal palliative care, the concept has existed for decades. In the 1970s the difficulties of withholding therapies in a special care nursery were described for the first time.[16] In this landmark article, the authors describe a collaborative process in which parents and physicians determined through joint decision-making the low likelihood of meaningful survival for 43 babies and opted to redirect care for them. They also acknowledge the difficulty of these decisions for parents and physicians, something that is still experienced today. Several years later, the notion of adapting concepts from an adult hospice model to address issues related to caring for a dying neonate in various settings was described.[17–21] Since this time, the importance of extending these concepts to the perinatal period and providing such care during pregnancy once a potentially life-limiting fetal diagnosis is made has been recognized.[22]

Paradigms of Palliative Care

There are multiple paradigms in which palliative care can be provided. As Fig. 23.1 illustrates, the first model represents the serial approach. This is congruent with the earliest view of palliative care. Once a terminal condition has been diagnosed, curative therapies continue until they are seen as futile. Then palliation and comfort measures are offered until the point of death. After death, bereavement support is offered. As the field of palliative care has evolved, adult palliative care has adapted the parallel model in which palliative care is introduced early in combination with curative therapies, and as the disease trajectory changes with time, the care model changes with it. Again, once death occurs,



• **Fig. 23.1** Various models for providing palliative care. (Modified from Milstein J. A paradigm of integrative care: healing with curing throughout life, "being with" and "doing to." J Perinatol. 2005;25:563–568.)

bereavement support is offered. The final model, the integrated model, is one that has been adopted most often by the growing field of pediatric palliative care and is most conducive to the distinctive environment of the NICU and neonatal-perinatal palliative care. When a life-limiting diagnosis is made, the concepts of palliation are often introduced at times along with curative therapies and the focus and goals change with the disease trajectory. With recognition that the family is experiencing loss on multiple levels at the time of diagnosis, bereavement and grief support are offered in a multidisciplinary fashion from the time of diagnosis, through the entire illness course, and after death.[23]

Because the perinatal-neonatal period is unique, it requires a different approach to palliative care from that in most other fields. The perinatal-neonatal period is usually a time of joy, anticipation of life, and hope for making many memories growing as a family. When a diagnosis of a life-limiting condition that results in a high likelihood of fetal or neonatal death is made, every milestone and stage of the pregnancy, birth, life, and death are very different from what was hoped for and anticipated.[11,24–29] In the NICU environment, clinicians are trained to offer intensive and invasive therapies with the goal of saving lives. It can be challenging to shift goals of care in the face of a life-limiting illness.[25,30–32] With increased recognition of the importance of neonatal-palliative care and the unique challenges clinicians face, the number of programs dedicated to providing this care continue to grow. There are marked differences in the structures of these programs and how care is provided.[33,34] While there is no standard for the components of a neonatal-perinatal palliative care program or how the care is delivered, a consistent and important theme is the focus on and goal of maximizing quality of life and providing support to families as they navigate through goals of care and complex medical decisions. Regardless of the specific make up, having formal programs in place leads to more comprehensive and cohesive care.[35]

Scope of the Problem

While neonatal–perinatal palliative care provides unique challenges for families and healthcare professionals alike, it is something that most individuals who care for neonates will be faced with. Despite the many advances in medical therapies and improvements in neonatal survival and outcomes, newborns will still die. At times, while the timing and cause of death may differ depending on the type of care provided, some babies will die regardless of the treatment options chosen by the family and medical care team. Each year, in the United States, there are more than 1 million fetal deaths, with more than 20,000 occurring after 20 weeks' gestational age.[36] Up to 3% of pregnancies are complicated by a life-limiting fetal diagnosis.[37–39] Expanded testing options and advanced technology have allowed for earlier and more frequent life-limiting diagnoses to be made in utero.[22,40] When such a diagnosis is made, between 20% and 40% of individuals choose to continue the pregnancy.[41] Some fetuses will die in utero. For others a live birth is followed by a death in the immediate neonatal period. Other newborns with a life-limiting diagnosis will survive for a period of time, even beyond the neonatal period, with interventions.[22,42–45] Yet there are more than 15,000 neonatal deaths each year, accounting for nearly 70% of deaths within the first year of life.[36] Roughly one-third of deaths in children's hospitals in the United States occur in the NICU setting.[46] As a result, most clinicians who care for neonates will be faced with the death of a patient and navigating goals of care with a family. Most infants who died within the first year experienced severe and chronic illnesses stemming from the time of birth. When a baby survives, there may be multiple treatment options or approaches to care. Some of the options may alter the disease trajectory but cannot correct the diagnosis or significantly impact the prognosis. It is important that families understand the prognosis, implications of interventions and care plans, and explore goals of care so that there is a focus on value-driven medical care when determining the most appropriate treatment options. With a value-driven approach, some families will seek a comfort measures-only approach to care while others, up to 50%, wish to explore varying degrees of life-sustaining medical interventions.[47]

Given the likelihood that neonatologists will care for patients with life-limiting illnesses throughout their careers, it is pertinent that they incorporate the precepts of palliative care in to daily practices to best care for patients and families. The uncertainty of prognosis, physical constraints of the NICU, time constraints, moral distress, and lack of education are known barriers to providing palliative care in the NICU.[28,31,32,48–50] In an intensive care setting it can be challenging to shift from cure-directed aggressive and invasive interventions to palliation and comfort care. However, early initiation of palliative care has been associated with improved memory making, decreased pain, decreased rates of invasive procedures that are unlikely to change the outcome, and increased parental satisfaction.[4,51–53] Consequently, it is imperative that all healthcare professionals view success for the unique subset of patients with life-limiting illnesses as eliminating unnecessary suffering, supporting families through navigating complex decisions, helping them find meaning in their baby's life, and ensuring that the care provided is in line with the values and goals of the family.[23,54] Failure in these situations is not the

inability to sustain life but rather unnecessary pain and suffering and an undignified death.

Which Patients Benefit From Neonatal-Perinatal Palliative Care?

Many neonates will respond to intensive care therapies, graduate from the NICU, and lead long, meaningful lives. Knowing this, which patients and families benefit from palliative care in the NICU setting? There is a role for palliative care in neonates with serious but possibly reversible or treatable conditions where intensive interventions will be beneficial. Arguably, many aspects of palliative care should be incorporated in to the multidisciplinary approach to care of any baby in the NICU. Any family member of a newborn requiring resuscitation or intensive interventions, regardless of the ultimate outcome, experiences grief and loss. The family has to alter its expectations for a healthy and uneventful pregnancy leading to a healthy baby. The family often requires spiritual, emotional, and psychosocial support to navigate goals and cope with this trauma both in the immediate period and moving forward.[55]

However, there is a special and more pronounced role for palliative care when the likelihood of long-term survival is uncertain or minimal. This is also true when there is a low likelihood of survival without severe morbidity or prolonged technology dependence. Decisions around the most appropriate treatment plan are often based around views of acceptable quality of life. At times it may help both clinicians and families who find it challenging to process this to a consider what the quality of each day is like in the NICU. When death is almost certain or the chance of survival without severe morbidity that would lead to an unacceptable quality of life is minimal, continuing life-sustaining treatments may prolong the suffering of the neonate and give the family false hope of meaningful survival. The care initiated and the treatments that follow may be aggressive and at the expense of the comfort of the neonate. Palliative care focuses on the prevention and relief of physical pain and suffering that can be experienced by periviable or extremely premature infants, actively dying infants, or those severe morbidities and possibly technology dependence, as well as on supporting the needs of the family.[56] Everyone involved in the care of these neonates, as well as the neonates themselves, can strongly benefit from this type of care. In these situations, regardless of whether a neonate dies, lives with a permanent impairment, or goes on to live a normal life, there is a necessary role for palliative care. Beyond addressing symptoms such as pain, this approach offers support to families and addresses short-term and long-term measures to ensure that there is value-driven medical care so the infant has the best quality of life for as long as they may live.[21,57–59]

While every patient and family dynamic is different and goals need to be explored for each patient, there are several categories where it is appropriate to discuss transitioning from life-extending therapies to comfort measures:[21,22,24,25,28,42,54,60,61]

1. Periviable or extremely premature newborns based on gestational age or birth weight, especially when they have significant complications.
2. Neonates with multiple congenital anomalies considered life-limiting or that may threaten vital functions. Under these circumstances, intensive care therapies may alter the time course and the baby's/family's life experience but likely will not change the long-term outcome. Individuals may be expected to have varying degrees of survival and severe morbidity. These conditions may involve any single-organ or multiple-organ systems and at times are the result of genetic abnormalities.
3. Newborns who have received intensive interventions and despite efforts are not responding to therapy, continue to decline, have continued life-threatening events, or interventions are seen as overly burdensome.

When these difficult situations arise, it is important to have a multidisciplinary approach to caring for the patient and family, starting from the time of diagnosis. The NICU provides a unique environment in which intensive life-sustaining therapies are provided with an emphasis on comfort of the patient and involvement of the family in the care process.[24] It is one of the few places in hospitals where a multidisciplinary team (physicians, nurses, social workers, chaplains, etc.) is focusing on multiple aspects of the total care of the baby–family unit. This lends itself nicely to the provision of palliative care because much of the framework already exists and allows for families to explore and share their hopes and goals while focusing on parenting their baby. Yet, while many clinicians in NICU settings feel they are comfortable and competent in providing palliative and EOL care for neonates, there is a wide variety in the care patients receive and in the discussions of goals of care with families.[24,52,62] Similarly, there may be attitudinal barriers to consulting palliative care clinicians. Evidence suggests, however, that once the need for and importance of palliative care in the NICU are realized, palliative care clinicians can be readily integrated into the management of neonates with life-limiting illnesses.[63]

Timing of the News

Families may learn of a life-limiting condition in the early prenatal period, late prenatal period, or early neonatal period.[8] While the job of the healthcare team remains the same in supporting the family members and helping them find meaning in their baby's life, the timing can slightly alter the approach to care.

Early Prenatal Diagnosis

If the news is given in the early prenatal period, parents often have time to begin grieving the loss of a healthy pregnancy and reframe their idea of hope. If palliative care is initiated early on, the family members may benefit from the involvement of palliative care clinicians who learn about the family and support them through the process. They can facilitate bonding and memory making throughout the pregnancy. They also have time to navigate their goals of care and develop a care plan that includes a birth plan and advance care planning known by all clinicians at the time of delivery. This allows the family and care team to explore desires for the pregnancy, labor and delivery, and care of the baby afterward. It can give the family a sense of control and ensures that the care team will know and attempt to honor their wishes. Box 23.1 highlights important components of a perinatal palliative care birth plan. The delivery room resuscitation plan should be discussed ahead of time with the family.[29,53,64–66] Staff need to remain ready to adapt such plans to the reality of the newborn's condition at birth. Families may change their minds, the newborn's condition may be better or worse than anticipated, and decision-making remains rather fluid. Ethics and palliative care support may aid parents and facilitate their navigation through difficult decisions and how they choose to spend whatever amount of time they have with their baby.

• BOX 23.1 Components of a Perinatal Palliative Birth Plan

Important Information for the Care Team
- The baby's name (if known)
- A diagnosis for the baby (if known) and pertinent medical information
- What the family has been told to expect
- Names and numbers for important providers

Clarify Maternal Goals
- Site of delivery
  Community or tertiary
- Fetal heart monitoring
- Mode of delivery
  Burdens, benefits, values
- Analgesia for mother
- Special requests during labor
- Who should be in attendance
- Who should cut the umbilical cord
- Preferences for if the baby is stillborn

Clarify Neonatal Goals
- Invasive interventions vs. comfort measures only
  Any specific interventions they would or would not want
  Specified components of resuscitation and care
    - Intubation, noninvasive respiratory support, medications, access
- Site of care for the baby
  Mother's room, nursery, intensive care unit, home
- Wishes for delaying routine procedures or providing them while the baby is in the parent's arms
- Wishes for feeding the baby
  Breast, tube, cup, finger, none
- Wishes for medications
- Wishes for additional testing
- Wishes for memory-making and support
  Photographs and videos
  Keepsakes: footprints, handprints, locks of hair, crib card, ID bands, blankets, clothing, heartbeat recording
  Wishes for bathing the baby and special outfits
  Family involvement
  Spiritual rituals/wishes

Goals for if the Baby Survives the First Day
- Intensive care versus take their baby home for end-of-life care versus receive end-of-life care in the hospital
- If they wish to be discharged home
  The name of the hospice/home care that will support them
  Anticipated care needs at home
  Information about whom to notify if baby dies at home

Wishes for if the Baby Dies Before Discharge
- Symptom management
- Wishes for organ/tissue donation if eligible
- Wishes for autopsy or further testing
- Funeral home information
- Special wishes about transportation of the body
- Any other additional requests the family may have

Late Prenatal Diagnosis

If the news of a life-limiting illness is discovered late in the pregnancy or just before delivery, it often results in a more chaotic and less formulaic approach. Teams are working rapidly to provide immediate care to the pregnant individual and fetus/neonate. There often is not time to have lengthy discussions or learn the parent's values. In the chaos, communication can be rushed and fragmented. It remains imperative to provide accurate information and support to the parents while making the environment as calm and private as possible, allowing the family members to express their emotions. In the time that follows, discussions can be continued and goals and treatment plans can evolve.

Postnatal Diagnosis

If the news of a life-limited illness is made after delivery, parents are often shocked. Most assume that if a pregnancy and delivery are uncomplicated, their baby will be healthy. For them it is an abrupt loss of the healthy baby they were holding moments before they received the news. It is vital to continue to promote bonding with the baby and ease suffering. The care team should use the medical information along with the knowledge of the family's values to shape the goals of care and aid in decision making. Extra time is often necessary in this situation, with care to provide support for the family and staff in redefining and redirecting hope and the goals for the newborn's care.

Parents note (especially with a postnatal diagnosis) that the delivery of the news and the discussions that follow shape their experience with the care team, their memories of time with their child, and their subsequent bereavement.[67] Some parents report that the manner in which news about a diagnosis, prognosis, or treatment options was given, was insensitive. They wish to be included in the decision-making process and know that their input as parents is what matters most. They also desire privacy, support, and time to come to terms with their loss.[51,68–74] They feel quality care was best provided when providers listened, aided in decision-making, supported their goals, explored the range of outcomes, included the family in care, asked about hopes and fears, and provided ongoing compassionate support.[75]

Components of Neonatal-Perinatal Palliative Care in the Neonatal Intensive Care Unit

The major components of neonatal-perinatal palliative care are a focus on communication, quality of life, family support, and grief support.[64,76] Ideally, clinicians who have become familiar with the family (including neonatologists, nurses, social workers, and chaplains) and have developed a rapport with them should initiate and be present for difficult conversations. Depending on the timing of diagnosis, these conversations may begin before the birth of the baby. Conversations with family members should be clear, direct, and honest.[77] The intent is to provide the necessary medical information and in that context explore goals of care. This is unlikely to be achieved in a single discussion. Rather, the clinician is engaging in a conversation that starts the journey they will be embarking on with the family. When important or new information is being conveyed, it should occur in a quiet environment where the family has the clinician's undivided attention. Parents wish that difficult news be delivered in an honest and compassionate manner that lets them know their child matters.[65,74,77,78] After the diagnosis and the relative certainty of the prognosis have been discussed, the focus of these conversations should shift to exploring the values of the family, wherein the care team can better understand the family, how the family members make decisions, what is important to them, and how in the context of their specific situation and values or goals the medical information is important.

In these conversations it is important to help the family identify—and often redefine or redirect—their hopes. By being open and compassionate, clinicians can give family members a sense of realistic hope during this difficult time without misleading them toward a false sense of the likelihood of survival.[51,65,74] It is often helpful to focus the conversation on what is meaningful to the family members in the context of given prognosis.[54] Their idea, or object, of hope may change over the course of the baby's life. With a better understanding of the family's values, the care team can participate with the family in shared decision-making and ultimately define or redefine the goals of care. These conversations and the decisions made are unique to each family regardless of the diagnosis. It is not uncommon for parents and the care team to have different agendas and priorities during this time.[79] It is critical to attend to the parent's priorities and ensure that they feel valued, listened to, and a member of the care team. It is the care team's job to understand the family's values and goals and to make medical recommendations based on those goals. There are times when the choices of the family may be in opposition to the views of the care team.[80,81] It is the team's obligation to continue conversations with the intent of better understanding the reasoning behind the family member's decisions/goals and support them through the process. The goal should not be to convince the family that an alternative approach is most appropriate. In partnership with the NICU team, palliative care clinicians may be instrumental in facilitating such ongoing conversations, especially as primary neonatal clinicians may change every week or every 2 weeks.

When the end of life (EOL) is approaching, it may also be important to have open conversations about organ or tissue donation, autopsy, and funeral, memorial, or burial services. Many NICUs have a team of social workers, chaplains, and psychologists to help attend to these needs. The addition of palliative care clinicians can bring expertise in providing additional support during these very unique and difficult times. When there is an anticipated compassionate withdrawal of life-sustaining medical treatment, it may be beneficial to contact a liaison with a regional organ procurement organization to engage families in discussions about organ/tissue donation.

Regardless of the family's care goals or treatment options, the baby's quality of life should remain paramount. Care should be taken to ensure that painful procedures are minimized, unnecessary tests stopped, the physical environment is made comfortable and conducive to family bonding, and that pain and symptoms are addressed with nonpharmacologic and pharmacologic management when necessary.

Neonatal End-of-Life Care

Palliative care is extremely important during EOL care, when a neonate is imminently dying. Every effort is made to ensure the family members can shape the death experience in a way that is most meaningful to them and that they are as prepared as they can be for the dying process. For some families, it is important that they understand the physiologic changes that they will likely see ahead of time. If family members wish to stay in the hospital for the death of their baby, every effort should be made to bring them to a private room within the NICU or to a different location within the hospital. The environment should be as peaceful and homelike as possible. Monitors and unnecessary equipment should be removed. Families should determine who they wish to have present and if they would like to hold their baby or perform any rituals. Care should be taken to respect any cultural or spiritual desires of the family. If parents desire to take their baby home for EOL care, clinicians should coordinate with local home hospice programs or home-health services to ensure that the family will have access to the care, support, equipment, and medication needed during this time. Parents should be reassured that regardless of location, they will not be abandoned and that care will be taken to attend to pain and symptom management. The focus should be on what the team can do for the baby and family as opposed to what the team can no longer achieve.[64,82,83] Neonates feel pain and other symptoms of distress, and those symptoms should be addressed accordingly.[84,85] Symptoms that should be anticipated, assessed for, and may require treatment at the EOL include pain, agitation, dyspnea, and secretions.[86–88] Nonpharmacologic interventions to address these issues, such as decreasing stimulation, a soothing environment, massage, kangaroo care, elevating the head, fluid restriction, and gentle suctioning, are important. At times, especially during the EOL, nonpharmacologic interventions will not be enough. It is important to anticipate this and to help alleviate the stress of the family by discussing it ahead of time. It is both clinically and ethically appropriate to provide pharmacologic symptom relief. While there may be a concern of hastening death with certain medications (e.g., opioids), at appropriate doses this generally does not happen. The intent to provide relief of pain and suffering, it can be argued, outweighs the small chance of respiratory depression. Tables 23.1 and 23.2 provide common pharmacologic and nonpharmacological symptom management at the EOL. It is important to note that for pharmacological management, the doses and the medication choices need to be altered based on the patient's previous medication exposure history. The medications used can be tailored based on the symptoms and symptom severity expected from the clinical situation and underlying disease process. Opioids, for example, are most effective for dyspnea and pain while benzodiazepines are effective for agitation. The route of administration will depend on the illness severity and location of end-of-life care.[87] Intravenous administration, especially in the NICU setting, is typically utilized. In the home or hospital setting, most of the medications can be administered via other routes. Most opioids can be administered orally or buccally. Fentanyl, midazolam, and dexmedetomidine can be administered intranasally.

In addition to the care provided to the baby surrounding the time of death, there are other considerations that will need to be addressed either before or after death occurs. If the mother has been providing breast milk, she will need to be educated about options including lactation suppression and milk donations. These are very personal decisions as some women find milk donation therapeutic or a way to have their baby's legacy continue, while others feel it is a painful reminder of their baby who is no longer able to breast feed.[35,47,89] Regardless of the decision around milk donation, she will need to be counseled about the options available as continuing to produce milk or trying to decrease the milk supply without guidance can be painful and lead to medical complications.

Other important topics to discuss are organ and tissue donation and a postmortem exam. Organ donation options for medical purposes and research are variable by area and often connection with a local organ procurement center will allow parents to know what options are available.[35,47] If they wish to pursue organ or tissue donation it is important that they are prepared for the necessary procedures ahead of time and how that might change the EOL experience they have with their baby and the time with the body afterward. Similarly, the discussion of a postmortem exam,

TABLE 23.1 Symptom Management for End-of-Life Care: Pharmacologic

Symptom	Medication	Class	Starting Dose with Route and Frequency
Pain	Acetaminophen	COX2 inhibitor	15 mg/kg PO/PR q6 6–8 mg/kg IV q8
	Dexmedetomidine	Selective alpha 2 agonist	0.5–1 mcg/kg IV/IN q2 0.5–1 mcg/kg/hr IV continuous
	Fentanyl	Opioid	0.5–2 mcg/kg IN/IV q2 1–4 mcg/kg/hr IV continuous
	Ketamine	Dissociative anesthetic	0.5–1 mg/kg PO/IV q2–4
	Methadone	Opioid	0.05–0.2 mg/kg IV/PO q12–24
	Morphine	Opioid	0.05–0.2 mg/kg IV/IM q2–4 0.15–0.5 mg/kg PO/Sublingual q2–4 0.01–0.05 mg/kg/hr IV continuous
Agitation (and neuroirritability)	Dexmedetomidine	Selective alpha 2 agonist	0.5–1 mcg/kg IV/IN q2 0.5–1 mcg/kg/hr IV continuous
	Fentanyl	Opioid	0.5–2 mcg/kg IN/IV q2 1–4 mcg/kg/hr IV continuous
	Gabapentin	Anticonvulsant	5–15 mg/kg PO q8
	Ketamine	Dissociative anesthetic	0.5–1 mg/kg PO/IV q2–4
	Lorazepam	Benzodiazepine	0.05–0.1 mg/kg PO/IV q4–6
	Midazolam	Benzodiazepine	0.05–0.1 mg/kg IV q2–4 0.2–0.3 mg/kg Sublingual q2–4
	Morphine	Opioid	0.05–0.2 mg/kg IV/IM q2–4 0.15–0.5 mg/kg PO/Sublingual q2–4 0.01–0.05 mg/kg/hr IV continuous
Dyspnea	Fentanyl	Opioid	0.5–2 mcg/kg IN/IV q2 1–4 mcg/kg/hr IV continuous
	Lorazepam	Benzodiazepine	0.05–0.1 mg/kg PO/IV q4–6
	Midazolam	Benzodiazepine	0.05–0.1 mg/kg IV q2–4 0.2–0.3 mg/kg Sublingual q2–4 0.25 mg/kg IN q2–3 0.05 mg/kg/hr IV continuous
	Morphine	Opioid	0.05–0.2 mg/kg IV/IM q2–4 0.15–0.5 mg/kg PO/Sublingual q2–4 0.01–0.05 mg/kg/hr IV continuous
Secretions	Atropine	Anticholinergic	0.01–0.02 mg/kg PO q2
	Glycopyrrolate	Anticholinergic	0.01–0.02 mg/kg IV q4 0.04–0.1 mg/kg PO q4

IN, Intranasal; *IM*, intramuscular; *IV*, intravenous; *PO*, per os; *PR*, per rectum; *q2*, every 2 hours; *q2–4*, every 2–4 hours; *q4–6*, every 4–6 hours; *q6*, every 6 hours; *q8*, every 8 hours; *q12–24*, every 12–24 hours.

or autopsy, is a delicate topic that requires care and attention. At times, an autopsy can provide valuable information to the family. It leads to a change in diagnosis or additional findings in 22% to 76% of cases.[90] Depending on the circumstances and what is already known, this can give insight and answers to families both for this baby and possibly future pregnancies. When a diagnosis is not known, given the potential to reveal additional information, it is suggested that clinicians encourage families to consent to an autopsy.[90–94] In order to do so, it is important that providers are knowledgeable about the exam itself and options available as there are stigmas and perceptions about the exam. Concerns with autopsy include the invasive nature, the timing of the autopsy, transporting the body, religious and cultural concerns, and poor communication about the potential value.[95] To address some of these concerns, it is important that clinicians having these discussions be familiar with what happens to the body, the timeframe, and the expectation/limitations.[92] There are options for full, limited, stepwise, or noninvasive post-mortem exams and

TABLE 23.2 Symptom Management for End-of-Life Care: Nonpharmacologic

Symptom	Action	Provider
Pain or agitation	Reduce ambient noise	Clinician
	Reduce procedural touching (handling, suctioning, laboratory tests, and imaging)	Clinician
	Reduce temperature swings	Clinician
	Swaddle; facilitated tuck	Clinician or parent
	Nonnutritive suckling	Clinician or parent
	Skin-to-skin contact	Parent
Dyspnea	Postural positioning (may be lateral, upright, or prone; may include neck, chest, or shoulder roll)	Clinician or parent
	Consider fan or humidified air	Clinician
Secretions	Oral and nasal suctioning	Clinician or parent

more recently genomic autopsies.[90] Over time, the rates of the postmortem exam for neonates has decreased, with over 50% of families declining the exam, prompting the need for other methods of providing information to families. When families decline a full autopsy, there are minimally invasive or noninvasive options such as tissue sampling, MRI, and x-ray that may yield some answers depending on the suspected diagnosis.[94–98] There are certain diagnoses that will not be discovered with these approaches, but oftentimes they can still yield some results.[95] Some may find benefit by contributing to medical knowledge, or altruism, but most who find benefit do so because it gives them answers as to the cause of death. It is important to have open, honest conversations about the exam itself and value of and limitations of such exams because there are times when autopsies can lead to answers and other times when it can contribute to further grief.[99] It is also important to provide a clear plan about how the details of the autopsy will be discussed with the family. It is also important that they know when to expect the information (often, results from a full autopsy take two or more months) and how it will be disclosed to them. In-person meetings at a neutral location where there is time to discuss the details of the exam and any questions the family may have is ideal. It may be important to follow up with them after the initial meeting to provide support as they continue to process the information.

Memory-making, Legacy Building, and Grief and Bereavement Support

Grief support should be initiated shortly after a life-limiting diagnosis. Initially such support may focus on grieving the loss of the healthy newborn and the life the family members anticipated in the context of still celebrating and enjoying their baby. Attention should be given to ensure the parents, siblings, grandparents, and extended family members are able to make memories with the baby. During this stressful time, especially if it is their first child, parents are often unable to think about what memories would be important for them to make. Some families wish to take pictures, have handprints/footprints or molds made, record heartbeats, bathe the child, sing/read to the child, take the child outside, have the child meet a family pet, taste foods, sleep in a bed, or participate in spiritual/religious ceremonies.[100] Legacy-making activities, while new to the neonatal population, can be meaningful for families to explore.[101] Every effort should be made to help these families maximize the experiences they have with their baby during what may be a brief life span. Bereavement support for parents, siblings, grandparents, and other family members should start before the death of the baby and continue for a period afterward.[53,102] Parents appreciate bereavement support and follow-up phone calls from or meetings with physicians who provided care for their child and other forms of acknowledgment such as condolence cards.[53] It is also imperative to recognize that staff members benefit greatly from grief support.[52,103,104] Often the NICU staff has been involved in the care of the baby for much of his or her life and has spent time becoming familiar with the family. News of a poor prognosis can be emotionally trying staff as well. Because the staff are attending to the immediate care needs of the baby, family, and other neonates in the unit, they may be unable to process such difficult news. It is important that we support them as well.[104,105]

Ethical Concerns

With advances in equipment and medicine, and more rigorously studied and implemented care practices, neonates are increasingly benefiting from their NICU stays. The gestational age of viability has dropped with continuously improving clinical capabilities and we are able to offer interventions for diagnoses for which there previously were no treatment options.[106,107] As we become better at saving one group, the limits are pushed for another group of neonates.[108] Yet these advances have come with a variety of uncertainties and outcomes. Difficulty remains around the borderlines of viability and diagnoses such as bilateral renal agenesis. When to offer resuscitation? When to forgo life-sustaining therapies? Whether or not to redirect care toward palliation? At 22 weeks' gestation, for example, some centers universally offer a trial of intensive care whereas others do not, or do so on only an individual basis.[109,110]

It is often difficult to predict a neonate's course or future complications. This uncertainty brings many questions and discussions among professionals and families about the neonate's response to intensive care, complications encountered, and what actions are in the neonate's best interests.[56,106,107,111–115] Families are often faced with decisions that are not seen elsewhere in medicine. These decisions are complicated by the urgency of need for action and the uncertainty of prognosis.[8] Many times both families and care teams feel that once care is initiated, everything possible must be done to save the neonate. However, there may come a point in time when certain life-sustaining interventions are considered acceptable by some families whereas others believe such continued intervention or technology dependence render a quality of life for their baby that they do not find acceptable.

Ethically and legally withholding an intervention or withdrawing one already started are considered equivalent.[116] In other fields of medicine this seems to be more readily accepted. The thought of withdrawing life-sustaining medical therapies (LSMT) from a neonate leads many parents to believe they are actively aiding in their baby's death. When discussions are based on notions or

descriptions of futility or a perceived quality of life, a contest of whose perception of these terms matters most (clinicians or families) often results. Consequently, physicians may have difficulties addressing these issues, discussions may be limited, and if they occur it is late in the treatment course.[48,49]

Withdrawing/withholding LSMT, while a relatively infrequent phenomenon in a given NICU, is the most frequent mode of dying in both neonatal and pediatric ICUs.[117–119] It is understandable that parents want to be sure that there is essentially no chance of survival with an acceptable quality of life before such decisions are made. This warrants open communication, transparency in prognostication, and attention to empathy.[108,120]

Barriers to Palliative Care in the Neonatal Intensive Care Unit

While palliative care is essential to individuals in the NICU, it is often overlooked and there are many barriers. The NICU environment is often noisy and crowded, with little room for privacy.[60] Also, with current advances in technology, death may be viewed as a failure. The technologic imperative is so operant that if a therapy is available, it must be used without full consideration of how burdensome it is or if it will ultimately benefit the neonate.[60,121,122] Often families are overwhelmed by the technology and feel an incredible burden that the decisions are solely on their shoulders. If the outcome is certain and the treatment is a standard of care, then this is often not an issue. However, there is rarely such certainty. Beyond that, the lack of training for clinicians and organizational barriers remain a challenge in establishing programs and providing quality neonatal-perinatal palliative care.[122] Some have suggested that using standard criteria to prompt the involvement of palliative care is effective at overcoming some of the barriers.[123]

Training in Neonatal–Perinatal Palliative Care

Clinicians' discomfort in addressing the limits of medicine and technology and to redirect the prevailing care paradigm from one that is disease based and cure oriented to one that is palliative and comfort oriented is likely contributed to by a relative lack of palliative care training in neonatal-perinatal training programs. One published study addressing the training of neonatal fellows in discussions with families and helping them make decisions regarding critically ill neonates was particularly revealing.[124] Fellows from 83% of the accredited programs participated. For the most part, all fellows felt confident in their medical training. However, more than 40% stated that they had not had any formal communication or clinical communication skills training. More than 90% felt that training in this area—specifically palliative care, spiritual needs, and managing conflicts of opinion—was lacking or nonexistent in their training program. It is imperative that neonatologists learn these skills because families who have lost a child often cite how important physician communication with them is. Parents desire to hear information in a manner they can understand/handle and have the physician address all of their palliative care and spiritual needs so that they can collaboratively develop a clear plan.[32,52,124–127]

Research Opportunities/Future Directions

While in recent years there have been many advances in the field of neonatal palliative care, there are still many exciting opportunities for research. Studies have yet to determine which interventions offer optimal pain and symptom management at the EOL. There are also many opportunities for research addressing communication training and quality improvement investigations looking at the timing and delivery of palliative care, which paradigm of delivery is most effective, and how it will alter the course of living with neonatal loss for these families. The goal of this much-needed research is to advance the evidence base to help develop best practice models and approaches to treatment and care.

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24

Risk Assessment and Neurodevelopmental Outcomes

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KEY POINTS

- Preterm and critically ill infants are at high risk for cognitive and motor development problems, neurobehavioral and executive function problems, learning and academic problems, neurosensory problems, and poor functional outcomes.
- Neonatal morbidities, socioeconomic factors, and early interventions can influence long-term outcomes for high-risk infants.
- While neurodevelopmental impairment (NDI) at 2 years corrected age is a common outcome in neonatal clinical trials, there is ongoing debate regarding the appropriateness of this outcome as a gold standard and limitations to interpretation of NDI data.
- Early neurodevelopmental outcomes do not consistently predict abilities and challenges in later childhood and beyond. Longitudinal assessments into school age, adolescence, and adulthood are essential to understand the lifetime trajectory of former high-risk infants.
- All high-risk infants and their families must have access to comprehensive, multidisciplinary developmental follow-up programs and early intervention services in order to optimize both short-term and long-term outcomes.

Outcome Assessment in High-Risk Infants

Who Is the "High-Risk" Infant?

The term *high-risk infant* has been defined in many ways and has been burdened with many negative connotations. However, one general goal for attempting to delineate infants who are high risk is to heighten awareness and focus on those children who may benefit from increased surveillance and early intervention across a number of domains. In a policy statement from the American Academy of Pediatrics (AAP),[1] high-risk infants were defined broadly as including[1] the preterm infant;[2] the infant with special healthcare needs or dependence on technology;[3] the infant at risk because of family issues; and[4] the infant with anticipated early death. Others have underscored the importance of critically assessing risk in any infant admitted to the neonatal intensive care unit (NICU) or special care nursery.[2] Many clinical conditions and risk factors seen in term-born infants—including but not limited to congenital heart disease (CHD) (3), need for extracorporeal membrane oxygenation (ECMO) in the neonatal period,[4] and hypoxic–ischemic encephalopathy (HIE) regardless of treatment with therapeutic hypothermia[5,6]—place them at high risk for neurologic, developmental, functional, and health outcome challenges in early childhood and beyond. Unfortunately, although the risks for postdischarge difficulties, in addition to medical morbidities, have been increasingly well-described in the literature for these and other predisposing risk factors, the neurodevelopmental needs of many of these term-born, high-risk infants may be overlooked for referral at hospital discharge, or they may not obtain or have access to follow-up even if referred.[7,8]

Preterm infants are the most recognized and targeted population of high-risk infants. Preterm birth, defined by the World Health Organization (WHO) as delivery before 37 completed weeks of pregnancy, remains a crucial global health challenge.[9] According to the Centers for Disease Control and Prevention National Center for Health Statistics, in the United States in 2019, the proportion of live births <37 weeks' estimated gestational age [EGA] was 10.23%.[10] This represents the fifth consecutive year of an increase in the preterm birth rate from the 2014 rate of 9.57%. The increase in total preterm birth rate from 2018 to 2019 was among infants born late preterm (34 to 36 weeks) (7.28% to 7.46%), while the early preterm birth rate (less than 34 weeks) of 2.77% in 2019 was essentially unchanged. The proportion of infants delivered very preterm in 2019 (VPT, <32 weeks' EGA) stands at 1.59%, which remains relatively unchanged. Similarly, the extremely preterm (EPT, <28 weeks' estimated EGA) birth rate is relatively stable; in 2019 the EPT birth rate was 0.66%, exactly the same as 2018, and minimally changed from 2014 (0.69%). Although the VPT and EPT birth rates seem to be miniscule, the impact in terms of total births is important. With nearly 3.75 million births annually in the United States, more than 380,000 neonates <37 weeks' EGA, 100,000 early preterm neonates, and 25,000 EPT neonates were born in 2019 alone.

What Is Meant by "Outcomes"?

As survival of even the most EPT and complex infants has improved over the decades, short-term mortality and morbidities have moved from being the only outcomes reported for high-risk infants to being only the first of many outcomes of interest. There is increasing recognition of the critical significance of understanding later outcomes in order to evaluate the true impact of interventions and management approaches in the NICU, to inform counseling, and direct early detection and preventive care.

Certainly, for some trials of treatments and management strategies designed to test a hypothesis of improved in-hospital morbidities, the primary outcome may best be short term.[11–13] However, longer-term follow-up or later primary endpoints may provide valuable additional outcomes data, safety assessments, or information about functional elements.[14–20]

What are these post-discharge outcomes of potential importance? How are they measured, and what are the barriers or benefits of focusing on various outcomes? The value placed on one outcome or group of outcomes may differ greatly for families, children, and adults who were born high risk, physicians, and other care providers, investigators, educators, and those involved in developing public policy.[21,22] The importance of various outcomes may also vary substantially among individuals within these groups and across different time points of their lives. Furthermore, later cognitive and behavioral outcomes are complex and influenced by the postdischarge environment, relationships, and biologic factors.[23,24] Functional and adaptive outcomes may be considered the most important in some scenarios as these skills are related to essential daily tasks, abilities, and interactions, and their assessment generally does not require detailed and normative evaluations.[25,26] Therefore the concept that there is one "best" outcome measure among those born preterm or at high risk is ill conceived.

In the following section, we provide an overview of some general later outcome categories that have been frequently reported and proposed for high-risk infants, particularly for those born extremely preterm or at very low birth weight (VLBW). We begin with special attention to early neurodevelopmental outcomes assessments (18 to 36 months) since these are most frequently reported in trials and prospective observational studies. We discuss challenges and strengths reported at this age, using frequently reported definitions of impairment and disability, and describe the usual battery of tests and assessments. Limitations of standard outcomes definitions and challenges to interpretation of early neurodevelopmental outcome studies are considered. We also highlight abilities and difficulties assessed through school age and adulthood.

Early Neurodevelopmental Outcome Assessments

For the vast majority of interventional trials and prospective observational studies involving high-risk infants, neurodevelopmental outcome at approximately 2 years corrected age, and usually death or "neurodevelopmental impairment" (NDI), is reported as a primary outcome. Although the rationale for the combined outcome is clear in the setting of competing outcomes, there is ongoing and substantial debate regarding the appropriateness of this outcome as a gold standard for all trials.[27] Furthermore, the NDI outcome is itself a composite outcome, composed of morbidities from neurodevelopmental and sensory domains with different risk profiles, causal pathways, and predictive validity. There are challenges to interpretation of these data, potential limitations in terms of comparisons across cohorts and across years, and concerns regarding the value of early neurodevelopmental outcomes to predict abilities and challenges in later childhood and beyond.

In this section, we will focus specifically on the neurodevelopmental components generally presented in studies of 2- to 3-year follow-up. The traditional battery of tests and assessments includes motor function, cognitive/developmental capabilities, and neurosensory outcomes including hearing and vision impairments. These general components have been proposed and recommended by expert panels and working groups,[28–30] and within the context of prospective studies and trials although the specific evaluations within each area differ among groups and over time.

Motor Function

Motor impairments including cerebral palsy (CP) are among the most frequently reported neurodevelopmental outcomes for high-risk infants. Motor difficulties may become evident over months or years, yet timely identification of motor difficulties may allow for interventions to improve outcomes, thereby reinforcing the critical importance of vigilant long-term follow-up. CP is defined as a disorder of movement and posture that involves abnormalities in tone, reflexes, coordination, and movement, delays in motor milestone achievement, and aberration in primitive reflexes that is permanent but not unchanging and is caused by a nonprogressive interference, lesion, or abnormality of the developing immature brain.[31,32] CP is also categorized by type (spastic, dyskinetic, or dystonic); topography (limbs involvement); and descriptors of extent and pattern of involvement (monoplegia, diplegia, hemiplegia, and quadriplegia). Previously, it was considered that CP could not be diagnosed prior to approximately 18 to 24 months corrected age. However, it is now clear that a child may receive an accurate diagnosis of CP or high-risk for CP before 6 months of corrected age using evidence-based and standardized clinical assessment tools and imaging appropriate for age.[33] In a systematic review utilizing Grading of Recommendations Assessment, Development, and Evaluation (GRADE) framework, Novak and colleagues presented specific essential and additional criteria, and detection pathway options based on level of risk. In terms of standardized assessment tools, they found that in combination with medical history and depending on age of assessment, magnetic resonance imaging, the Prechtl Qualitative Assessment of General Movements, the Hammersmith Infant Neurological Examination, and the Developmental Assessment of Young Children were most predictive for detecting risk for CP. Of note, successful deployment of these guidelines for early diagnosis and risk stratification has been demonstrated across diverse high-risk infant follow-up clinic populations.[34] The goal of early identification of children with CP or high-risk for CP is to allow for early and targeted intervention, thereby maximizing neuroplasticity in the developing brain, and minimizing adverse impact of CP to muscle and bone growth and development.[35,36] There is evidence that involving and supporting parents as a critical part of this intervention process also leads to improved parent outcomes as well as parent-child interactions.[36–38] International clinical practice guidelines have recently been published to guide early intervention approaches for CP across the domains of motor function, cognitive skills, communication, eating and drinking, vision, sleep, managing muscle tone, musculoskeletal health, and parental support.[38]

The Gross Motor Function Classification System (GMFCS)[39–41] provides a valid and reliable system to classify the extent of activity limitation in CP. It is a five-level system (I to V) used to categorize children up to 18 years of age based on their usual performance, with a focus on functional capabilities, including sitting, mobilizing, walking, and need for assistive devices. Such classification helps clinicians communicate information about severity, choice of interventions, and prognosis in a standardized, easy-to-use, valid, and reliable way. Higher level on the GMFCS is associated with increasing functional difficulty, but, of note, distinctions between levels I and II are not as significant as differences between other levels, particularly for younger children. Children classified

as level I overall are able to walk without restrictions but may have difficulty with the speed, balance, and coordination required for higher-level skills; between 2 and 4 years, they are able to floor sit with both hands free to manipulate objects, move in and out of floor sitting and standing without adult assistance, and they walk as the preferred method of mobility without the need for any assistive mobility device. In contrast, children classified as level V are profoundly impaired with no means for independent mobility; between 2 and 4 years, this is described as physical impairments that restrict voluntary control of movement and the ability to maintain antigravity head and trunk postures and functional limitations in sitting and standing that are not fully compensated with the use of adaptive equipment. GMFCS categorization in children less than 2 years old depends predominantly on the amount of support required for the child to sit and also considers more advanced skills such as crawling and walking. Although some children diagnosed before 2 years of age will require reclassification, most are not reclassified by more than one level. The positive predictive value (PPV) of a classification of GMFCS level I, II, or III (child will walk with or without aids) as compared with level IV or V (child will probably need a wheelchair for mobility) is very high (0.96). Thus the GMFCS provides a sound approximation, rather than a definitive final categorization, in this age group.[42]

Although CP with severe functional limitations is of great concern, it is relatively rare. An Australian CP registry review from the 1970s to 2004 showed increasing prevalence of CP in the 1970s and 1980s attributed to the increasing survival of EPT infants; however, CP rates stabilized or decreased between the early 1990s and 2004.[43,44] Analysis of the Danish nationwide CP registry revealed a significant decline in the rate of CP in birth years 2005–2007 explained primarily by fewer cases of severe bilateral spastic CP among term children between these periods, although the rate of CP among premature infants had been shown to dramatically decline in previous periods.[45] Among infants born < 27 weeks' GA in the National Institute of Child Health and Human Development (NICHD) Neonatal Research Network (NRN) and assessed at 18 to 26 months' corrected age, there was a decrease in the rate of any CP (16% in 2011 compared with 12% in 2014), with a decrease in severe CP (26% to 16% of all CP cases) during the study period.[46]

There are numerous motor and coordination challenges apart from CP that are reported in high-risk children, including those born preterm. Developmental coordination disorder (DCD) is diagnosed when (1) coordinated motor skills are substantially below those expected given the individual's chronological age; (2) the motor skills deficit significantly and persistently interferes with the activities of everyday living appropriate to chronological age and impacts school, play, and leisure activities; (3) the motor skills deficits are not better accounted for by any other medical, neurodevelopmental, psychological, social condition, or cultural background; and (4) the onset of symptoms was during childhood.[47] Current recommendations for diagnosis of DCD include a score less than the 16th percentile on the Movement Assessment Battery for Children[48] or equivalent test such as the Bruininks–Oseretsky Test of Motor Proficiency.[49] A recent longitudinal study of children born <30 weeks' GA demonstrated that at 5 and 7 years of age, nearly 1 in 3 had DCD, with an approximately fourfold increased odds for DCD compared with term-born children.[50] Although the motor difficulties associated with DCD are usually considered "minor" motor impairments, particularly in comparison with disabling CP, nonetheless they can have significant impact on the child. These difficulties may include important functional skills such as fine motor skills, speed and accuracy in motor planning, balance, and coordination. Children with DCD or probable DCD have been shown to be at increased risk for executive function and cognitive challenges, social–emotional and behavior problems, speech and language impairment, and other issues.[51,52]

Cognitive Assessment

A central component of a high-risk infant neurodevelopmental follow-up visit has been administration of a standardized developmental test. These tests are intended to provide a measure of "cognitive" function, although there are widely acknowledged limitations including the evolution of test versions making it difficult to compare across cohorts, preclusion of extrapolation of 2- to 3-year results to intelligent quotient (IQ) at later time points, and challenges to interpretation of results in the preterm population using standardized "cut points" alone and in the absence of a contemporaneous normal birthweight (NBW) term control group (see later section Limitations and Challenges to Interpreting Early Neurodevelopmental Outcomes Studies).

The Bayley Scales of Infant and Toddler Development (BSID), with a test age range of 1 to 42 months, is now the most widely used developmental test for high-risk infants across the United States and Europe. The original version, released in 1969, was revised in 1993.[53] The BSID-II had two developmental scores: the Mental Developmental Index (MDI), a composite of cognitive and language tasks, and the Psychomotor Developmental Index (PDI), a composite of fine and gross motor skills. This perceived drawback, as well as the usual drive to revise editions due to the "Flynn effect,"[54] contributed to the development of BSID-III,[55] which contains three main domains, (1) a cognitive composite score, (2) a language composite score (with receptive and expressive subscores), and (3) a motor composite score (with gross and fine motor subscores), in addition to social–emotional and adaptive behavior domains. The goal of the BSID-III was to allow identification of delays, as well as relative strengths and challenges, in specific developmental domains, and to target interventions to areas of need. However, in part because of a change in approach to norming the BSID-III, and also possibly because of separation of the cognitive and language scales, cognitive scores on the BSID-III are substantially higher than anticipated among both preterm high-risk children and term control groups.[56,57] These findings also led to concern that the BSID-III underestimates developmental delay if utilizing normative test means alone, which has serious implications for both clinical and research endeavors. Previously, commonly used "cut points" for categorization of "moderate" and "severe" developmental delay or disability were 2 to 3 standard deviations (SD) and greater than 3 SD below the normative mean, respectively; thus for BSID-II, MDI 55 to 70 was considered a moderate delay whereas an MDI less than 55 was considered a severe delay.[57] However, in the absence of contemporaneous term control groups, commonly used cut points shifted in the era of the BSID-III. Some have recommended that BSID-III cognitive and language scores less than 85 or "combined BSID-III" scores less than 80 provide the best definition of "moderate-to-severe" delay for equivalence with BSID-II MDI less than 70,[58] whereas others have modified the threshold for cognitive delay categorization to define moderate delay as 70 to 84, severe delay 55 to 69, and profound delay as less than 54.[16,59,60] The Bayley Scales of Infant and Toddler Development, Fourth Edition (Bayley-4) was published in 2019.[61] The Bayley-4 has more contemporary normative data; notably, this sample does not include the 10% of at-risk children that had been included in the BSID-III normative sample.

The Parent Report of Children's Abilities-Revised (PARCA-R) is a parent questionnaire assessing a child's cognitive and language development, but is limited to evaluation at 24 to 27 months of age or corrected for prematurity.[62] The PARCA-R is available for free (https://www2.le.ac.uk/partnership/parca-r), in multiple translations, and has concurrent validity with examiner-administered developmental tests, and excellent test-retest reliability.[63,64] Age- and sex-adjusted standard scores were developed using data from over 6,000 children, with a normative mean of 100 and SD of 15 and allow an assessment of development ranging from < −3SD to > +3 SD.[62] Norms and percentile ranks can therefore now be used to aid in identifying children with either advanced or delayed cognitive and/or language development. The PARCA-R has been used as an outcome measure in observational studies and clinical trials and has been recommended to screen children born preterm in NICE (United Kingdom) developmental follow-up guidelines.[28] Of note, this instrument has also been used widely in telehealth-based assessments.

There are numerous cognitive development tests available to provide full-scale IQ or equivalent that have been used for later childhood assessments, including the Wechsler Preschool and Primary Scale of Intelligence (age range: 2 years 6 months to 7 years 7 months), now in the fourth edition (WPPSI-IV),[65] the Differential Ability Scales (age range: 2 years 6 months to 17 years 11 months), now in the second edition (DAS-II),[66] and the Wechsler Intelligence Scale for Children (age range: 6 years 0 months to 16 years 11 months), now in the fifth edition (WISC-V).[67] The Wechsler Individual Achievement Test (age range: 4 years 0 months to 50 years 11 months), now in the fourth edition (WIAT-4),[68] identifies *academic* strengths and weaknesses, and has been used in clinical, research, and educational settings.

Hearing and Vision Outcomes

Severe neurosensory impairments, including profound hearing and vision impairment, among preterm infants are now low in incidence but have important long-term consequences. Rates of blindness and significant hearing impairment are inversely related to gestational age.[69] Both moderate-to-severe vision and hearing impairment are more common among high-risk infants and with neonatal morbidities including bronchopulmonary dysplasia (BPD), brain injury, and seizures.[70–72]

Early detection of hearing impairment is vital for optimizing speech and language development, and guidelines and recommendations reflect the importance of recognizing potential problems as early as possible. As stated in the AAP Joint Committee on Infant Hearing position statement,[73] infants admitted to the NICU for more than 5 days are to have auditory brainstem response included as part of their predischarge screening so that neural hearing loss will not be missed; for those who fail, referral should be made directly to an audiologist for rescreening and, when indicated, comprehensive evaluation for hearing loss. Reevaluation should occur, regardless of initial evaluation results, based on individual risk factors and readmissions. All infants, including well infants, should have a hearing screening by 1 month of age, with rescreening and referral for audiology evaluation by 3 months of age for those who do not pass the initial screen. In addition, a validated global screening tool is administered to all infants at 9, 18, and 24 to 30 months or sooner when there is concern about hearing or language.

Prematurely born children have an increased risk of various ophthalmic and visual dysfunctions and abnormalities, in particular those children with a history of severe or treatment-requiring retinopathy of prematurity (ROP) and those with severe brain injury. These functional visual challenges include strabismus, problems with acuity, convergence and visual fields, and retinal morphology.[74] It is important to recognize that a short-term outcome of severe ROP in the NICU may not result in the most severe functional vision outcomes in early childhood.[12,17] Even children born preterm without a history of ROP or with only mild ROP also have an increased risk of problems. Among EPT children in the population-based Extremely Preterm Infants in Sweden Study (EXPRESS) at 6.5 years of age, 55% had poor visual-motor integration (VMI) performance.[75] Compared with term-born controls, VMI scores of EPT-born children were lower, and were associated with challenges in everyday school activities. In addition, VMI was most impaired in the children with the lowest. Recommendations from the AAP and the American Association of Pediatric Ophthalmology outlines a detailed construct for the termination of acute retinal examinations based on age and retinal ophthalmoscopic findings; therefore, if hospital discharge is considered before appropriate retinal development, follow-up must be arranged with an ophthalmologist trained in ROP care prior to discharge.[76] Furthermore, regardless of whether infants have required treatment for ROP, ophthalmologic follow-up is indicated within 4 to 6 months after discharge because they are at increased risk for other seemingly unrelated visual disorders, such as strabismus, amblyopia, high refractive errors, cataracts, and glaucoma.[76]

For early neurodevelopmental outcomes studies among preterm infants at 18 to 30 months' corrected age, criteria for "profound" or "severe" disability in the hearing domain have generally included some definition of "no useful hearing" even with aids or "some hearing but loss not corrected by aids," whether accompanied by specific decibel hearing loss (dBHL) audiologic evaluation cut points (profound >90 dBHL; severe 70 to 90 dBHL) or by examination and observation of bilateral hearing loss not correctable by amplification. Similarly, "severe" disability in the visual domain has generally been defined as functional bilateral blindness, including examination consistent with presumed visual acuity less than 20/200, or inability to perceive light, or only able to perceive light or reflecting objects. Definitions of "moderate" hearing and vision impairment differ among studies.

Neurodevelopmental Impairment—Difficulties and Realities of a Composite Outcome

Neurodevelopmental impairment is a composite outcome, combining criteria and cut points from several domains as presented above, including neuromotor, cognitive, hearing, and vision. The relative prevalence of each of these outcomes is not consistent, and rates of each outcome may respond differently to an experimental therapy. This necessarily leads to a number of difficulties in overall interpretation, as well as challenges in generalizability and counseling. Use of composite outcomes during counseling in the prenatal or postnatal setting may not be meaningful to parents and families. The value of each of the components may vary broadly for each individual family, outcomes may be conceived differently, and statistics are probably difficult to grasp.[77,78]

In addition, significant center variation in 18- to 22-month neurodevelopmental outcomes has been demonstrated even after adjustment for demographic variables, prenatal interventions, and neonatal clinical factors, thus presenting challenges to accurate counseling from multicenter datasets.[69,79] Unfortunately, robust data from a single center on outcomes of specific high-risk groups are generally unavailable. Furthermore, the largest contributing

component to the composite outcome is that of "cognitive" delay or impairment. As explored previously and discussed in more detail later in the section School-Age Outcomes After Prematurity, developmental tests at 18 to 30 months are intended to assess cognitive abilities. However, in children born at extremely low birth weight (ELBW) and EPT, scores on these early administered tests predict cognitive scores at school age poorly.[15,80–83]

Death or NDI at 2 to 3 years is a primary or main secondary outcome in many clinical trials and prospective studies in neonatal medicine, particularly for EPT infants.[27] This combination is understandable because death and NDI are competing outcomes; it is assumed in this context that adverse outcomes (death and NDI) will not be influenced in opposite directions by an intervention and ideally that components of the combined outcome will carry similar value; these tenets may not always be true. Because the incidence of the composite outcome is greater than any individual component, death or NDI may also be a logical statistical choice for powering a trial; however, it may not be the most biologically plausible target. Moreover, although neurodevelopmental outcome at 2 to 3 years is critically important for any prospective observational study or clinical trial of high-risk infants, it may not be the most appropriate primary outcome for every trial depending on the intervention under evaluation.

Limitations and Challenges to Interpreting Early Neurodevelopmental Outcomes Studies

As described previously, early childhood follow-up visits for high-risk infants at 18 months to 3 years typically assess outcomes in multiple domains. Although these evaluations are important and informative, interpretation and comparison of studies are difficult. Many studies report outcomes by categories and frequently include *any* adverse finding to yield an "impaired" or "unimpaired" status. The definitions of "adverse" outcomes may not be consistent across studies; indeed, the definitions of the individual components of "impairment" such as CP, blindness, deafness, and developmental delay often differ across studies. A literature review highlighted the impact of varying definitions of NDI, applying definitions from several international neonatal follow-up networks to infants 23 to 28 weeks' GA in the Canadian Neonatal Network from 2009 to 2011 with follow-up at 18 to 22 months' corrected age.[84] A fourfold difference in severe NDI incidence was noted, ranging from 3.5% to 14.9%. In addition, differences in risk factors associated with severe NDI were found depending on the definition applied. Furthermore, not all prospective studies have enrolled contemporaneous term, NBW controls. Comparing test scores from a VPT or EPT study group with standardized norms instead of scores from a peer, term-born control group has been demonstrated to substantively limit the relevance and veracity of the findings and may have important public policy and resource implications.[85–88]

Very early childhood developmental and neurologic outcomes evaluations should only be considered as a first step to comprehensive follow-up; assessments into school age, adolescence, and adulthood are critically important to understand the lifetime trajectory and functional and societal outcomes of former high-risk infants.[89–91] Concerns regarding changes in cognitive abilities over time, and an increasing recognition that physical and environmental effects as well as early intervention approaches may modify recovery, underscore the need for later assessments. Some neurocognitive, executive function, and behavioral challenges may only be detected at school age; even recognizing that such learning and attention problems may occur in preterm infants is a critical step to ensuring adequate support and services for families and teachers to help children achieve their best possible outcomes. Evaluation of neuromotor outcomes throughout childhood is also critical. Although most toddler-age and very early childhood outcome studies focus narrowly on the diagnosis of CP, later neuromotor and coordination problems, such as DCD, are prevalent among school-age children born EPT compared with term and can be associated with other functional challenges and academic difficulties. Furthermore, parental perceptions of DCD are not reliable, yet interventions may be able to remediate the functional limitations of DCD, reinforcing the importance of ongoing clinical assessments throughout childhood.[92]

Similarly, there are substantial concerns regarding the ability of developmental or cognitive tests at toddler age, particularly the BSID, to detect developmental delay when using standardized test norms. In a group of EPT and ELBW infants at 2 years' corrected age, Anderson et al.[56] found mean BSID-III cognitive scores of 96.9 and motor scores of 100.4 but also substantially higher than expected scores among a term-born control group. If normative BSID-III cut-point criteria alone were applied to the scores of this cohort, it would severely underestimate moderate-to-severe cognitive and motor delay relative to the control group. Vohr et al.[57] compared mean BSID-II versus BSID-III scores at 18 to 22 months' corrected age for prematurity among infants less than 27 weeks' EGA born in National Institute of Child Health and Human Development (NICHD) Neonatal Research Network (NRN) hospitals over two adjacent periods and found mean cognitive composite scores on the BSID-III to be 11 points higher than mean BSID-II MDI scores. Even after adjusting for differences between groups, BSID-III was a significant factor in a perceived decrease in neurodevelopmental impairment in the more recent time period. Dilemmas also exist with regard to meaningful utilization of BSID-III motor composite and fine motor scaled score normative data and cut points in the context of other gross motor assessments at 18 to 24 months corrected age and the value of these early evaluations to predict later outcomes and intervene appropriately.[93] In a prospective, longitudinal EPT cohort from the Victoria Infant Collaborative Study Group (VICS Group), the BSID-III motor scale normative cut points for impairment at 2 years seriously underestimated rates of motor impairment at 4 years.[94] In addition, although the BSID-III cognitive and language scales at 2 years were associated with cognitive functioning at 4 years as assessed by DAS-II, developmental delay at 2 years as determined by BSID-III reference data and normative cut points had low sensitivity in predicting future cognitive, verbal, and nonverbal reasoning impairments at 4 years on the DAS-II.[82] All of these findings have important implications for resource availability and public policy. In countries, states, or regions without specific policies advocating for ongoing, longitudinal assessments and services for children born EPT, the 2-year or 3-year evaluation may be the final opportunity to identify challenges before transitioning to the school system. If ongoing needs are determined only by scoring below a normative cut point on the BSID-III, many children at significant risk for future impairments could be left behind.

Despite these many challenges and provisos, a substantial body of literature exists on early neurologic and cognitive outcomes. An understanding of the range of initial neurodevelopmental outcomes, and the factors associated with adverse outcomes, is crucial for both the family and medical care team. In later sections of this chapter, we review early neurodevelopmental outcomes of recent

high-risk cohorts, including those born EPT and selected groups of high-risk late preterm and term infants.

Focus on Functional and Adaptive Outcomes

The WHO has defined "disability" as an umbrella term, covering impairments, activity limitations, and participation restrictions. An impairment is a problem in body function or structure; an activity limitation is a difficulty encountered by an individual in executing a task or action; and a participation restriction is a problem experienced by an individual in involvement in life situations. According to the World Report on Disabilities,[95] approximately 95 million infants, children, and youths 0 to 14 years of age worldwide (5%) live with a disability, of whom 13 million (0.7%) are considered to have severe disabilities. Nearly 800 million (~16%) individuals more than 15 years old live with a disability. The WHO International Classification of Functioning, Disability, and Health (ICF) provides standard language and a framework for the description of health and health-related states that are focused on functioning rather than diagnosis alone.[96] It is a classification of health and health-related domains that describes what a person with a health condition can do in a standard environment, as well as how he or she can perform in his or her usual environment. These domains are classified from body, individual, and societal perspectives organized in two parts, each comprising two components. Part 1—Functioning and Disability—includes Body Functions and Structures and Activities and Participation; Part 2—Contextual Factors—incorporates Environmental Factors and Personal Factors. The ICF is meant to be universally applicable and useful in a range of different sectors, including individual assessments, public policy, and research. The focus of the ICF is on health and functioning, rather than on disability. In most studies and approaches, particularly in early childhood and even through school age, impairments are identified through tests or examinations, and an individual is classified with regard to that finding alone. In contrast, the ICF approach is to measure functioning in society, regardless of underlying impairments. This allows for a broader view than a traditional classification of health and disability. The ICF shifts the focus from diagnosis or label to impact on function, which may be affected by environment, intervention, and other factors.

In 2011, the WHO approved a "derived" classification, the International Classification of Functioning, Disability and Health for Children and Youth, which conforms to the common classifications of the ICF, with a goal of creating a comprehensive, lifespan approach to describing health and functioning. However, attempts to apply these functional outcome classification approaches to very early childhood follow-up studies of high-risk infants have been limited, with some notable exceptions, including Msall and colleagues, who have championed the framework of function, even at early preschool age, as a highly relevant and measurable outcome.[25] Among the several instruments available, the Vineland Adaptive Behavior Scale (VABS) is an interview survey for assessing adaptive behavior (Sparrow et al., 2005).[97] In children less than 6 years old, the domains include communication (receptive, expressive language), daily living skills (self-care/personal), socialization (interpersonal relations and play), and motor skills (gross and fine). There is a classroom edition for children 3 years 0 months to 12 years 11 months. The Pediatric Evaluation of Disability Inventory (PEDI) assesses self-care, mobility, and social functional activities in children 6 months to 7 years[98] and has been used extensively in children with severe perceived or known disabilities including those with CP and other physical impairments. The Warner Initial Developmental Evaluation of Adaptive and Functional Skills (WIDEA-FS) is a measure of multidomain development, including adaptive and functional skills.[25] The WIDEA-FS is a 50-item parent questionnaire designed to assess the adaptive skills of a child in several domains, including mobility, communication, social cognition, and self-care. The WIDEA-FS takes 10 to 15 minutes to administer, can be conducted by phone, and is available in English and Spanish. WIDEA-FS scores were significantly associated with Bayley-III scores in all domains. Studies have demonstrated concurrent validity between the WIDEA-FS and BSID-III[99] as well as with the Capute Scales.[100] Lower scores on the WIDEA-FS were significantly associated with an increased risk of adverse developmental performance on all BSID-III scales, and cut points for WIDEA-FS scores with at least 90% sensitivity have been published.[99] The WIDEA-FS has been used in studies where in-person evaluations are not feasible, including in an international follow-up study of outcomes after Zika exposure.[101]

Outcomes of Preterm Infants Across the Life Spectrum

Early Neurodevelopmental Outcomes of Extremely Preterm Infants

Early neurodevelopmental outcomes of several large cohorts of EPT infants around the world have been reported. These are summarized in Table 24.1 and presented in detail later.

The Victoria Infant Collaborative Study Group

The VICS Group has reported on a series of birth cohorts of EPT neonates born in the state of Victoria (Australia) from 1991 to 1992, in 1997 and 2005, and most recently from April 1, 2016, to March 31, 2017.[86,102,103] Neonates born alive at 22 to 27 completed gestational weeks and surviving to follow-up received neurodevelopmental assessment at 2 years of corrected age (1991–1992, $n = 219$, 97% of survivors; 1997, $n = 149$, 99% of survivors; 2005, $n = 163$, 95% of survivors; 2016–2017, $n = 174$, 81% of survivors).[103] Importantly, contemporaneous term controls were also enrolled and evaluated for each cohort. Children were evaluated for blindness, deafness (hearing loss requiring amplification or worse), and developmental delay. To account for the different versions of the Bayley Scales used, developmental delay was classified relative to the MDI or combined cognitive and language composite scores for the contemporaneous term controls, rather than the normative test scores and cut points alone. Developmental delay was defined as less than −1 SD relative to the mean of the controls and categorized as mild (−2 SDs to < −1 SD), moderate (−3 SDs to < −2 SDs), or severe (< −3 SDs). Neurologic examination for CP was also performed, utilizing the GMFCS, describing "severe" CP as unlikely ever to walk and "moderate" CP as unable to walk at 2 years but likely to walk. Overall disability was considered "severe" for children with severe CP, blindness, or severe developmental delay; "moderate" with moderate CP, deafness, or moderate developmental delay; and "mild" with mild CP or mild developmental delay. "Major" disability comprised either the moderate or severe category.

Neurodevelopmental outcomes of the 2016–2017 VICS cohort at 2 years' corrected age[103] are shown in Table 24.1. The birth cohorts, rates of any disability, and moderate-severe

TABLE 24.1 **Early Neurodevelopmental Outcomes: Selected Extremely Preterm Cohorts**

	VICS 2016–2017 (Cheong 2021)	NICHD NRN (Adams-Chapman 2018)	NICHD NRN (Younge 2017)	Japan NRN (Kono 2018)	CNFUN (Synnes 2017)	EPICure 2 (Moore 2012)	EXPRESS (Serenius 2013)
Study group description	22–27 $^{6}/_{7}$ week EGA	<26 $^{6}/_{7}$ week EGA	22–24 $^{6}/_{7}$ week EGA	22–24 $^{6}/_{7}$ week EGA	<29 weeks EGA	22–26 week EGA	<27 week EGA
Birth years	2016–2017	Evaluated 4/2011–1/2015	2008–2011	2008–2012	2009–2011	2006	2004–2007
Age at follow-up corrected for prematurity	2 years	18–26 months	18–22 months	36–42 months (chronological)	18–21 months	3 years	2.5 years
# (% follow-up of eligible survivors)	174 (81%)	2113 (87%–90% over study years)	487 (92%)	832 (60.7%)†	2340 (83%)	576 (55.3%)†	415 (90%)
Outcomes							
Blind	0.6	1.1% (bilateral)	0.4% (bilateral)	4.4%‡	1.6%	1%	0.9%
Deaf/require aids	2%	3%	2.9%	Amplification required: 1%	2.6%	Not improved by aids: 0.2% Improved by aids: 5%	Not improved by aids: 0.2% Improved by aids: 0.7%
*Developmental/cognitive	Any: 41% Mild: 26% Moderate: 11% Severe: 5%	Bayley-III Cognitive <85: 28% <70: 10%	BSID-II <70 or Bayley-III Cognitive <85: 41%	KSPD DQ <70: 37.3%	Bayley-III Cognitive <85: 14.7% <70: 3.3%	Predicted MDI 70–84: 34% <70: 30%	*Cognitive/language None: 55% Mild: 25% Moderate: 11% Severe: 9%
Cerebral palsy (CP) or motor delay	Any CP: 6% Moderate: 2% Severe: 1%	Moderate: 4.7% Severe: 2.1%	Moderate: 6.2% Severe: 5.3%	CP with GMFCS >II: 9.5%	Any CP: 6.4%	Any CP: 14% Moderate motor: 3% Severe motor: 5%	Mild: 2.9% Moderate: 2.9% Severe: 1.3%
Disability or impairment	None: 49% Mild: 26% Moderate: 11% Severe: 5%	NDI using Bayley-III Cognitive as: <85: 32% <70: 19%	Moderate-Severe NDI: 43%	NDI: 39.1%	NDI: 46% Significant NDI: 16.5%	None/mild: 75% Moderate: 12% Severe: 13%	None: 42% Mild: 31% Moderate: 16% Severe: 11%

*For VICS: developmental delay was classified relative to contemporaneous term control group; for EPICure 2: Predicted MDI (Mental Developmental Index) BSID ed 2 from Bayley-III; for EXPRESS: aggregated Bayley-III cognitive and language score information, with mean and SD relative to a contemporaneous 37- to 41-week GA control group; for Japan NRN: formal evaluation by the Kyoto Scale of Psychological Development (KSPD) was available for 472.
†Multiple imputation from perinatal, neonatal, and sociodemographic information estimated outcomes for the entire cohort.
‡Defined as blindness or no functional vision in one or both eyes.

disability at 2 years were not significantly different across eras. However, survival with no major disability increased over time from 42% in the 1991–1992 cohort to 62% in the 2016–2017 cohort (odds ratio [OR] 1.3 per decade, 95% confidence interval [CI] 1.15 to 1.48, p<0.001). Of note, for the 1991–1992, 1997, and 2005 cohorts, rates of any CP were 11%, 12%, and 10% respectively, whereas for the 2016–2017 cohort the rate of any CP had decreased to 6%. Moderate or severe developmental delay were similar overall across cohorts, with a decrease noted between 1997 and 2005 but an increase in 2016. In terms of 2-year outcomes by gestational ages, mortality and survival with no major disability appeared stable across birth cohorts for those born at 22 and 23 weeks. Mortality improved consistently for those born at 24 weeks from 1991–1992 to 2016–2017. A trend toward improving rates of survival with no major disability was observed across cohorts particularly for those in the more advanced gestational age week groups, with approximately 80% of those born at 26 weeks and 27 weeks in the 2016–2017 cohort having survived with no major disability at 2 years' corrected age. Although the follow-up rate was lower for the most recent birth cohort compared with previous cohorts, multiple imputation and sensitivity analysis was performed, which resulted in the same results and conclusions.

EPICure 2

The EPICure 2 Study is a population-based mortality, morbidity, and neurodevelopmental outcomes study.[104] All neonates born at 22 to 26 $\frac{6}{7}$ weeks' gestation in the United Kingdom during 2006 were identified and data about death and morbidities were collected from the delivery room through hospitalization.[105] Comparisons between EPICure 1[106] (1995 birth cohort) and EPICure 2, both on short-term and neurodevelopmental outcomes, were limited to those 22 to 25 $\frac{6}{7}$ weeks' gestation and born in England. For EPICure 2, follow-up was hampered by changes in national research governance procedures in the United Kingdom and increased privacy restrictions by the National Health Service. Therefore, tracking success was substantially limited in comparison with the previous study, and multiple imputation was therefore used to estimate outcomes of patients lost to follow-up. Follow-up assessments in EPICure 2 were with the BSID-III at a target age of 36 months, and at a target age of 30 months with the BSID-II in EPICure 1. In order to directly compare results from the two cohorts, combined BSID-III scores were converted to "predicted MDI."[107] "Severe impairment" was defined as nonambulant CP (GMFCS III to V), blindness, profound hearing loss, or developmental quotient less than 3 SD below the mean for age. "Moderate impairment" was defined as ambulant CP (GMFCS 2), functionally impaired vision, hearing loss improved by aids, or developmental score of 2 and 3 SD below the mean.

Of the 1031 children in EPICure 2 who survived to follow-up (1041 survivors to discharge, 10 died after discharge), study examiners evaluated 576 (55.3%, median 34 months), and information from an additional 191 were available from local records (18.3%, median 25 months) (see Table 24.1). The groups seen in person and not seen in person had similar baseline perinatal and neonatal characteristics, but socioeconomic factors differed; children from families with greater social disadvantage were less likely to have a formal study evaluation.

Among the EPICure 2 group with formal study evaluations, 75% were free from impairment or had mild impairment. Survival without disability for the entire EPICure 2 cohort, including imputed outcomes, among those admitted to the NICU demonstrated increases with each week in gestational age: 22 weeks, 5% (95% CI, 0% to 26%); 23 weeks, 15% (95% CI, 10% to 21%); 24 weeks, 30% (95% CI, 25% to 35%); 25 weeks, 49% (95% CI, 43% to 55%); 26 weeks, 62% (95% CI, 57% to 67%). In addition, 66% had no hearing, vision, or communication disability, but only 36% of children were reported to have no developmental disability (BSID-II scores >85). However, robust comparisons are difficult, given that patient numbers were quite small, particularly for the less than 23 weeks' EGA group. This finding underscores the grave prognosis for survival among the most premature infants in this cohort; only 18% of less than 23 weeks' EGA neonates admitted alive to an NICU survived to discharge, compared with 48% of 25 weeks' EGA neonates.

Extremely Preterm Infants in Sweden Study

The Extremely Preterm Infants in Sweden Study (EXPRESS) cohort (birth year 2004–2007, <27 weeks' gestation) was followed to a median of 30.5 months, at which point BSID-III, neurologic examinations, and parental questionnaires were administered.[108] Of the 707 liveborn infants, 491 survived to follow-up (69%), 415 of 461 who were eligible for inclusion were assessed (90% follow-up rate), and 399 of these completed at least part of the BSID-III. Chart review was available for 41 additional children. The group was skewed to higher gestational age, with only 52 (11%) of the cohort born at 22 or 23 weeks' gestation. Of importance, a control group (37 to 41 weeks' gestation, matched 2:1 with the preterm group) was recruited by random selection from the Swedish Medical Birth Registry. Severe disability was defined as BSID-III composite cognitive, language, or motor score greater than 3 SD below the mean relative to the control group, severe CP, or bilateral blindness or deafness. Moderate disability was defined as scores between 2 and 3 SD from the mean of any of the BSID-III scales, moderate CP, and moderate visual or hearing impairment. Mild disability was defined as scores between 1 and 2 SD from the mean of any of the BSID-III scales or mild CP.

As summarized in Table 24.1, of those children born EPT, 11.3% had moderate or severe cognitive disability by BSID-III, compared with 0.5% of controls. When considering cognitive, language, or motor scores, 15% of EPT and 3% of control had moderate disability, and 8.9% of EPT and 0.3% of controls had severe disability. The proportion of children with mild or no disabilities increased from 40% at 22 weeks to 83% at 26 weeks. There was a significant decrease in severe disabilities with each increase in gestational week, with an OR of 0.58 (99% CI, 0.39 to 0.86), $P < .001$. Overall, 42% of the EPT children in this group had no disability at 30 months, compared with 78% of control children. The majority of the 58% disabled EPT children had mild disability (31%). In further analyses, Serenius et al.[109] explored whether intensity of perinatal care in regions across Sweden was associated with an increased risk of death or NDI at 2.5 years. The investigators found significant variation in obstetric and neonatal intervention practices. In regions with more aggressive perinatal intervention practices, the risk of death or NDI at 2.5 years was reduced, but only for the 22 to 24 weeks group. Furthermore, there was no increase in NDI among survivors associated with more aggressive perinatal practices.

Another national Swedish birth cohort, EXPRESS 2, included all births in Sweden at 22 to 26 weeks' gestational age during a three-year period between January 1, 2014, and December 31, 2016.[110]

Japan Neonatal Research Network

The NRN of Japan reported outcomes of neonates born alive at 22 to 24 $^{6}/_{7}$ weeks' gestation during two periods (period 1, 2003–2007 and period 2, 2008–2012) from 52 tertiary centers with at least 10 infants of that gestational age range inborn during each period.[111] Neurodevelopmental assessments were performed at 36 to 42 months' chronologic age by a trained pediatrician and consisted of a neurologic examination, functional assessment for vision and hearing, and cognitive evaluation by the Kyoto Scale of Psychological Development (KSPD). A developmental quotient (DQ) was derived, with a mean SD score of 100.6 ± 13.4. NDI for this study was defined as any CP with GMFCS II to V, hearing impairment (amplification was required), visual impairment (blindness with no functional vision in at least one eye or bilateral amblyopia), or KSPD DQ score of less than 70. Of note, this group has previously published that KSPD DQ <70 is equivalent to BSID-III cognitive score < 85.[112]

Highlights of outcomes are shown in Table 24.1. Active treatment, defined as receiving any of tracheal intubation, ventilator support including continuous positive airway pressure, surfactant therapy, or parental nutrition at delivery or in the NICU period, was reported for 98.6% of infants in period 1 and 98% of infants in period 2. Infant survival to 3 years increased from 66.5% in period 1 to 74.6% in period 2. At 3 years, of 983 survivors in period 1 and 1372 survivors in period 2, 631 (64.1% of survivors) and 832 (60.7% of survivors) had some neurodevelopmental outcome data. Among infants with actual evaluations, CP with GMFCS ≥II (15.9% vs. 9.5%, p<0.01), visual impairment (13.6% vs. 4.4%, p<0.01), and hearing impairment (2.6% vs. 1%, p<0.05) decreased significantly from period 1 to period 2. The proportion of DQ of KSPD <70 increased from 32.8% to 37.3% although this was not significant. No significant changes were noted in NDI between period 1 and 2 among infants with full evaluations (39.4% vs. 39.1%) or in those with incomplete evaluations (45.7% vs. 38.1%). Multiple imputation was also used to account for missing data with the assumption that data were missing at random, modelled to adjust for potential confounding demographic and perinatal factors between periods. Results after multiple imputation indicated that death or NDI decreased between periods 1 and 2 from 53.7% to 46.9% (aOR 0.83, 95% CI 0.71 to 0.97), but both KSPD DQ <70 (26.7% vs. 27.8%) and NDI (30.3% vs. 28.8%) were not significantly different.

Eunice Kennedy Shriver NICHD Neonatal Research Network Follow-Up Study Group

The NICHD NRN was initiated in 1986 as a multicenter effort in the United States with the main objective of providing a registry of uniformly collected baseline and morbidity and mortality data information to provide the basis for planning and implementing clinical trials. The NICHD NRN Follow-Up Study Group was later added to provide neurodevelopmental follow-up for trials and for those meeting NRN Follow-Up Study criteria, which now includes infants less than 27 weeks' gestation. Highlights of selected works noted below are summarized in Table 24.1.

The NICHD NRN reported on the spectrum of neurodevelopmental outcomes of neonates born < 27 weeks' GA at 18 to 26 months' corrected age with follow-up assessments completed April 2011 to January 2015.[46] The follow-up visit included the BSID-III, as well as standardized neurosensory and motor examinations. Predefined criteria were utilized for neurologic outcomes including normal, suspect (isolated abnormal neurologic findings without functional impairment), abnormal but without CP (neurologic findings and functional impairment), and CP. Severity of CP was defined as mild with GMFCS level I, moderate with GMFCS level II or III, and severe with GMFCS level IV or V. For the primary analyses, NDI was defined by presence of 1 or more of moderate to severe motor impairment (CP or non-CP) with a GMFCS level ≥II, a BSID III cognitive score of <70, severe visual impairment (absence of functional vision in both eyes), or bilateral severe hearing loss (permanent, not allowing for ability to understand spoken words with or without amplification). Analyses defining moderate-severe NDI were also performed using a BSID III cognitive score cutoff of <85. The results of this analysis were important, as they demonstrated the range of neurologic outcomes among extremely preterm infants, with 59% with normal neurologic exams, 19% suspect, 9.8% abnormal but non-CP, and 11.7% with CP. Overall, CP was mild in 41%, moderate in 40%, and severe in 18%; the diagnosis of CP decreased from 2011 (16%) to 2014 (9%), and among those with CP the proportion with severe CP decreased (26% to 16%). For the entire cohort, the rate of NDI with BSID-III cognitive cutoff <70 was 19% (28% among 22 to 24 weeks' GA, 14% among 35 to 36 weeks GA). The rate of NDI with cutoff <85 was 32%. The rate of NDI by either definition did not decrease significantly over the years encompassed in this analysis.

The NICHD NRN also reported on the neurodevelopmental outcomes at 18 to 22 months' corrected age among children born at 22 $^{0}/_{7}$ to 24 $^{6}/_{7}$ weeks across three birth epochs (2000–2003, 2004–2007, 2008–2011).[113] As these epochs spanned BSID editions, moderate-severe NDI was defined as any of BSID-II MDI <70 or BSID-III cognitive composite score <85, GMFCS level II or greater, profound hearing loss bilaterally, or profound visual impairment. A significant decline in mortality across epochs for this population was accompanied by an increase in both the rates of survival without and with NDI. Among survivors to follow-up in epochs 1, 2, and 3, rates of NDI (49%, 46%, 43%) and moderate-severe CP (15%, 11%, 11%) were not found to have decreased significantly. However, profound visual impairment (2%, 2%, <1%) decreased significantly across epochs (p = 0.04).

As with *p*revious analyses from the NICHD NRN,[79] center differences in outcomes were observed in these studies, suggesting that variability beyond what can be explained by perinatal and neonatal risk factors plays an important role in outcomes. Between-hospital variation in survival and NDI at 18 to 22 months was also explored by Rysavy et al.[114] among infants inborn at an NRN site before 27 weeks between 2006 and 2011. Outcomes were evaluated in relation to hospital rates of "active treatment," defined as potentially lifesaving treatments initiated after delivery. For all infants, overall mean rates of survival without severe impairment were 3.4%, 17.9%, 44.7%, 61.1%, and 75.6% for 22, 23, 24, 25, and 26 weeks' gestation, respectively. However, there were significant differences among hospital rates of active treatment for those born at 22 to 24 weeks, which accounted for a substantial proportion of variation in outcomes. In an update to and validation of the NICHD Extremely Preterm Outcomes Tool (https://www.nichd.nih.gov/research/supported/EPBO), Rysavy and colleagues demonstrated that among actively treated infants born <26 weeks' GA, rates of moderate-severe NDI at 18 to 26 months' corrected age were 21% among those with an estimated survival probability greater than 80%, and 78% among those with an estimated survival probability of 11% to 20%.[60] No actively treated infants with a probability of survival ≤10% survived to follow-up.

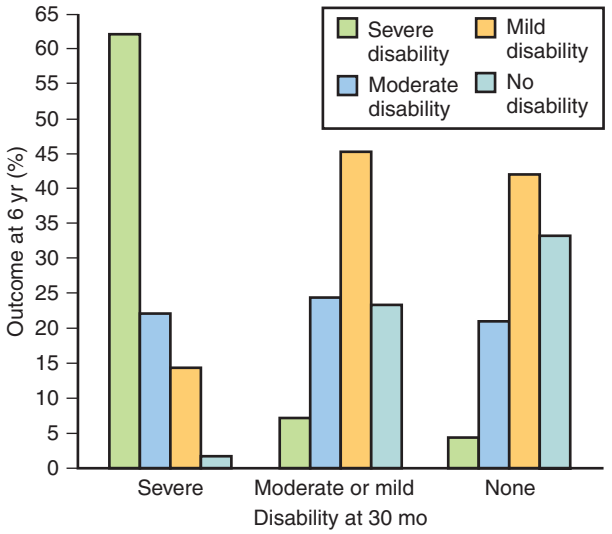
Canadian Neonatal Follow-Up Network (CNFUN)

The Canadian Neonatal Follow-Up Network (CNFUN) is a collaboration between Neonatal and Perinatal Follow-Up Programs in Canada and their multidisciplinary team members, developed in coordination with the Canadian Neonatal Network (CNN) and the Canadian Institutes of Health Research (CIHR) Team in the Maternal-Infant Care (MiCare) study. The CNFUN developed a national standardized assessment and data collection database for infants at 18 to 21 months' GA, with the ability to link data to the CNN database to advance research and knowledge translation to improve care and outcomes.

The CNFUN reported on neurodevelopmental impairment (NDI) and significant NDI (sNDI) and their determinants at 18 to 21 months' corrected age among infants born between 2009 and 2011 at <29 weeks' gestational age, admitted to one of 28 CNN NICUs.[69] Infants were excluded if they were moribund on admission or had life-threatening congenital anomalies. The NDI definition included any of CP with GMFCS I or higher; BSID-III motor, language, or cognitive composite < 85; any sensorineural or mixed hearing loss; or unilateral or bilateral visual impairment. Significant NDI included any of CP with GMFCS III or higher; BSID-III motor, language, or cognitive composite <70; need for hearing aid or cochlear implant; or bilateral visual impairment. Of 3700 eligible infants admitted to NICUs, 592 died prior to discharge, 276 were not linked to CNFUN, and there were 22 post-discharge deaths; 2340/2810 survivors (83.3%) had assessment at 18 to 21 months' corrected age. Among those, 16.5% were categorized with sNDI and 46.0% with NDI. Rates of NDI and sNDI were inversely correlated to GA, with sNDI was present in 37.1% of those both ≤23 weeks' GA but only 8.4% of those 28 weeks' GA, and NDI present in 62.7% of those both ≤23 weeks' GA and 36.2% of those 28 weeks' GA. For the entire cohort, a diagnosis of CP of any severity was made in 6.4%, ranging from 4.8% for those 28 weeks' GA, to 13.1% for those ≤23 weeks' GA. Factors independently associated with sNDI included lower GA, sex, higher SNAP score, ROP, brain injury, and infection. As in other multicenter studies, substantial center variation was also noted in NDI, sNDI, death or sNDI, and the components of NDI.

School-Age Outcomes After Prematurity

School-age survivors of prematurity have high rates of neurodevelopmental impairment, with CP rates 9% to 10% and cognitive disability rates of 4% to 36%, depending on the population evaluated, outcome definitions, and age at assessment.[115] Rates of major neurosensory disability and average IQ at school age among former extremely preterm infants have not improved significantly since the 1990s.[116] Infants who have *severe* cognitive, motor, or neurosensory impairments in early years (2 to 3 years of life) often have moderate or severe impairments at school age.[85,117,118] In the EPICure cohort of infants born ≤25 weeks' gestational age, 86% of children with severe disability at 30 months continued to have moderate or severe disability at 6 years (Fig. 24.1).[85] In the more recent Extremely Low Gestational Age Newborn (ELGAN) cohort of infants born <28 weeks of gestation, 64% of children classified as profoundly impaired at 2 years had moderate, severe, or profound disability at 10 years.[118] Most commonly, though, school-age survivors of ELBW or very preterm birth have more mild impairments that are difficult to identify or predict at earlier ages. These impairments include mild cognitive impairment (IQ 1 to 2 SDs below the mean, or 70 to 84); learning, emotional, behavior, motor coordination, and executive function disorders; and poor academic achievement.



• **Fig. 24.1** Severity of disability at 6 years, based on classification at 30 months corrected age. Data are based on 236 children who were born at 25 or fewer completed weeks of gestation in the United Kingdom and Ireland in 1995. (From Marlow N, Wolke D, Bracewell MA, Samara M, EPICure Study Group. Neurologic and developmental disability at six years of age after extremely preterm birth. *N Engl J Med*. 2005;352:9–19.)

Cognitive delay is the most common impairment in children who were born very preterm. In a 2018 systematic review of 71 studies that included 7752 preterm infants and 5155 controls at 5 to 20 years of age, preterm born children had 0.86 SD lower intelligence quotient (IQ) than controls.[119] The most immature infants have the highest risk for poor cognitive outcome.[85,120] For example, at 6 years 49% of boys and 32% of girls in the EPICure cohort (≤25 weeks' gestation at birth) had cognitive impairment.[85] Yet, even moderate and late preterm infants have an increased risk for poor cognitive functioning compared to full-term peers at school age.[121] Biologic factors such as sex, birth weight, and race are predictive of early cognitive outcomes.[122] As children enter school age, parental education is more predictive of cognitive outcome than biologic or perinatal factors. Furthermore, children with social advantage (two-parent household, educated parents, employed parents) demonstrate more cognitive gains through early childhood than those without social advantage.[123]

Similarly, in 14 studies of school-age academic achievement, very low birth weight or very preterm infants have scores on math, reading, and spelling tests that are one-half to three-quarters of an SD below term peers.[124] Performance on academic assessments is also closely correlated with both birth weight and gestational age.[120,124,125] Thus, the most immature infants born at or before 25 weeks of gestation have eight times higher odds of serious impairment in academic achievement than matched term controls.[85]

Measures of executive function (verbal fluency, working memory, and cognitive flexibility) also demonstrate decreases of about a half SD between very preterm or very-low-birth-weight infants and term controls.[120,124,126] Unlike cognitive performance, executive functions may not be influenced by socioeconomic status and dysfunctions in some domains such as working memory and

organization may be increasing among the children born most recently.[126]

At school age, children who were born preterm are more frequently reported by both their parents and their teachers to have behavior problems. Very preterm and even late preterm infants are more likely to have internalizing or externalizing symptoms on the Child Behavior Checklist than term counterparts.[125,127,128] Internalizing symptoms manifest as shyness, social maladaptation, anxiety, and withdrawn behavior.[129,130] It is critical to note that behavioral problems commonly co-occur in children with cognitive disabilities. In the French regional EPIPAGE cohort of children born <33 weeks, there was a direct correlation between cognitive performance and behavioral problems.[128] Children with IQ more than 2 SDs below the mean had 2.6 times higher odds of behavior problems than those with IQ less than 1 SD below the mean. However, cognitive disabilities did not entirely explain the higher rates of behavior problems in preterm-born children, because preterms remained at higher risk even after adjustment for cognitive performance. Behavioral problems in this cohort were more common in boys and in children with more complex medical histories, socioeconomic risk factors, and poor maternal well-being.

Neurobehavioral or psychiatric problems including autism spectrum disorders (ASDs) and attention deficit-hyperactivity disorder (ADHD) are reported with higher frequency in school-aged former preterm children than in the general population.[125] In the Extremely Low Gestational Age Newborn (ELGAN) study of infants born before 28 weeks, 7% were diagnosed with ASD at school age.[131] Boys were twice as likely as girls to have ASD in this cohort. Moderate and late preterm infants may also be more likely to have ASD: one study reported that these children have 1.55 higher odds of being diagnosed with autism by 10 years than full-term-born children.[132] In preterm-born children, risk of ASD seems to be particularly increased among those who were small for gestational age, those with congenital malformations, low 5-minute Apgar scores, intracranial bleeding, cerebral edema, or seizures in the neonatal period, and those born to mothers with preeclampsia.[132]

In a systematic review, preterm-born children had a relative risk for being diagnosed with ADHD of 2.64 (95% CI 1.85 to 3.78) when compared to term-born children.[125] In a large Swedish cohort, odds of ADHD at school age decreased with increasing gestational age until term-corrected age.[133] Odds of ADHD were 2.1 among the most immature (23 to 28 weeks' gestation) infants and 1.1 among early term (37 to 38 weeks' gestation) infants. In the most preterm children, risk of ADHD does not seem to be significantly impacted by socioeconomic factors. However, sociodemographic risk—particularly low maternal education—may increase risk for ADHD in moderately preterm children born to women with lower education.[133] Across the entire gestational age spectrum, school-age boys are about four times more likely to be diagnosed with ADHD than girls. Children with multiple psychiatric disorders, such as ADHD and ASD, have significantly higher likelihood of repeating a grade, requiring an aide in school, or being placed in a special classroom setting and have lower quality of life.[134]

Because adverse developmental and neurobehavioral outcomes are considerably more common among preterm infants, it is essential for the pediatrician caring for former preterm school-age children to have a high index of suspicion for these problems. Many developmental and behavioral problems are more common among preterm-born male children than female children. Further, while risk is highest in the most extremely preterm infants, moderately preterm and late preterm infants are also at higher risk for adverse outcome at school age than term-born counterparts. School-age problems in the older preterm infant include fine and gross motor coordination problems, academic difficulties, attention problems, and need for special support in school.[135] As moderate and late preterm infants far outnumber very preterm infants, it is critical to be aware of the likelihood of such outcomes and address any concerns or potential problems expeditiously. Early access to services is essential for optimizing outcomes in these children. Cognitive and neurobehavioral problems in all high-risk children significantly increase special education costs and resource utilization at school age and strongly influence outcomes later in life.

Adolescent and Adult Outcomes After Prematurity

The advent of assisted ventilation, surfactant, and antenatal corticosteroids in the late 20th century led to the first substantive generations of extremely low birth weight (ELBW) and extremely preterm-born survivors. These surviving preterms are now adolescents and adults, whose outcomes are of importance not only to pediatricians, but also to the adult physicians who will care for them for the remainder of the life course. The associations between increased risk for all categories of adverse outcomes and decreasing gestational age continues into adolescence and adulthood.[136] Medical sequelae of prematurity include impacts on respiratory, cardiovascular, and renal function. Surviving ELBW infants are more likely to have CP, blindness, and deafness than term-born matched controls.[137] As these are typically fixed impairments, rates in adulthood closely mirror rates at school age. Further, over time, ELBW survivors have higher rates of other visual impairments, including refractive errors and late retinal detachment.[138] In adulthood, preterm survivors are more likely to receive disability pensions than term-born controls.[137]

Very preterm, very-low-birth-weight, and moderately preterm-born adolescents are less likely to complete high school than adolescents born full term.[139–141] However, the majority do not require special education support and are able to complete high school.[136] Very-low-birth-weight or very preterm-born adults are also less likely to seek education beyond high school.[139,141,142] In adulthood, preterm-born cohorts have worse performance on tests of academic achievement than term-born controls.[139] Few studies have closely evaluated cognitive function in preterm survivors. IQ is generally reported to be about a half a SD below term controls, but differences depend on the population studied. Predictors of adolescent and young adult IQ include maternal educational level, birth weight, gestational age, and small for gestational age birth.[139,143,144]

Even in adults without neurosensory or cognitive impairment, impairments in learning or executive function may interfere with educational and vocational achievement. Executive function deficits primarily involve impairments in response inhibition and mental flexibility.[145] As discussed above, autism and attention deficit disorders are more common among both very preterm or low-birth-weight children and even moderate-/late-preterm children; these disorders likely persist into adulthood.[146,147] Reports of increased rates of schizophrenia, anxiety, and depression among

preterm-born adults are inconsistent and conflicting.[147,148] In a large Norwegian study, relative risk of autism spectrum disorder was 9.7 times higher among adults born before 28 weeks as compared to those born at term.[147] Furthermore, relative risk of having a disorder of psychological development, behavior, and emotion resulting in a need to claim disability benefits was 10.5 times higher among those born before 28 weeks.

Adults who were born at 23 to 27 weeks' gestation are 7.5 (95% CI 5.5 to 10.0) times more likely to have a medical disability affecting the ability to work than those who were born full term.[147] Among those who do not have a medical disability, very preterm-born adults have significantly lower educational level, have lower income, and are less likely to get married and become parents.[90,147] Relative risk of having at least one child among men born at 22 to 27 weeks is 0.24, relative risk among women is 0.33.[149] Further, preterm-born women are more likely to have preterm babies. Preterm-born adults are less likely to move outside their parents' home or cohabitate with a partner.[141,149] Net income is significantly lower among very preterm-born adults.[90,147] On the positive side, preterm- and very-low-birth-weight-born adults are consistently reported to have lower rates of risk-taking including smoking, alcohol, and drug use, and delinquent behavior.[90,148] This is likely related to persistent tendencies toward internalizing behaviors and fewer externalizing behaviors throughout life.[150]

Health-related quality of life is an important consideration as our most fragile and vulnerable patients age into adulthood.[151,152] Only a few small studies have evaluated the long-term impact of prematurity on self-reported quality of life. Saigal and colleagues assessed health-related quality of life in a population of ELBW infants born in 1977–1982 when the participants were 24 years old. Despite more functional limitations (cognition, sensation, mobility, and self-care), the ELBW participants did not report significantly different health-related quality of life from term-born controls. Later, in their 30 s, the same cohort indicated lower self-esteem along with lower measures of multiple functional and health-related outcomes.[90] Similarly, in a small Danish cohort of young adults, objective quality of life (based on societal standards) was lower in those born very preterm or with a chronic health problem.[152] However, subjective quality of life (based on individual life preferences and experiences) was not significantly different.

In summary, the impact of prematurity on medical, developmental, psychological, and functional domains persists throughout the life course. It is critical to note that the most extremely preterm (22 to 23 weeks' gestation) infants are only now surviving into adulthood. Adolescent and adult impact of survival at the limits of viability remains to be fully described in the current era of neonatal medicine.

Risk Factors for Adverse Outcomes in Preterm Infants

As described above, preterm infants as a group are at risk for multiple adverse outcomes throughout childhood and into adulthood. These risks are modified by patient characteristics, morbidities, and complications that occur during the neonatal period. Important neonatal morbidities that directly impact outcomes of preterm infants include brain injury, bronchopulmonary dysplasia (BPD), ROP, infection, necrotizing enterocolitis (NEC), and poor growth and nutrition. We end this section by addressing the relationships between socioeconomic factors and childhood outcomes.

Brain Injury

Cranial Ultrasound

Cranial ultrasound (CUS) has been used to image preterm infant brain injury since the late 1970s.[153,154] With the development of a standardized grading system for intracranial hemorrhage (ICH),[155] CUS quickly became and remains the neuroimaging standard of care for preterm infants.[156] Although many still seem to rely heavily on simply the presence of grade 3 ICH, intraparenchymal hemorrhage (IPH), or cystic periventricular leukomalacia (PVL) to counsel families about the neurodevelopmental outcomes of their preterm infants, the complexity of interpretation of CUS findings, and limitations of prediction of outcomes with any single neuroimaging or other finding, should give clinicians cause for prudence and careful consideration.

Virtually every major study of early neurodevelopmental outcomes among preterm and ELBW infants has confirmed a strong association between major CUS abnormalities and adverse neurologic and developmental outcomes. Definitions of CUS abnormalities as well as specific outcomes differ among studies. Traditionally, adverse neuroimaging findings have included Papile ICH grade 3 or 4, regardless of laterality. Other studies consider intraparenchymal hemorrhage (IPH), ventriculomegaly (VM), or cystic changes, also often without respect to laterality or extent of the findings, to be severe abnormalities. In some, persistence of periventricular echodensity or "flaring" is included.[157–159] The diagnosis reported in studies is frequently based on the results from a single CUS, either the "worst" or the "final" imaging study, but some prospective cohorts include serial imaging.

The focus of many studies has been on exploring the association of major CUS findings with CP. The ELGAN study followed infants of less than 28 weeks' gestation from 14 institutions across five states in the United States from 2002 to 2004.[160,161] Three study CUS were performed during hospitalization and by multiple radiologists. BSID-II and standardized neurologic examinations for CP were performed at 2 years. There were strong independent associations between CUS findings and CP with about half of the children with CUS echolucency or VM going on to develop CP. Late occurrence of VM, bilateral echolucency, and IPH or PVL were strongly predictive of quadriparesis. However, almost half of the children with CP at 2 years had completely normal CUS, and the PPV of VM or echolucency for moderate or severe CP was poor. Isolated intraventricular hemorrhage (IVH) was not strongly predictive of CP. In three birth cohorts (1991–1992, 1997, 2005) born in Victoria, Australia, grade 3 or 4 ICH on neonatal CUS was independently associated with ninefold increased odds for CP and more than fourfold increased odds for any motor dysfunction at 8 years.[162] Of note, the rate of any motor dysfunction among those with no IVH was 24%, compared with 43% among those with grade 3 IVH and 92% among those with grade 4 IVH. Grade 1 and 2 IVH were associated with a twofold increased odds for CP.

However, CUS findings alone are poorly predictive of early developmental outcomes or later childhood cognitive and learning outcomes,[163] and 30% to 40% of those with "normal" CUS have neurodevelopmental challenges at 18 to 30 months.[164] The ELGAN study group showed that IVH was associated with increased risk for motor or developmental impairment at 2 years *only* when accompanied or followed by white matter lesions,[165] highlighting the limited predictive value of both early CUS findings and IVH alone. Children in the ELGAN cohort were also evaluated for neurological and cognitive outcomes at 10 years.[166] The presence of white matter lesions without IVH was found to

be associated with a more than threefold increased odds of cognitive impairment and sevenfold odds of epilepsy; similar association were found for white matter lesions accompanied by IVH. However, isolated IVH was not significantly associated with cognitive impairment or epilepsy. In later childhood follow-up studies of the EPIPAGE cohort, including infants born at 22 to 32 weeks in nine regions in France in 1997, approximately 40% of those with major neonatal CUS abnormalities had no significant cognitive or learning challenges identified at 8 years, whereas 30% to 40% of those with no neonatal CUS abnormalities had moderate-to-severe challenges.[167,168] In the Victoria, Australia birth cohorts followed to 8 years of age, neonatal CUS with grades 3 and 4 IVH were each independently associated with higher risk for poorer intellectual and academic outcomes at 8 years.[162] Of note, among those with grade 3 IVH, 22% and 24% had scores <2 SD below the term comparison mean for IQ and for any academic skill, respectively; this compared with 12% and 16% among those with no IVH. Lower IVH grades were generally benign with respect to cognitive outcomes. These findings taken together underscore the need for truly long-term surveillance through childhood for all born EPT, allowing for consideration for interventions to improve outcomes.

Nevertheless, in skilled hands, with meticulous technical attention and serial CUS imaging, much can be seen beyond ICH by CUS. In a single center, deVries et al.[157] reported 76% sensitivity and 95% specificity of CUS abnormalities for CP at 2 years for patients of less than 32 weeks' EGA. Of importance, among those with major CUS abnormalities who developed CP, approximately 30% were noted only after 28 days. Major CUS abnormalities were also not strongly associated with cognitive delay at 2 years. More recent findings from this group, using magnetic resonance imaging (MRI) at term-equivalent age (TEA) to refine specific CUS findings, have resulted in PPV and negative predictive value of 96% and 69%, respectively, for CP at 2 years among preterm infants.[158]

CUS is an operator-dependent modality, imaging procedures and views differ among institutions and studies, and there is no uniform approach to serial imaging protocols. Although interrater reliability and accuracy are very good to excellent for severe ICH, agreement is only fair or poor for subtler findings and PVL alone.[169] Cerebellar hemorrhage, a finding that may be missed without appropriate CUS views, is associated with neurodevelopmental disabilities in children born preterm,[170–172] and more detailed understanding of size of cerebellar hemorrhage is increasingly recognized as important for prognosis.[173,174] Transient lesions may be missed, including echodense periventricular lesions or collapsing small cystic lesions.[175] Isolated IPHs and of course large IVHs can be seen by CUS; however, not all "severe" hemorrhages can be considered equivalent in terms of association with early neurodevelopmental outcomes. Characteristics of the hemorrhage including laterality, midline shift, and extent of hemorrhage,[176,177] as well as the presence or absence of other adverse clinical factors,[178,179] impact prediction of neurodevelopmental outcomes.

Magnetic Resonance Imaging

Brain MRI has been used more extensively in recent years among very and extremely preterm infants, both for research and for clinical indications. MRI provides a more comprehensive and detailed picture of the brain, with better delineation of deep structure and cortical injury. Potentially most importantly, MRI provides improved detection of white matter injury (WMI), which is common among preterm infants at term-corrected age. Identification of WMI is critically important to understanding the structure–function relationship of the developing preterm brain, influences on later neuromotor and cognitive outcomes, and developing future neuroprotective strategies.[180] Subtle WMI on MRI is associated with reduced total brain and gray matter volumes, reduced cerebellar volume, and reduced basal ganglia and thalamic volume, which in turn are associated with childhood developmental impairments among preterm infants. These findings and others provide evidence that WMI in the preterm is associated with brain maturational disturbances, suggesting an overall link to impaired neural connectivity.[181]

A number of single and multicenter efforts have investigated whether MRI may provide enhanced information by which to predict outcomes for very and extremely preterm, particularly in comparison with CUS. Scoring systems were developed,[182,183] and larger cohort studies have been published, initially implementing primarily qualitative brain MRI at near term. Among the first of the larger multicenter studies compared CUS with near-term MRI findings and their association with 2-year outcomes in 167 infants of less than 30 weeks' EGA in Australia and New Zealand.[184] This study demonstrated that moderate-to-severe WMI on term-equivalent age (TEA) MRI was significantly associated with neuromotor delay and CP, independent of CUS findings and other risk factors. Increasing WMI severity was also related to lower BSID-II MDI scores, but an independent association of moderate-to-severe WMI with severe cognitive delay was not detected. However, CUS was assessed only with respect to early findings including grade of ICH and periventricular cystic changes, and a substantial proportion of infants with moderate-to-severe WMI by MRI did not have adverse 2-year outcomes. The NICHD Neuroimaging and Neurodevelopmental Outcomes study was a prospective study of early and late CUS and near-term MRI, which included 480 infants of less than 28 weeks' gestation, with outcomes including BSID-III assessed at 18 to 22 months.[185] In multivariable models, both late CUS findings reflective of WMI and MRI findings of significant cerebellar injury remained independently associated with adverse neurodevelopmental outcomes. In models that did not include late CUS, MRI findings of both moderate-to-severe WMI and significant cerebellar lesions were independently associated with adverse outcomes. Early CUS findings were not associated with adverse outcomes when any late neuroimaging was taken into account. In 6- to 7-year follow-up of that cohort, severe but rare adverse late CUS findings were most strongly associated with IQ <70 and the composite outcome of disability, which included moderate-severe CP. Significant cerebellar lesions seen on MRI were associated with disability.[18] Similarly, in a prospective study of serial CUS and near-term MRI among neonates of less than 27 weeks' gestation in Sweden, associations between MRI findings and 30-month outcomes were demonstrated, but the investigators determined that any substantial abnormalities on MRI were detected by the late CUS done on the same day.[186] Diffuse excessive high signal intensity on TEA MRI has been shown not to be associated with adverse early childhood outcomes in several studies[187,188] nor in later childhood or early adolescence.[189] In contrast, longitudinal brain MRI studies of very PT infants using a more comprehensive scoring system,[190] taking into account WMI and also measures of cortical gray matter, deep gray matter, and cerebellum, demonstrated that moderate-severe global abnormality on TEA MRI was associated with a reduction in IQ, math, and motor scores at 7 years independent of the other perinatal and neonatal confounders including severe abnormalities on neonatal CUS.[191] However, in a Dutch cohort of infants born extremely preterm, the prognostic value of that

MRI scoring system for 2-year outcomes was limited.[192] Other studies have pointed to associations of TEA MRI findings and growth of specific brain regions and outcomes, including that WMI, deep gray matter nuclear injury, and earlier gestational age are associated with smaller TEA cerebellar volume and growth trajectory through childhood, which in turn is associated with poorer IQ, language, and motor function.[193] A recent systematic review compared CUS and MRI to detect brain injury and predict outcomes,[194] which concluded that MRI provided more detailed information on the severity and the extent of preterm brain injury, particularly for white matter injury and cerebellar hemorrhage. The authors suggested that prediction of outcomes may be enhanced with MRI, citing improved negative predictive value compared with CUS, but acknowledging both lower PPV and wide heterogeneity among studies.

Despite what appears to be substantial experience with CUS and conventional brain MRI in preterm infants, controversies and questions remain as to which studies to perform, when to perform them and under what circumstances, and relative values in prognosis. These are not simple questions, as the value of additional information may vary by clinical circumstances and for individual parents and physicians.[195] Guidelines published in 2002, prior to the vast majority of large studies related to CUS and MRI comparisons, did not recommend TEA MRI for routine PT infant neuroimaging.[156] In 2015, the "Choosing Wisely Top Five for newborn medicine" suggested avoidance of routine term-equivalent conventional brain MRI for screening purposes because there was "insufficient evidence that the practice improves long-term outcomes."[196] In 2020, the AAP Committee on Fetus and Newborn, Section on Neurology, and Section on Radiology published a joint clinical report as guidance for routine neuroimaging for the PT infant.[197] The document included recommendation for routine CUS with anterior and mastoid fontanelle views by 7 to 10 days for all infants ≤30 weeks' EGA, with repeat CUS at 4 to 6 weeks and at TEA or before hospital discharge. The guidelines indicated that TEA MRI for infants ≤30 weeks is "not indicated as a routine procedure" although may be offered for the "high-risk infant" after counseling with the family regarding prognostic limitations of MRI. Of note, what is considered "high-risk" was not described, and certainly may be interpreted variably across institutions, among neonatologists, and by individual parents and families. Furthermore, careful counseling and discussion with a family about prognostic limitations of a test should not be limited to TEA MRI. The same careful review and acknowledgements should accompany discussion of CUS findings, or for that matter any other test.

Scientific conversations will certainly continue on the topic of appropriate and "best" neuroimaging for PT infants in specific circumstances, particularly as the evolution and advances in quantitative MRI continues. Future studies may focus on identifying high-risk groups of preterm infants to allow for neuroprotective or interventional trial risk stratification, and approaches to improve positive prediction of outcomes of importance both to parents and investigators. Investigations with advanced magnetic resonance techniques, including diffusion tensor imaging, functional connectivity MRI, surface morphometry, and volumetric methods, hold enormous promise to help to explore these questions.[198]

Bronchopulmonary Dysplasia

Bronchopulmonary dysplasia (BPD) is the most common morbidity of prematurity. BPD occurs in nearly half of infants born ≤28 weeks' gestation, and rates may be increasing over time.[199] Historically, BPD was most commonly defined as oxygen dependence at 36 weeks postmenstrual age (PMA). However, it is now increasingly recognized that grading of BPD according to level of respiratory support at 36 weeks PMA is more closely associated with risk for respiratory and neurodevelopmental sequelae through 2 years' corrected age.[200] BPD is associated with adverse respiratory, developmental, educational, and health economic outcomes. Extremely preterm infants with BPD have increased coughing, respiratory medication use, hospitalizations, and impact of respiratory disease on the family at 18 to 22 months corrected age, compared to those without BPD.[201] A meta-analysis of pulmonary function testing in children born preterm with BPD, those born preterm without BPD, and children born at term demonstrated significantly decreased $\%FEV_1$ in those born preterm, with a further decrease in those with BPD.[202]

MRI studies have shown changes in brain structure and function that may underpin the differences in development that are observed in children with BPD.[203–205] Several investigators have reported adverse neurodevelopmental outcomes in toddlers with a history of BPD, when compared to preterm-born toddlers without BPD.[179,206–211] BPD is a consistent independent predictor of risk for developmental impairment at 2 and 5 years.[179] Adjusted odds of adverse outcomes at 5 years are 2.3 (95% CI 1.8 to 3.0) times higher among very preterm-born children with BPD than among those without BPD.[179] Reports of cognitive outcomes at 8 years suggest that preterm-born children with BPD have lower IQs than those without.[212,213] In a large meta-analysis, BPD explained about 65% of the observed variance in cognition among children who were born prematurely.[119] BPD was associated with about a 1 SD decrease in childhood intelligence. A second meta-analysis reported that BPD is also associated with CP at school age, and infants receiving mechanical ventilation at 36 weeks' PMA (Jensen grade 3) have the highest risk for CP.[214]

Multiple large, randomized trials have evaluated strategies to decrease BPD in extremely preterm infants by applying interventions or drugs either in the delivery room or during the neonatal hospitalization. While some interventions or medications successfully improve rates of BPD, few have demonstrated improved developmental outcomes. Vitamin A reduces BPD or death in extremely-low-birth-weight infants (relative risk 0.89) but does not improve developmental outcomes at 18 to 22 months.[215] Postnatal corticosteroids reduce BPD, but significantly increase risk for CP if given in the first 4 days of life. Use of systemic corticosteroids after 7 days of life reduces BPD without increasing risk for CP.[216,217] Use of inhaled steroids after 7 days of life does not reduce BPD and developmental impacts are unknown.[218] Neither is recommended for use before 7 days.[219,220] Caffeine reduces BPD at 36 weeks, CP at 2 years, and severity of motor impairment at 5 and 11 years.[11,15,221,222] In multiple randomized trials, surfactant therapy for respiratory distress syndrome reduces both air leak syndromes and mortality.[223] It may reduce mild disability at 1 year but does not reduce moderate or severe disability at 1 year or any adverse outcomes at 2 years.[224]

Lastly, although antenatal corticosteroids reduce many complications of prematurity including mortality and respiratory distress syndrome, it is unclear whether they improve rates of chronic lung disease.[225,226] In the most immature (≤25 weeks) infants, antenatal steroids may increase rates of BPD, perhaps by leading to increased survival of high-risk infants.[226] Yet, antenatal steroid treatment is associated with a reduction in developmental disability or developmental impairment in childhood survivors.[225,226]

This includes decreased rates of cognitive impairment and CP among infants ≤25 weeks gestation who are exposed to antenatal steroids.[226]

Numerous neonatal trials have evaluated various methods of respiratory support in the delivery room and during the hospitalization. Early extubation to or complete reliance on noninvasive support such as continuous positive airway pressure (CPAP) has become standard of care.[227] However, none of these respiratory interventions has clearly led to measurable improvements in developmental outcomes.[17]

Retinopathy of Prematurity

ROP occurs in about 60% of neonates born at less than 28 weeks' gestation in the United States, and stage 3 or higher ROP disease occurs in about 15% of those neonates.[199] Incidence of both ROP and high-grade ROP possibly requiring treatment increase significantly with decreasing gestational age.[199] With the evolution of cryotherapy, laser therapy, and antivascular endothelial growth factor (VEGF) pharmacologic treatment, blindness caused by ROP in the developed world is now rare. Nevertheless, on a global level, as many as 20,000 children annually are blind from ROP.[228] Prematurely born children who are treated for ROP are at clear risk for long-term visual morbidities. However, infants with ROP that do not require therapy or lead to blindness are also at risk for long-term visual problems. At a 6.5-year follow-up of the Swedish EXPRESS cohort of neonates born at less than 27 weeks' gestation, 38% had at least some ophthalmologic abnormality, including blindness, strabismus, and refractive errors.[229] Visual problems were strongly associated with ROP treatment and lower gestational age.

Like BPD, ROP is associated with abnormal brain development in very preterm infants.[209,230] This may explain why severe ROP is associated with both visual and non-visual disabilities.[230] For example, at 5 years, stage 4 or 5 ROP or treated ROP is an independent predictor of poor neurodevelopmental outcome.[179] This effect is similar in magnitude to and additive to the risks associated with severe brain injury or bronchopulmonary dysplasia (see above). These infants have more than four times higher odds of motor and cognitive disability than infants without severe ROP.[231] In addition, they are more likely to have impairment in multiple domains.

The primary strategy utilized by neonatologists to reduce ROP has been restriction of supplemental oxygen. In a meta-analysis of five oxygen saturation targeting trials in EPT infants, targeting lower oxygen saturations (85% to 89%) as compared with higher oxygen saturations (91% to 95%) was associated with a somewhat lower risk of ROP (risk ratio 0.72, 95% CI 0.50 to 1.04).[232] Offsetting this potential benefit, the low saturation targeting strategy was also associated with an increase in risk of death before discharge (risk ratio 1.18, 95% CI 1.03 to 1.36) though not a significant difference in death by 18 to 24 months. Thus careful targeting of oxygen saturations alone is insufficient to eliminate risk of ROP in EPT infants, and low oxygen saturation targeting is unlikely to prevent enough ROP to justify potential risks.

The effect of ROP treatment itself may also have important implications for both visual and developmental outcomes. Laser therapy is the current standard treatment, but injection of antivascular endothelial growth factor (VEGF) agents is increasingly used to treat acute ROP. In one small randomized trial, an anti-VEGF agent appeared to be superior to laser therapy for treatment of Zone 1 ROP.[233] However, due to concerns about systemic absorption of the drug, late proliferative vascular changes that lead to retinal detachment, and only minimal differences in refractive outcomes at 2.5 years, many neonatologists and ophthalmologists remain apprehensive about use of these drugs.[234] Such concerns are augmented by inconsistent reports about neurodevelopmental outcomes of infants treated with anti-VEGF drugs as compared to laser therapy.[235,236] Recent meta-analysis suggests that anti-VEGF treatment does not increase risk for poor outcomes, though existing studies are small and generally rated as low quality of evidence.[237] Further research is needed to identify both novel strategies for prevention of ROP as well as treatments that are safe and lead to improved visual outcomes without adverse developmental impacts.

Infection

Preterm infants are at high risk for both perinatally acquired and postnatally acquired infections. Due in large part to the introduction of peripartum prophylaxis against group B streptococcal infections coupled with intense efforts to reduce iatrogenic late-onset sepsis, rates of neonatal infection have fallen in recent years. Nevertheless, about a quarter of extremely preterm infants (born ≤28 weeks) have culture-positive bacterial sepsis while in the intensive care unit.[199,238] Like other neonatal morbidities, risk for late-onset sepsis increases significantly as gestational age decreases. Infection is associated with poor growth, and poor head growth in particular, which is an independent predictor of adverse outcome in preterm infants (see below).[239] Furthermore, neonatal bacterial infection is associated with increased risk for low cognitive performance, CP, and vision impairment at 2 years' corrected age.[238,240–242] Yet, the overall risk for neurodevelopmental impairment (any one of cognitive impairment, CP, or vision or hearing impairment) associated with neonatal bacterial sepsis is slightly less significant than risks associated with severe IVH, ROP, or BPD.[238,243] By 5 years of age, both early- and late-onset sepsis in very preterm infants remain associated with significantly increased odds of CP; associations with cognition are less certain.[244]

In addition to bacterial infections, infections with other types of pathogens can also have significant adverse effects on neurodevelopment, particularly in preterm-born children. Infants with candida sepsis and/or meningitis and infants with bacterial meningitis experience a more significant increase in risk for poor developmental outcomes in early childhood than those with bacterial sepsis alone.[243,245] Additionally, many viral infections acquired in utero or during the perinatal period are associated with significant brain injury and developmental aberrations, both in term infants and preterm-born infants.[246]

Necrotizing Enterocolitis

Necrotizing enterocolitis (NEC) is diagnosed in about 7% to 13% of infants born ≤28 weeks.[199] As with other morbidities of prematurity, risk for NEC increases with decreasing gestational age and birth weight. The bowel injury and necrosis pathognomonic of necrotizing enterocolitis trigger a systemic inflammatory response which is thought to be injurious to the premature brain.[247] Infants with NEC are at increased risk for death, cognitive delay, CP, severe vision or hearing impairment, and the composite outcome of developmental impairment at 18 months.[243,248] In one large observational study, nearly all of this increase in risk for adverse outcome was associated with NEC requiring surgical

intervention.[249] Surgical NEC and spontaneous intestinal perforation are associated with more than doubled odds of neurodevelopmental impairment among survivors.[250] However, similar to infection, the overall risk for adverse outcome associated with NEC is less significant than risks associated with severe IVH, ROP, or BPD.[243]

Antenatal corticosteroid treatment leads to a significant reduction in necrotizing enterocolitis.[225,226] Despite this reduction in NEC, as noted above, the impact of antenatal steroids on developmental outcomes remains uncertain. Exclusive maternal milk feeding reduces risk for NEC and improves developmental outcomes, though it is unclear that these two benefits are causally related to one another. When compared to preterm formula, donor human milk leads to decreased incidence of NEC but no change in cognitive, language, or motor development at 2 years corrected age.[251] Additional interventions such as lactoferrin and probiotics show promise for further decreasing rates of NEC, but there is currently insufficient data about long-term impact on developmental outcomes.[252,253]

Growth and Nutrition

A focus on growth and nutrition both in the NICU and after discharge is increasingly recognized as essential for optimization of longer-term developmental outcomes. In a large cohort study, infants born 501 to 1000 g were divided into quartiles of in-hospital growth velocity.[239] Quartile of in-hospital growth velocity was associated with risk for CP and developmental outcomes more than two SDs below the mean at 18 to 22 months' corrected age, even after adjustment for other factors predictive of poor growth and development. Preterm infants who fail to thrive in the first 8 months after discharge have poor developmental outcomes compared with those who demonstrate catch-up growth or maintain an appropriate growth trajectory.[254] Greater head growth over the first 2 years is associated with higher school-age IQ and lower risk for poor executive function or motor function at school age.[255]

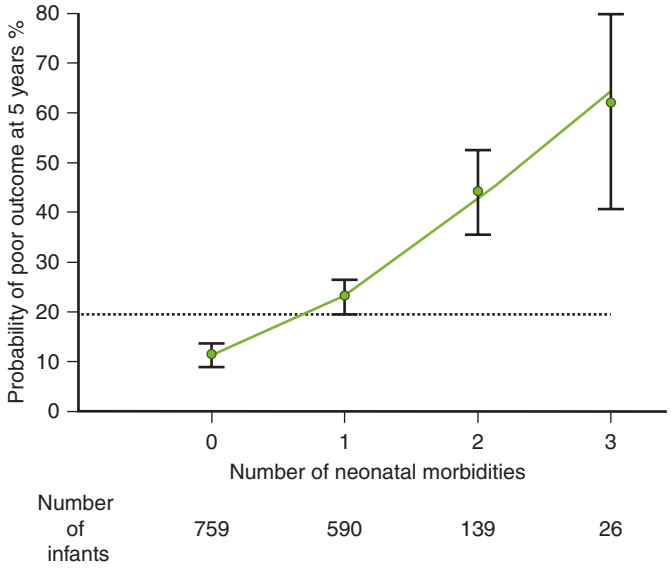
Because of the strong associations between nutrition and growth during the first year of life and developmental outcomes, multiple strategies to improve growth have been evaluated. Early aggressive nutrition in the NICU currently includes early administration of parenteral nutrition, early enteral nutrition with maternal or donor human milk, and fortification of enteral milk feedings. While many of these strategies successfully improve in-hospital growth, there is little evidence to date that any of these interventions improve long-term growth or developmental outcomes.[251,255–258]

Socioeconomic Status

The critical importance of socioeconomic factors in the long-term development of high-risk infants cannot be over-emphasized.[259] Socioeconomic factors that influence cognitive outcomes include parent education, having two parents in the household, neighborhood, and social and racial backgrounds.[123,129] Furthermore, these factors influence the chance that a child's cognitive status will improve over the first years of life.[123] Similarly, adverse socioeconomic factors such as younger maternal age and poor maternal well-being are strongly associated with increased behavior problems in preterm-born children.[128]

As high-risk children get older, perinatal factors have progressively less influence on outcome, while parental education remains highly important.[122] Older children with functional limitations are more likely to live in families with limited resources, have less access to health care, and be exposed to less healthy home environments.[260] This is associated with decreased access to necessary health care due to cost. Therefore, as with biologic risk factors, social risk factors including poor parental education, single-parent household, low social class, and maternal well-being must be taken into consideration when assessing an individual child's risk for adverse developmental and behavioral outcomes.

In summary, multiple adverse events during the neonatal course can impact longer-term developmental risk. Each of these events is associated with a unique level of risk and may impact development in somewhat different ways. These morbidities interact with one another to additionally increase risk. After gestational age and birth weight, the most significant risk factors for poor developmental outcomes in very preterm infants are IVH, ROP, or BPD (Fig. 24.2).[179] Each of these morbidities is associated with a linear, additive increase in risk for adverse developmental outcome. Neonatal infection—particularly fungal infection and meningitis—and necrotizing enterocolitis—particularly surgical NEC—add to the prediction of poor outcome. Poor growth is independently predictive of adverse outcome. Additional neonatal factors that increase risk for poor outcome include surgical ligation of the patent ductus arteriosus, general anesthesia, seizures, serious pulmonary hemorrhage, small size for gestational age, and congenital abnormalities. Lastly, the family's socioeconomic status is of increasing importance as children grow. Assessment of the number and severity of neonatal risk factors will aid in counseling



• **Fig. 24.2** The probability of neurodevelopmental impairment (NDI) in the Caffeine for Apnea of Prematurity Trial participants (n = 1514) at 5 years corrected age, based on the number of neonatal morbidities. Morbidities included were severe brain injury, bronchopulmonary dysplasia, and severe retinopathy of prematurity. The error bars represent 95% confidence intervals, the sloping green solid line indicates predictions based on a fitted morbidity count model, and the horizontal black dotted line indicates overall probability of NDI in the entire cohort. (From Schmidt B, Roberts RS, Davis PG, et al. Prediction of late death or disability at age 5 years using a count of 3 neonatal morbidities in very low birth weight infants. *J Pediatr*. 2015;167:982–986.)

of families about likely developmental outcomes and determination of possible need for services during early childhood.

Other Infants at High Risk for Adverse Outcomes

Though the primary focus of the current chapter is risk assessment and outcomes of preterm and low-birth-weight infants, select groups of late preterm and full-term infants also have significantly increased risk for poor outcomes and are addressed below.

Hypoxic–Ischemic Encephalopathy

Infants with perinatal depression or HIE are at risk for poor outcomes due to a peripartum or immediate postnatal neurologic insult. Diagnosis of HIE is made by a standardized neurologic examination consistent with encephalopathy, history of an acute perinatal event, low Apgar scores, and evidence of acidosis from either the umbilical cord or an infant blood sample obtained soon after birth.

More than 1000 full-term and near-term infants with moderate or severe HIE have been enrolled in randomized trials of therapeutic hypothermia or "cooling" for HIE. These trials have demonstrated that cooling significantly reduces mortality (RR 0.75, 95% CI 0.63 to 0.88) and decreases rates of survival with major disability at 18 months of age (RR 0.68, 95% CI 0.56 to 0.83).[261] Benefits of cooling persist at least until school age. Follow-up of two randomized trials of therapeutic hypothermia for HIE reveals lower rates of death or severe disability, CP, and moderate or severe disability, in addition to improved motor function scores, at 6 to 7 years.[6,262] Despite the success of this intervention, about half of children with moderate or severe HIE still die or suffer long-term neurologic impairment. CP is diagnosed in nearly 20% and developmental delay in more than 22% of surviving cooled infants.[261]

The relationships between several clinical factors and outcomes of infants with HIE have been studied. Certainly, the degree of encephalopathy based on the original clinical assessment is closely correlated with risk for both mortality and developmental impairment. Other early clinical signs predictive of poor longer-term outcome are administration of chest compressions for greater than 1 minute at birth, onset of spontaneous breathing greater than 30 minutes after birth, and base deficit of greater than 16 at any time.[263] Serial clinical examinations are more predictive of outcome than any single examination. Improvement in the clinical examination during cooling or by 72 hours of life and normal examination at discharge are associated with decreased rates of death or disability by 18 months.[264,265]

Children undergoing cooling are often monitored with either electroencephalography (EEG) or amplitude-integrated electroencephalography (aEEG). Prolonged discontinuity on EEG is associated with brain injury on MRI and poor developmental outcomes.[266] Burst suppression, low voltage, and flat trace predict developmental outcomes at greater than 12 months.[267] However, it is uncertain whether aEEG or EEG data add significantly to the initial clinical assessment based on severity of encephalopathy in the era of therapeutic hypothermia.[268,269] Particularly in cooled infants, *serial* evaluation of aEEG background may improve prediction of poor outcome just as *serial* clinical examinations improve prediction.[270] About half of newborns with HIE have seizures on EEG.[271] When managed with cooling, infants with HIE have lower overall seizure burden and shorter duration of seizures. The AAP recommends that centers offering therapeutic hypothermia have monitoring with aEEG or EEG available.[272]

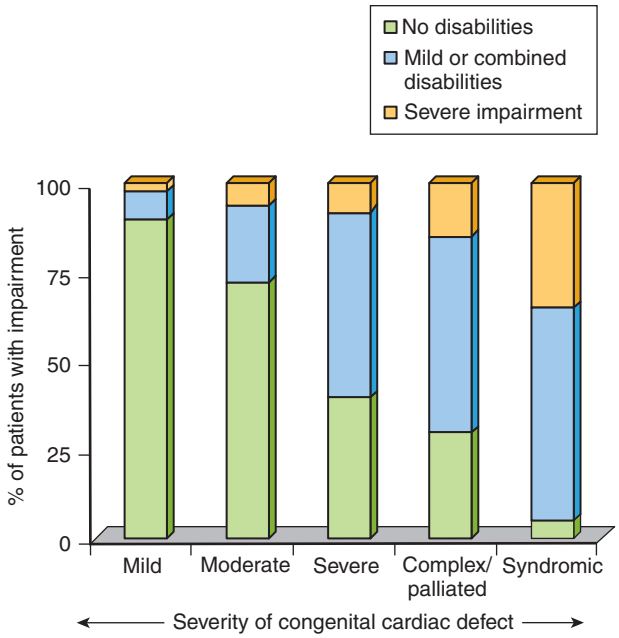
MRI-detected patterns and extent of brain injury are predictive of developmental outcome both at 2 years and at school age in HIE.[273] In particular, injury to the basal ganglia and/or thalami is highly predictive of poor outcome.[274] Interpretation of MRI findings depends on timing of the examination after birth, because sequelae of the initial insult may evolve over the first days to weeks of life. Infants treated with cooling are more likely to have normal MRI than those who are not cooled, but an MRI without abnormal findings does not guarantee "normal" developmental outcome after HIE.[275] Therefore all hospitals that provide cooling should also be capable of providing longitudinal neurodevelopmental monitoring and care.[272]

With therapeutic hypothermia established as standard of care and adjunctive agents under study, attention is turning to evaluation of whether these strategies are also safe and effective for more immature infants with HIE.[276] In addition, infants with mild HIE have not been evaluated in prior therapeutic trials; however, data suggest that these children may also be at risk for poor neurodevelopmental outcomes.[277] Thus, identification and optimal treatment of infants of any gestational age with mild HIE are critical areas for future investigation.

Congenital Heart Disease

Over the past several decades, improved surgical techniques and medical management have resulted in significantly improved survival rates for the 0.6% to 0.9% of infants who are born with CHD.[3,278] This has led to increasing interest in the longer-term developmental and quality-of-life outcomes of children and young adult survivors of CHD. A 2012 position statement from the American Heart Association and the AAP stratifies children based on risk for poor outcome, and suggests age-based approaches to surveillance, screening, and evaluation of children with CHD.[3] Noncardiac complications of CHD include cognitive, academic, financial, and psychological problems. Prevalence of poor outcome increases with severity of the heart lesion.[279] Furthermore, children who have genetic abnormalities or prematurity in addition to CHD have increased risk for developmental delays compared with those without these confounding conditions (Fig. 24.3).

Infants with CHD are often born with brains that are both small and immature for their gestational age,[280] which is thought to lead to increased vulnerability during the perioperative period. On MRI imaging, brain injury is detected both preoperatively and postoperatively, and predominantly consists of white matter injury. However, degree of white matter injury has not yet been correlated with developmental outcomes in the CHD population.[278] In children with CHD, gross motor problems are often evident in early infancy and during the toddler period. However, rates of CP are low, especially in comparison to other high-risk groups such as extremely preterm infants or full-term infants with HIE. Intelligence in children with a history of CHD is decreased from expected values by 5 to 10 points and is <70 in about 10% to 20% of these children. This is associated with increased rates of language and learning problems at school age, emotional and behavioral problems in up to 40%, visuomotor and fine motor coordination problems, and a significant impact on executive function. Behavioral problems, ADHD, depression, and anxiety



• **Fig. 24.3** Relationship between severity of congenital heart lesion in children with and without syndromes and severity of neurodevelopmental impairment. (Modified from Wernovsky G. Current insights regarding neurological and developmental abnormalities in children and young adults with complex congenital cardiac disease. *Cardiol Young*. 2006;16:92–104.)

are also common in children with CHD and quality of life is lower than among children without CHD.[281,282]

Adverse neurologic or developmental outcome in congenital heart disease is associated with multiple factors that are known in the neonatal and perioperative period.[281] Just as clinical examination in HIE is predictive of longer-term outcome, so too is a careful neurologic examination of the infant with congenital heart disease.[283] Neurobehavioral problems are often evident even before surgical palliation of the CHD. Seizures occur in more than 10% of infants who undergo bypass surgery and are associated with poor outcomes including mortality.[284,285] Duration of intensive care stay is a strong risk factor for adverse cognitive outcome up to 8 years, likely because it is a surrogate measure of overall severity of illness in this population.[286] Similarly, need for repeat hospitalizations and surgical procedures are associated with risk for adverse outcomes.[281]

Survivors of CHD often require habilitative services including therapies and special education. As in adults who were born preterm, persistence of these problems over time is likely to impact academic achievement, employment, and quality of life. Because the first generations of survivors of CHD are now entering adulthood, more work to describe the developmental, functional, and quality-of-life outcomes of CHD across the lifespan will continue to be essential.

Extracorporeal Membrane Oxygenation

Extracorporeal membrane oxygenation (ECMO) is a lifesaving therapy for patients with severe cardiac or respiratory failure that cannot be managed with conventional medical therapies. Almost half of all patients treated with ECMO are neonates, and about three-quarters survive to discharge.[287] Both survival and longer-term developmental outcome after ECMO depend on the original indication for ECMO, pre-ECMO course, and complications while on ECMO. Infants with medical indications for ECMO such as meconium aspiration syndrome have higher survival rates than those with surgical indications such as congenital diaphragmatic hernia. Lower gestational age and birth weight are associated with higher risk of both complications and mortality. The primary neurologic complication of concern while on ECMO is intracranial bleeding.

Children who were treated with ECMO during the neonatal period are at higher risk for later respiratory morbidity; hearing loss; poor motor, cognitive, and visuomotor performance; and behavior problems. Developmental delays are observed in multiple domains at least through 2 years.[288] Infants with CHD who are supported with ECMO have a higher risk for poor neurodevelopmental outcomes than those not treated with ECMO.[289] A randomized trial of therapeutic hypothermia during ECMO failed to demonstrate any neuroprotective effects at 2 years.[290] However, even without ECMO these children are likely to remain at high risk for many of these outcomes and are likely to have higher rates of mortality. For example, infants with congenital diaphragmatic hernias who do not undergo ECMO have lower developmental outcomes at one year than expected norms, and 13% have severe cognitive, motor, or language delays.[291]

In summary, infants in each of these high-risk categories are at high risk for problems in multiple neurodevelopmental domains throughout childhood. They should all be followed throughout the early childhood years by a multidisciplinary neurodevelopmental follow-up clinic that is capable of comprehensive surveillance, screening, diagnosis, and management. Monitoring should include traditional cognitive and motor assessments, behavioral evaluations, and surveillance for neurosensory deficits. This proactive approach will ensure that these high-risk infants achieve their greatest potential as they mature through childhood and adolescence.

Postdischarge Management of the High-Risk Infant

Discharge Planning for the High-Risk Infant

Proper discharge planning will ensure a smooth transition home from the hospital both for the high-risk infant and for the family. A pre-discharge conference to review the infant's hospital course and plans for discharge will provide an opportunity to discuss the infant's progress and goals for discharge, identify risk factors for developmental challenges, assess the parents' understanding, and make clear plans for post-discharge care. In review of the infant's various risk factors, an honest but sensitive discussion about the range of possible outcomes will help put risk for neurodevelopmental disabilities into perspective. Parents should be reassured wherever possible and given opportunity to hope.

Discharge teaching includes routine well-baby care, cardiopulmonary resuscitation, use of any special equipment or medication, and anticipatory guidance about car seats, safe sleep, and other common topics. When infants are discharged on medication or equipment, parents must demonstrate safe administration of the medication and use of the equipment. A pediatrician must be identified before discharge, and the inpatient team must personally communicate all relevant data about the child's hospital

• BOX 24.1 Considerations for the Primary Care Physician Caring for the High-Risk Infant After Discharge

1. *Growth*—Monitor weight, length, and head circumference in all children and especially in children with BPD. Follow feeding skills closely.
2. *Neurosensory*—Diagnostic audiology assessment by 24 to 30 months (Harlor and Bower, 2009). Continued ophthalmology screenings and evaluation postdischarge for children with or at risk for ROP (Fierson, 2013).
3. *Immunizations*—Routine immunizations according to birth date, rather than corrected age. Influenza immunization yearly for child and caregivers. Palivizumab for all high-risk infants and young children per AAP recommendations (AAP, 2014).
4. *Neurodevelopmental*—Screen for neurodevelopmental problems, including ADHD and ASD, behavioral problems, and developmental delays.
5. *Motor*—Perform careful neuromotor examination and refer to physical or occupational therapy if not meeting milestones or abnormal examination.
6. *Learning*—Refer for evaluation for learning and executive function disorders if children struggle in school.
7. *Sociodemographic*—Assess for sociodemographic factors that may adversely impact outcomes, including parental anxiety and depression. Provide access to social supports as indicated.

AAP, American Academy of Pediatrics; *ADHD*, attention deficit hyperactivity disorder; *ASD*, autism spectrum disorders; *BPD*, bronchopulmonary dysplasia; *ROP*, retinopathy of prematurity.

American Academy of Pediatrics Committee on Infectious Diseases, American Academy of Pediatrics Bronchiolitis Guidelines Committee. Updated guidance for palivizumab prophylaxis among infants and young children at increased risk of hospitalization for respiratory syncytial virus infection. *Pediatrics*. 2014;134:415–420.
Fierson WM, American Academy of Pediatrics Section on Ophthalmology, American Academy of Ophthalmology, American Association for Pediatric Ophthalmology and Strabismus, American Association of Certified Orthoptists. Screening examination of premature infants for retinopathy of prematurity. *Pediatrics*. 2013;131:189–195.
Harlor AD, Bower C. Hearing assessment in infants and children: recommendations beyond neonatal screening. *Pediatrics*. 2009;124:1252–1263.

course and postdischarge plans with the pediatrician. The AAP has established a policy for the screening and surveillance of developmental concerns in the primary care setting.[292] General recommendations for primary care givers who care for high-risk infants after discharge are shown in Box 24.1. Most premature infants will require ongoing ophthalmologic surveillance for progression and/or sequelae of ROP. Infants who have had complications during the hospital course may require ongoing care from various subspecialists. Lastly, all high-risk infants should be evaluated on an intermittent basis by a comprehensive developmental follow-up clinic and early intervention program. These services are an essential part of the continuum of care provided to high-risk infants and their families.

Referral for Early Intervention Services

All preterm children and high-risk children with abilities in any domain that are consistently below expectations based on their age should be referred for early intervention. Eligibility for surveillance or services for high-risk infants or children differs by geography. In the United States, the Individuals with Disabilities Education Act requires states to provide early intervention services for infants and toddlers up to 3 years of age with developmental delays and with conditions that lead to developmental delays. However, states differ in definitions of delay, standards regarding correction for prematurity, and services offered.[293]

Infants may be referred to early intervention by the NICU, follow-up clinic, pediatrician, or family. Each child who is referred is entitled to a multidisciplinary assessment and a service coordinator to facilitate the assessment and services. Early intervention programs recognize the importance of (1) viewing each child as a unique individual, (2) evaluating not only needs but also strengths, (3) including the family in the planning process, and (4) coordinating all intervention services. The choice of interventions is determined by the individual child's developmental profile and health, the needs of the family, and available resources.

Early intervention helps ensure that children reach their maximum potential but does not necessarily prevent disability.[35] Research about the efficacy of early intervention includes many different interventions, applied with different intensity, in different populations, and for different lengths of time. Hearing and visual impairments are responsive to early intervention, and early therapies can significantly improve long-term functioning and quality of life. In general, early intervention programs have been shown to have positive effects on developmental and neurologic outcomes for preterm infants during infancy and early childhood.[35] Specifically, early intervention is associated with improved behavioral outcomes, reduced anxiety and depression for primary caregivers, and cognitive benefits through preschool age. Importantly, early intervention programs focused on the parent–child relationship are more effective than programs focused on the child or the parent alone.

Early intervention has less of an impact on motor outcomes and no impact on rates of CP. However, a few recent small studies of focused, intensive, goal-oriented therapy for infants at high risk for CP have demonstrated improved motor outcomes.[294,295] No long-term studies of such interventions for infants at risk for CP have been completed to date. Thus, it remains uncertain whether any specific forms of early intervention or disability-focused therapies have significant long-term impact on functional motor outcomes at school or at home. However, once a child has a diagnosis of CP or DCD, targeted interventions are then aimed at prevention of further delay and compensating for deficits, to optimize the child's function and independence.[209] The most effective therapeutic approach to teaching motor skills to children with DCD is the task-oriented approach, which includes cognitive approaches with a focus on specific aspects of a motor skill.[296] Continued research is essential to identify the best therapies, including timing, intensity, and duration, to optimize outcomes for preterm and high-risk infants and children.

What Is Multidisciplinary Follow-Up Care for the High-Risk Infant?

Challenges of the Current State

In 2008, the AAP Committee on Fetus and Newborn released a policy statement to guide hospital discharge planning and postdischarge care for infants deemed "high-risk."[1] However, the definition of high-risk infant and the criteria for referral to comprehensive developmental follow-up vary widely, based on state and regional requirements often linked with NICU certifications or approvals, available resources in the community, and funding. Furthermore, the nature of care provided in "NICU follow-up" settings vary widely. In 2006, an expert panel from the AAP, NICHD, Vermont Oxford Network (VON), and the California Children's Services (CCS) published quality-of-care indicators for

high-risk infant follow-up, developed through a modified Delphi process, resulting in 70 indicators including general care; physical, neurosensory, behavioral and developmental, and psychosocial assessments, and many interventions.[30] Although comprehensive, the metrics and indicators are extensive, and implementation across numerous post-discharge care settings would be required in most areas, thus would be difficult to enforce. Some NICU follow-up clinics focus on neurodevelopmental and behavioral evaluation only, while others may provide truly comprehensive care encompassing primary care, complex medical follow-up and coordination as well as neurodevelopmental assessment and interventions. In reality, care for the NICU graduate is usually very fragmented, with numerous subspecialties involved due to medical morbidities, technology, and therapies; nutrition and laboratory follow-up; coordination of appointments; as well as neurodevelopmental and behavioral care, depending on the needs of the child.[297] The substantial bulk of the complex coordination, surveillance, and communication will fall to the primary care provider and parents or primary caregivers, ideally in close collaboration in the setting of a family-centered approach. The AAP has recognized and endorsed the medical home model for primary care of all children and youth, and particularly for those with special health care needs,[298,299] and certainly a medical home would serve as an outstanding family-centered location from which to optimize the patient's medical and neurodevelopmental outcomes. However, access to and engagement with a true medical home is not consistent, with disparities reported based on special health care needs and sociodemographic factors.[300–302] Clinics and teams focused more narrowly on neurodevelopmental follow-up for NICU graduates very often attempt to fill the gap around coordination at least in part. However, NICU follow-up clinics are not uniform, and themselves struggle with numerous personnel, funding, and other barriers. In a survey of NICU follow-up directors of academic (55% response rate) and private institutions (45% response rate), less than 20% reported providing primary care, many reported significant challenges in supporting needs of the most complex medical conditions, and very few reported availability of services to support financial or legal challenges for the family.[303] Others challenges reported by follow-up clinics suggest a large burden on administrative assistants to improve workflow in the setting of a lack of adequate administrative resources and frequent cancellations, limited clinic time and space to serve their patients and families, limited availability of various personnel in clinic, and substantial funding challenges.[304,305] There is also a clear recognition by follow-up clinics of the significant parent and family challenges in getting to what are often many appointments requiring time off work, and particularly for those who live at a distance from the clinic.[304] Sociodemographic, program-level, and rural residence disparities in follow-up for both very preterm and high-risk term infants have also been demonstrated.[8,306–308] Taken together, the current challenges and confusion for families, providers, and clinic systems present make consistent and comprehensive follow-up care difficult in many instances, and highlight gaps in efforts to achieve ideal support for high-risk infants and their families.

Goals for the Future State

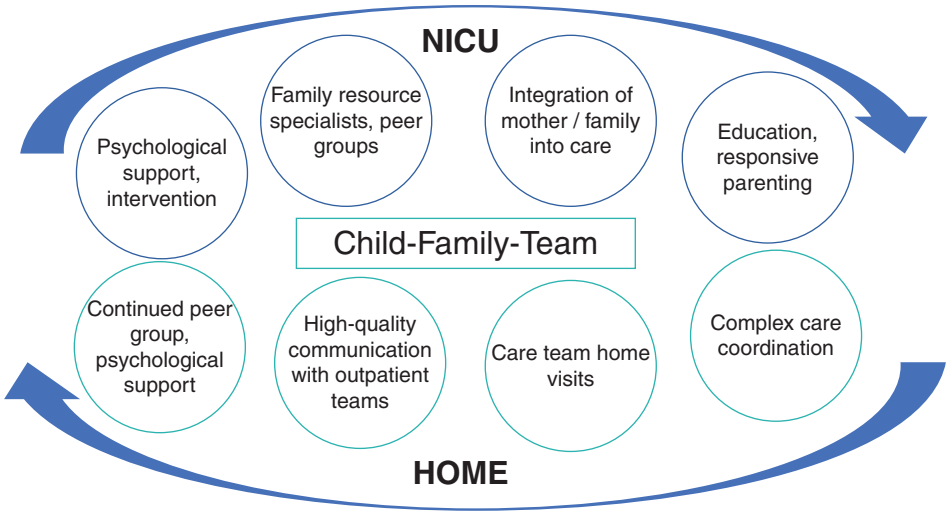
A recent perspective authored by Vermont Oxford Network (VON) leadership takes a more encompassing stance to the concept of "NICU follow-up" by proposing the construct of "follow-through" for high-risk children and their families.[309] This framework highlights the responsibility of all care providers to participate in care for the high-risk infant and their family from prenatal care through NICU to childhood and beyond. The perspective presents multiple potentially better practices (PBPs) in six categories (Table 24.2). Although implementation of the dozens of PBPs within these categories cannot be achieved for all programs in the short term, the perspective presents a clear view of the life-course challenges and barriers that high-risk infants and their families face, and the imperatives to build toward a cohesive, consistent, and equitable construct for high-risk infant follow-up care.

These broad concepts may be considered as opportunities for quality improvement efforts for high-risk infants and their families.[310] Certainly, NICU follow-up structures must continue to include serial neurodevelopmental and behavioral assessments, and the personnel and expertise to provide recommendations and interventions. However, multiple interdisciplinary connections between families, providers, and communities beginning in the NICU and continuing through childhood are needed (Fig. 24.4). Attaining this broad goal may include increasing alignment toward a comprehensive complex care outpatient model for children with special health care needs, including high-risk infants after NICU discharge. Although this type of "enhanced medical home" may

TABLE 24.2 Potential Better Practices for NICU Follow-Through

Promote a culture of equity	Establish cultural sensitivity; acknowledge and manage implicit and explicit personal biases; facilitate nurse-led rounds
Identify social risks of families and provide interventions to prevent and mitigate those risks	Screen for social determinants of health; provide support when necessary such as assistance with housing, meals, and transportation and counseling for mental health, drug or alcohol problems, or smoking cessation; include social workers and legal specialists on teams
Take action to assist families after discharge	Provide carefully tailored discharge teaching; use home visiting and social media; establish meaningful clinical-community partnerships
Maintain support for families through infancy	Use parent coaches and innovative medical visit structures; provide contraception, family planning, and high-quality obstetric care; provide evidence-based early intervention programs
Develop robust QI efforts to ensure equitable, high-quality NICU and follow through care to all newborns by eliminating modifiable disparities	Establish measurable aims; engage all disciplines, parents, and primary care providers; obtain support from organizational leaders through a formal charter
Advocate for social justice at the local, state, and national levels	Ensure that social justice is part of every organization's mission; advocate that health care organizations accept and act on their responsibility for the populations and neighborhoods that they serve; speak out!

From Horbar JD, Edwards EM, Ogbolu Y. Our responsibility to follow through for NICU infants and their families. *Pediatrics*. 2020 December;146(6):e20200360.



• **Fig. 24.4** Conceptualizing transition from NICU to home and community. (From Litt JS, Hintz SR. Quality improvement for NICU graduates: feasible, relevant, impactful. *Semin Fetal Neonatal Med*. 2021;26[1]:101205.)

be considered costly and challenging to create, and potentially not feasible in some settings, it has been shown to reduce emergency room visits, total hospital admissions, ICU admissions, number of days in the hospital, and costs.[311,312] However, even in the absence of this structure, the goal toward a connected, family-focused, continuum of care approach must be the driving goal for the future state of NICU follow-up. Achieving the best possible outcomes for high-risk infants necessarily means enveloping parents, families, physicians and providers, and community in the "follow-up" process, from early in the NICU course to discharge and well beyond. NICU follow-up programs have expanded to fill this role, engaging closely and often daily with parents and families, NICU teams, primary medical care providers, and community resources in creating parent-integrated "transition to home" programs.[313,314] Team personnel may vary depending on the approach, but involve physicians, APPs or clinical nurse specialists and educators, coordinators, clinical social workers, and peer parent, and family specialists, and include home visits as well as phone availability for some period after discharge. Programs may have differing bundles of interventions including parent education in complex and general medical care issues, nutrition, developmental progress, as well as training in community resources, positive parenting, and enhancing self-efficacy. But they have demonstrated positive results including reduced ER visits and hospitalizations, reduced Medicaid costs,[315,316] and better cognitive, motor, and symbolic communication skills at 2 years of corrected age.[317] As parents of NICU babies are at substantially increased risk for anxiety, depression, and trauma with potentially significant later impacts on child and family function, integration of parental mental health support and appropriate interventions during NICU stay and beyond is crucial.[318–321] The previous models of preventative early intervention are fortunately beginning to change, as research has demonstrated that programs with individually family-centered and home-based components, enhancing parent responsiveness and resilience, and combining child health and service support have the most comprehensive effect on outcomes.[35,322]

Challenges to and Importance of Follow-Up

In the research setting, there is a growing reliance on complete evaluation and reporting of neurodevelopmental outcomes in prospective studies and trials and an increasing and appropriate recognition that it is crucial to fully understand outcomes beyond the initial hospitalization and even beyond early childhood. Yet, the challenges of following a cohort for years or decades and the potential barriers to achieving reliable results are numerous. Long-term follow-up requires time, dedication, and persistence from both follow-up staff and from families. Achieving a high follow-up rate is essential to limit bias. Social and demographic disparities and population differences have long been shown to be associated with increased attrition,[7,305,323,324] which is of concern not only because of the potential bias introduced but also because those children and families lost to follow-up could potentially benefit most from supports and services. Questions related to generalizability of findings may be inherent, regardless of outstanding follow-up rates. Thus population-based or large regional-based cohorts may be considered the ideal model of prospective observational studies of high-risk infants. Caution is advised when comparing outcomes of different cohorts over time, because of differences in the enrolled populations and attrition rates, changes in versions of or use of different instruments, and varying approaches to use of term control groups. However, it is also important to broaden the scope of outcomes considered for study and trial endpoints beyond the traditional 2-year neurodevelopmental outcomes.[325] Inviting parents to meaningfully participate in research, from evaluating consents, questionnaires, and information documents to identifying research targets and planning studies, may also contribute to increasing the value of research to families and to the children and adults who were high-risk infants.[326]

Outside the scientific framework of follow-up for research purposes, there is a substantial *clinical* need for high-risk infant follow-up. The purpose and potential value of this difficult undertaking are manyfold. As survival of even the most extremely preterm and complex infants has improved, there is increasing recognition of the importance of neurodevelopmental outcomes, rather than short-term endpoints alone, as a measure of the impact of interventions and management strategies in the NICU. Much has been invested in assuring the survival of these high-risk infants and must be invested similarly to assure that all of these children reach their best potential. In addition to traditional quality improvement measures to decrease in-hospital morbidities that

are associated with adverse neurodevelopmental outcomes, early detection, preventive care, and intervention programs inclusive of both infants and families hold the best promise for changing the trajectory of outcomes.

Within an ecologic framework, the home environment and relationships are the most immediate and proximal influences on child development.[327] More distal factors, such as family income and broader community factors, influence children's development both directly and indirectly through interaction with other proximal environmental factors. In understanding the developmental systems approach and recognizing the profound potential to influence brain development, positive outcomes can be understood in terms of improvements in developmental pathways associated with parental sensitive–responsiveness and child participation in intensive intervention-oriented child care.[328–330]

Without a doubt, implementing a comprehensive system of NICU-to-community early and preventive interventions for high-risk infants is a time-intensive and resource-intensive undertaking. But we are unlikely to truly transform long-term care and improve the lifetime outcomes of high-risk infants without such an investment in their future.

Suggested Readings

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25 The Human Genome and Neonatal Care

C.M. COTTEN

KEY POINTS

- Twenty percent of infant deaths in the United States and a larger portion of infant deaths in the NICU have been attributed to chromosomal and congenital anomalies, with the prevalence increasing with the expanded use of genetic diagnostic tools.
- Thousands of individuals have had their entire genomes or exomes sequenced and shared, along with corresponding phenotype information. This comprehensive, linked information enhances our ability to identify genetic links with previously unexplained structural and functional disorders among newborns.
- Linking genetic variations to disease is a key step to advancing understanding of pathophysiology and development which will lead to better treatment and management strategies.
- Studies evaluating use of rapid next-generation gene sequencing in diagnosis of infants with diseases of unknown etiology have begun in NICUs, and early results reveal increasingly higher rates of diagnosis with time to result now in days to weeks rather than months to years.
- Ethical issues related to incidental findings arising from sequencing must be addressed as these approaches are applied in the NICU.
- Identifying genes and variants associated with the more common complex disorders of prematurity (ROP, IVH, NEC, BPD) remains a challenge.
- Application of genomics and genetics to clinical neonatology is evolving rapidly as the prices drop and the speed of sequencing, identifying, and analyzing variants for their association with a growing number of gene-disease databases accelerates.

Within 50 years of the discovery of the double helix structure of DNA, the Human Genome Project achieved the major milestone of identifying an almost complete sequence of the approximately 3 billion nucleotides in the human haploid genome.[1,2] Advances in sequencing technology have enabled the reporting of over 100,000 complete genomes and the characterization of millions of genetic variants in millions of individuals since the publication of these single, consensus genomes. Linking genetic variations to disease is a key step to advancing understanding of pathophysiology and development which will lead to better treatment and management strategies.[3] Clinicians in neonatal intensive care units (NICUs) are partnering with experts in genetics, gaining expertise and utilizing tools such as variant panels and next-generation sequencing to diagnose causes of birth defects and other systemic disorders that become apparent in infancy. This chapter will review concepts about the human genome relevant to neonatal care providers, including (1) the approach and evolving genome-focused tools that clinicians are using to diagnose neonates with genetic disorders; and (2) a summary of work seeking to identify associations between relatively common genetic variations and complex disorders associated with preterm birth. To facilitate reading, Table 25.1 lists and defines common terms applicable to genetics and genomics in the NICU.

The Human Genome Project and the Big Picture of Genomic Medicine

The Human Genome Project was an international effort that included a focus on identifying the chromosomal location of normal and disease-causing genetic variants.[4] The international effort continues with some countries' population-based studies applying whole genome or exome sequencing to tens of thousands of individuals, mostly in relatively high-resource countries with overrepresentation of communities with European ancestry.[5] The Global Genomic Medicine Collaborative (G2MC) was formed in 2015 to facilitate implementation of genomic medicine worldwide to improve individual and population health, with the imperative for inclusion of geographic and population diversity.[6] More global efforts in accumulating cohorts with extensive genomic information have begun,[7] and the G2MC has prioritized the need for provider education programs, as this is likely to be a rate-limiting step in appropriate allocation and utilization of genomic testing.[8]

By participating in collaborative efforts, clinicians and researchers have published studies of comprehensive views of genetic variation for thousands of individuals with type 2 diabetes, breast cancer, or traits such as height or birth weight. These collaborative international efforts have created the framework for one of the greatest successes of the Human Genome Project—that is, information generated relevant to the human DNA sequence and its variation, held in public trust with open access to the scientific community through dozens of accessible databases and analytic platforms.[9]

One major hub for clinicians that contains both clinical and scientific descriptors of genetic findings on a disease basis is located on the website Online Mendelian Inheritance in Man (www.ncbi.nlm.nih.gov/omim/)—a comprehensive catalogue, updated daily, of more than 16,000 described genes, including the ever-growing total of over 7000 genes with phenotyping associations with a known molecular basis, and over 4500 genes with phenotype-causing mutation.[10] The online version, a compendium of human genes and genetic phenotypes, also provides links to online resources that provide information about genetic variants, their

TABLE 25.1 Glossary of Terms Used in Genetics and Genomics

Allele: variant form of a gene at a certain locus

Autosomal: gene is located on one of the numbered (nonsex) chromosomes

CNV: copy number variant)– INDELS (insertions/deletions)

Curation: a term used to describe the analysis of the existing data and evidence about a specific gene or gene variant and its relation to a specific phenotype

Exome: the sum of all exons (coding sequence, splice sites, 5′ and 3′ UTRs, miRNAs) in the genome ~ 50 Mb or 2% of genome)

FISH: fluorescence in situ hybridization (FISH), which determines the presence or absence of discrete segments of DNA

Genome-wide association: in studies testing thousands or millions of variants for association with a phenotype, the statistical standard for likelihood a variant contributes to risk of the condition in question. In genome wide association studies (GWAS) is often set at $p < 10^{-8}$

Heritability: a quantitative measure of the extent to which genetic factors account for phenotypic variance

Human Genome: 3 billion nucleotides, with approximately 25,000 genes (+ 2× more highly conserved regions)

Linkage: cotraveling of alleles usually near one another—when you find one, you usually find the other; multiple alleles traveling together would be haplotype

Locus: position on a chromosome

NGS: next-generation sequencing, which includes whole exome sequencing (WES), and whole genome sequencing (WGS)

SNP: single nucleotide polymorphism, a variation in gene sequence that is the most common type of genetic variation among people. On average, each person has 4–5 million SNPs in their genome; about one occurs every 1000 nucleotides

VUS: variants of unknown significance

associations with disease, and multiple other aspects of these genes such as their commonality across species and the differences in variant prevalence between populations of different ancestry, proteins and variations in protein structures, and which genes are in which biological pathways (http://omim.org/help/external; www.ncbi.nlm.nih.gov/omim/).

The Clinical Sequencing Exploratory Research (CSER) Consortium, funded by the National Human Genome Research Institute (NHGRI) and the National Cancer Institute (NCI), is exploring analytic and clinical validity and utility, as well as the ethical, legal, and social implications of sequencing via multidisciplinary approaches.[11] OMIM also includes a link to ClinVar, a freely accessible, public archive of reports of the relationships among human variations and phenotypes, with supporting evidence (https://www.ncbi.nlm.nih.gov/clinvar/intro/). These and some of the other online tools that provide information about genetic variations and links to phenotypes are listed in Table 25.2.

TABLE 25.2 Online Genomics Resources

ACMG Recommendations for Reporting of Incidental Findings in Clinical Exome and Genome Sequencing https://www.ncbi.nlm.nih.gov/clinvar/docs/acmg/. Annually updated minimum list of genes that should be evaluated in individuals undergoing clinical ES/GS based on the medical actionability of the associated condition

ClinGen: a National Institutes of Health (NIH)-funded resource dedicated to building a central resource that defines the clinical relevance of genes and variants for use in precision medicine and research: https://clinicalgenome.org/

ClinVar: freely accessible, public archive of reports of the relationships among human variations and phenotypes, with supporting evidence: https://www.ncbi.nlm.nih.gov/clinvar/

Database of Genotype and Phenotype (dbGaP): an archive of data from genome-wide association studies on a variety of diseases and conditions accessible through this NCBI: https://www.ncbi.nlm.nih.gov/gap/

DECIPHER: a database of reported copy number variants and linked phenotypes: https://decipher.sanger.ac.uk/

Ensembl: a genome browser for vertebrate genomes that supports research in comparative genomics, evolution, sequence variation, and transcriptional regulation. Ensembl annotates genes, computes multiple alignments, predicts regulatory function, and collects disease data: https://www.ensembl.org/index.html

GeneMatcher: web site that enables connections between clinicians who have a patient with a candidate or ultra-rare gene and researchers who have an interest in that gene: https://genematcher.org/

GeneReviews: a clinical resource for many genetic conditions that provides clinically actionable information including diagnosis, inheritance, and management as well as a differential diagnosis of related conditions: https://www.ncbi.nlm.nih.gov/books/NBK1116/

GenomeConnect: GenomeConnect is an online registry designed by the Clinical Genome Resource (ClinGen) for people who are interested in sharing de-identified genetic and health information to improve understanding of genetics and health: https://www.genomeconnect.org/

Human Phenotype Ontology (HPO): a standardized set of phenotypic terms; a widely used resource for capturing human disease phenotypes for computational analysis to support differential diagnostics: https://hpo.jax.org/app/116

Online Mendelian Inheritance in Man (OMIM): a searchable database of clinical features, phenotypes, and genes: https://omim.org/

Unique: a website with patient-/family-facing resources regarding chromosome and gene disorders: https://www.rarechromo.org/

University of California Santa Cruz Genome Browser: a website created initially to ensure public access to the initial human genome assembly, has now evolved to include a broad collection of vertebrate and model organism assemblies and annotations, along with a large suite of tools for viewing, analyzing, and downloading data: https://genome.ucsc.edu/

VVP (VAAST Variant Prioritizer) rapidly prioritizes genetic variants: https://github.com/Yandell-Lab/VVP-pub

The Genome and Genomics

Genetics focuses on the study of single genes and their effects. Genomics is defined as the comprehensive study of the functions and interactions of all the genes in the genome. The Human Genome Project provided key information about the genome. It quantified the total number of base pairs (approximately 3 billion), defined genes, and provided more information about how genomic DNA might be classified.[1,2] The genomic DNA of

eukaryotic organisms includes exons (regions of DNA that are "expressed," or translated into protein) and introns (intervening regions not translated into protein). Despite their apparent importance, exons account for less than 2% of the DNA in the entire genome. The regions outside of the exons are increasingly recognized to play critical roles in how and when the genes are expressed. The definition of what a "gene" is changes regularly as we learn more about distant regulatory elements, DNA modifications, chromatin structure, gene/gene interactions, exon skipping, post translational modification, and a host of other factors that determine what a "gene" does.

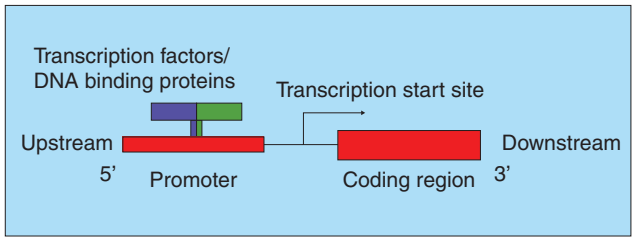
Understanding how conserved "noncoding" sequences influence human health and disease continues to be an important area of study. Even before the sequencing of the complete human genome, we knew that there were regulatory regions of the genome close to exons, sometimes called promotor regions. These are sites where transcription factors can bind to "turn on" or "turn off" a gene's expression. The regulatory region is usually a different set of base pairs than the actual transcription start site where transcription of mRNA is initiated. We also know that certain combinations of mRNA result in "stop codons," marking the point in the sequence where transcription stops (Fig. 25.1).

https://www.ncbi.nlm.nih.gov/Class/MLACourse/Modules/MolBioReview/alternative_splicing.html[12]

In addition to describing functional aspects of the genome, the origins of the human genome are also intriguing. Almost half of the genome is derived from foreign DNA.[1] These entered the genome via germ cells as transposable element DNA, first described in maize by Barbara McClintock.[13] Interestingly, we have learned that approximately 8% of the intronic base sequences are the products of human endogenous retroviruses (HERVs). The segments are DNA-based copies of their own viral RNA genetic material inserted into the human genome over millennia.[14] Evidence is accumulating to suggest a potential functional role of these HERVs in numerous pathologies including neurodegenerative diseases, autoimmune disorders, and multiple cancers.[15]

As our understanding of the genome has expanded, we know that many more RNAs and proteins can be made from the DNA in the genome. While approximately 20,000 protein-coding genes are known, the principle of alternative splicing (i.e., mRNA comprised of different exons from the same gene coding different proteins) increases the estimate of the number of proteins from the 20,000 one would imagine to well over 100,000. This is particularly important in the developing neuronal system, in which over 90% of multiexon genes are alternatively spliced throughout development (Fig. 25.2).[16–20]

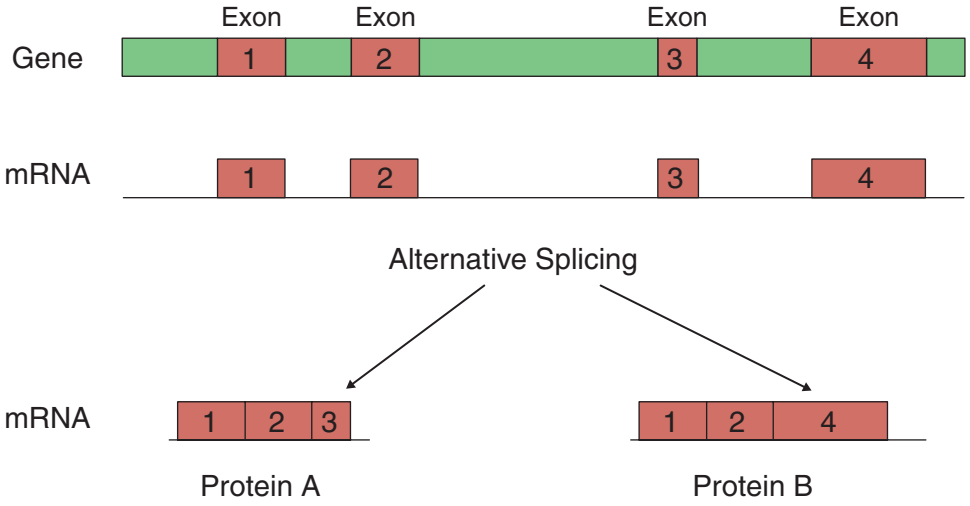
https://www.ncbi.nlm.nih.gov/Class/MLACourse/Modules/MolBioReview/alternative_splicing.html[12]



• **Fig. 25.1** Gene Control: Regulatory Regions. A diagrammatic depiction of gene structure, incorporating the exon, or coding region, as well as the promoter region, which is intronic, but key to a gene's function.[117] (https://www.ncbi.nlm.nih.gov/Class/MLACourse/Modules/MolBioReview/gene_control.html.)

Mitochondrial Deoxyribonucleic Acid

Mitochondria are the energy-processing organelles within each cell. Each mitochondrion has its own genome, distinct from the nuclear genome and thought to arise from incorporation of bacterial DNA. The mitochondrial genome is approximately 16,500 base pairs in length and encodes 37 genes. A wide range of disorders have been associated with variation in mitochondrial sequence. In addition, because mitochondria reside in the cytoplasm and are not found in sperm, they have a unique pattern of maternal-only inheritance, in which mothers pass their mitochondria on to all their offspring, with the daughters then passing their mothers' mitochondrial DNA on to subsequent generations. The knowledge that variants in mitochondrial DNA can cause disease plus the progress made in techniques for molecular and cellular manipulation have led to embryonic mitochondrial transplantation, where "healthy" mitochondria are transplanted into



• **Fig. 25.2** Alternative Splicing. A single gene can produce multiple related proteins, or isoforms, by means of alternative splicing of different exons from the same gene.[117,118] (https://www.ncbi.nlm.nih.gov/Class/MLACourse/Modules/MolBioReview/alternative_splicing.html.)

the cytoplasm of an early-stage embryo identified to have a lethal mitochondrial variant.[21,22]

Variations in the Human Genome

Genetic variations are differences in the DNA sequence and structure among individuals. Variant types include single nucleotide polymorphisms (SNPs), copy number variants (CNV), insertions and deletions (indels), polymorphic repeats, and microsatellite variants. Additionally, there are differences in the amount of chromosomal material, termed aneuploidies.

Single Nucleotide Polymorphisms

Single nucleotide polymorphisms (SNPs) were the first commonly characterized contributors to human genetic variation. SNPs are specific nucleotide sites in the human genome, where it is possible to have two (or even three or four) different nucleotides at a specific position on a chromosome. For example, there might be either a T or a G at a specific point in an individual's genomic sequence, with differences in populations in which 40% of the individuals have a T at the specific locus, and 60% have a G. These variant sites are common, with up to 1% of the 3 billion base pairs of human DNA sequence varying between any two individuals, resulting in millions of SNPs across the genome. Most variation is found across all human populations, although some variants appear to be highly population or ancestry specific. Chip-based DNA sequence detection allows assays of greater than 1 million SNPs simultaneously on one individual at a cost approximating $100. Since 1999 over 500 million SNPs have been catalogued in the online public-domain resource, the Single Nucleotide Polymorphism database (dbSNP: http://www.ncbi.nlm.nih.gov/snp). The vast majority of SNPs do not appear to be disease-causing, although they may lie adjacent to DNA changes that do contribute to disease predisposition and can be detected using the phenomena of linkage disequilibrium, in which the genotype of one SNP correlates highly with the genotype of nearby SNPs.[23]

Copy Number Variants

Copy number variants (CNVs) are another form of genetic variation that can contribute to inherited disorders. Although both small and large deletion and duplication events in human DNA sequence have been known since the 1970s, only recently has the widespread role that these play in disease been recognized. CNVs can range in size from a few nucleotides to thousands of base pairs in length, resulting in the possibility that two healthy people have genomes with total numbers of base pairs that differ by millions.[24] These variations may contain one or multiple genes that can exist in two, three, or more copies arrayed in tandem at particular chromosomal positions. When these tandem arrays of largely identical sequences align themselves during meiosis, there is occasionally a misalignment that can result in deletion or duplication of one or more of the copies. This event in turn can create a range of the number of copies present from zero to many.[25] When functional genes are contained within the copied or deleted element, the amount of gene product made may be higher or lower than a reference level.

Examples in which CNVs contribute to disease include the Di George syndrome 22q-deletion and deletions associated with spinal muscular atrophy (SMA) and Charcot-Marie-Tooth disease. Microdeletion syndromes are now characterized with great precision using array-based DNA analysis. The recognizable syndromes with an identifiable phenotype such as 22q- and 15q11–q13 associated with Prader-Willi and Angelman syndrome, are complemented by rare deletion or duplication events that result in congenital anomalies, developmental delay, or both, and where their etiologic nature can be inferred from the normal structure of the parental chromosomes. Microdeletion duplication events are also a likely explanation for the sometimes sporadic, as well as familiar, nature of disorders such as autism.

CNVs are estimated to cause approximately 10% of all known variant-contributed disease. Chromosomal microarrays have become the most commonly used clinical test to identify CNVs.[26] Despite their importance, technical and clinical issues related to their identification and meaning must be resolved given their prevalence among healthy individuals and uncertainty regarding disease causation when a CNV in a genomic region is found in a patient with a specific phenotype with no clear link between genes involved and the patient's presentation.[27–29]

Variant Counts in Individuals

In the years since completion of the human genome map, the number of identified variants in the map has exploded. In 2015, results of the 1000 Genomes project were published. The report provided information from whole genome sequencing of over 2500 individuals of varying ancestry and identified over 88 million variants. Almost 85 million of the identified variants were SNPs, 3.6 million were insertion/deletions, and 60,000 were structural variants. Approximately 64% of the autosomal variants identified across all individuals had population frequencies of less than 0.5%. Many of these were observed to be much more common in at least one specific geographically defined ancestry group. A "typical" single individual was likely to have 4 to 5 million variants, with most being SNPs and short insertion/deletions, with only 1 to 5% of these (40,000 to 200,000) being "rare variants" with a frequency less than 0.5% in the population. Most of the identified variants do not appear to be linked with disease, either by being predicted as protein-truncating variants or peptide-sequence altering variants, or variants overlapping known promotor regions or other functional genomic regions.[30]

Linking Genes and Diseases

One hundred and one years prior to the sequencing of the human genome, Archibald Garrod hypothesized that alkaptonuria, which he noted to occur at high prevalence among children born to parents who were first cousins, was likely a disorder of metabolism inherited according to Mendel's laws of heredity. It was the first disorder in humans attributed to what would become genetics. He also noted that the chemical basis for darkening the urine could be a sign of human biological diversity, one which could be harmless, but for other traits, might be manifest in lethal disease. He coined the term "inborn errors of metabolism" to describe diseases caused by errors and variations in chemical pathways.[31,32]

Over the next hundred plus years, facts about DNA and its impact on human development and disease have come to light, including the identification of the double helix as the structure that allows for transcription and translation.[33] Since then, we know that the estimated gene number located on the 23 human chromosomes has been clarified to be approximately 20,000, with only 2% to 3% of the base pair composition of DNA comprising

specific protein-encoding sequences, or exons, while the vast majority of base pairs are considered "introns."[12] From this basic material, normal and abnormal development occurs.

We estimate that 5% to 10% of newborns have suspected disease-causing chromosomal disorders. For decades, 20% of infant deaths in the United States, and a larger portion of infant deaths in the NICU, have been attributed to chromosomal and congenital anomalies. With the expanded application of genetic sequencing linked with powerful informatics tools, prevalence of genetic variant-linked disease is increasing.[34–38] In addition to mortality risk, infants with complex congenital anomalies and chromosomal disorders who survive beyond the first days are vastly overrepresented among hospitalizations in pediatric wards and intensive care units. Affected children are hospitalized more often, and have hospitalizations that are longer and more expensive than children without underlying chromosomal anomalies or congenital defects.[39–42] As more genetic variants are linked with disease, our hope is that our ability to provide care based on better understanding of the pathophysiology of the disease, and susceptibility to complications that arises from awareness of the genetic variations at the heart of the problem will guide preventive and treatment strategies that will improve outcomes.[43]

Making a Diagnosis in the Post Human Genome Era

In infants suspected of having a genetic disease, knowing the etiology can inform subsequent care, both for treatment and prevention of problems associated or anticipated.[44] The broadening availability and reductions in cost of sequencing since completion of the Human Genome Project have increased the availability of extensive sequencing and has led to questions of when such sequencing should be done relative to more familiar testing including karyotypes and chromosomal microarray.[45] Of note, this will continue to be examined and change, as we are likely less than a decade away from a cost of only $20 to sequence the entire genome.[46]

Tools most commonly used for genetic testing include approaches that assess the amount, structure, and sequence of an individual's genome (Table 25.3). Traditionally, genetic testing began with a karyotype, providing information on chromosomal quantity and structure, and at times, a very targeted sequencing of specific regions and genes that the clinician had rounded up as the usual suspect gene(s) for a given phenotype. These first-line tests included a karyotype, and/or fluorescence in situ hybridization (FISH) testing for aneuploidy, or other chromosomal anomalies such as chromosomal rearrangements and large gains and losses in chromosomal material.

Currently, clinicians often order a karyotype and chromosomal microarray (CMA), with CMA able to identify specific variants as well as CNVs and much higher resolution gains and losses in genetic material such as insertions and deletions (indels).[47] CMA are used to assess infants with multiple anomalies (including verifying suspected aneuploidies). Much progress has come as the CMA has evolved to include oligonucleotide and single nucleotide probes that identify both single nucleotide variants as well as CNVs (including microduplication and microdeletion syndromes with high sensitivity and specificity). Today, CMAs may identify up to 20% of potentially causative genetic variations which have previously been associated with disease phenotypes.[47] This traditional, step-by-step approach is in transition as the cost and time to complete whole exome and whole genome sequencing are both rapidly decreasing, but not so drastically as to eliminate the utility of karyotype, microarray, and use of specific gene and variant panels.[48]

TABLE 25.3 Testing for Chromosomal and Molecular Syndromes

Test	Discoverable Aberrations	Clinical Indications
Karyotype	Large structural changes, aneuploidies, translocations, large insertions and deletions	Suspicion of chromosome syndrome Infertility or recurrent miscarriage Rule out structural variant after microarray finding
FISH (fluorescent in situ hybridization)	Specific probes used to identify aneuploidies, copy number variants, translocations, inversions, insertions	Prenatal or postnatal tests for suspected aneuploidies or deletions/insertions
SNP Array (single nucleotide polymorphism array)	Copy number changes associated with unbalanced structural changes Regions of homozygosity	Congenital anomalies, intellectual disabilities
NGS Panel (next-generation sequencing panel)	Single nucleotide variants, indels, copy number changes	Phenotype specific gene panels (hypotonia, seizures, etc.)
WES (whole exome sequence)	Variation in exon sequence, to individual variant level, may miss indels	Suspected genetic-related disease phenotype
WGS (whole genome sequence)	Tests the order of all the nucleotides in an individual's deoxyribonucleic acid and can determine variations in any part of the genome. Captures most indels	Suspected genetic-related disease phenotype

Adapted from Lalonde E, Rentas S, Lin F, Dulik MC, Skraban CM, Spinner NB. Genomic diagnosis for pediatric disorders: revolution and evolution. *Front Pediatr*. 2020;8:373. doi:10.3389/fped.2020.00373.

Clinical Application of Next-Generation Sequencing

Whole exome sequencing (WES) and whole genome sequencing (WGS) are now being applied clinically. Initially, approximately 10 years ago, due to cost and time to return results, WES and WGS were used only in cases where a genetics cause was suspected but where karyotyping, FISH, or chromosomal microarray with CNV analysis did not provide a specific answer for the clinical phenotype. The potential for NGS to reduce the time to diagnosis and meaningfully redirect care was clearly demonstrated in one of the first case reports of use of extensive exome sequencing to

identify a specific disease-causing variant leading to a change in treatment, dramatically altering a child's course. The 15-month-old male patient first presented at 15 months of age with failure to thrive and a perianal abscess. The child was treated for months with presumptive diagnosis of Crohn disease. His fistulae persisted, and he underwent a colostomy. The child was treated with multiple medical regimens for several years, including with cyclophosphamide. Simultaneously, he underwent extensive immunologic function testing, specific genetic testing for known defects, and CNV assessments suggested by experts, but all were negative. Ultimately, given the complexity of his course, the lack of an identified diagnosis after multiple tests, and his poor prognosis, the care team decided to take the relatively newly available agnostic approach to try to identify a causative mutation, and used 5 micrograms of the patient's DNA to sequence exons from approximately 180,000 protein coding exons and 700 miRNA exons. Exome sequencing identified 16,124 variants. Further analysis identified a novel, hemizygous missense mutation, a G to A substitution at a highly conserved position, in the X-linked inhibitor of apoptosis gene (XIAP), resulting in a hemizygous cysteine to tyrosine amino acid substitution. The team reporting the case did not find this variant at this position in a search of over 2000 human control sequences or in orthologous genes from other species, a key step in the analysis to identify potentially pathogenic variants. Functional assays demonstrated pathology consistent with disease phenotype, and maternal testing confirmed her as a carrier. Based on the findings, an allogeneic hematopoietic progenitor cell transplant was performed to prevent the development of life-threatening hemophagocytic lymphohistiocytosis, in concordance with the recommended treatment for X-linked inhibitor of apoptosis deficiency. Six weeks post transplant, the child was able to eat and drink, and had no recurrence of gastrointestinal disease.[49]

Since this landmark report, multiple groups have tested the utility of NGS in children. These NGS studies rely on rapidly evolving genetic sequencing methodologies and advanced automated analysis of rapidly accumulating genotype and linked phenotype data. These groups use either selected panels of genes, or the entire genome (WGS), or selectively sequence the exons that make up the vast majority of the genes in the genome (WES). Sequencing is followed by in silico analysis, or analysis by a computer, comparing the generated sequence against a reference human genome to identify variants. Variants are further analyzed with tools that predict protein structure and function that would result from the sequence change, and assess their frequency in populations of varying ancestry. With this information, plus the growing information about genes identified to be disease causing, variants can be labeled using standard nomenclature on a 5-point scale as pathogenic, likely pathogenic, a variant of unknown significance (VUS) or likely benign, or benign. This is usually followed by review by experts in the disease phenotype and by genetic epidemiologists to affirm the classification for the variant in particular infants. The overall process, inclusive of the in silico analysis and expert review of the sequence variants relative to the phenotype, is sometimes called "curation."[48]

Because there are multiple software tools to do this type of analysis, there can be discrepancies in how different reporting laboratories classify variants.[50] The effort to build consistency into up-to-date classification of the gene-disease and variant-disease relationship is through the efforts of the NHGRI-funded Clinical Genome Resource (ClinGen).[51] ClinGen has developed tools to evaluate clinical validity of gene-disease associations and pathogenicity of genetic variants for use in clinical care. ClinGen's work is made publicly available via another resource, ClinVar, the NCBI archival database that aggregates information about genomic variation and relationships to health that are provided by researchers, clinical sequencing laboratories, expert groups, clinics, and patient registries. While single investigators may submit a variant suspected of association with specific disease, ClinVar submissions are scored based on the number and types of sources submitting the same data, and validation by working groups and expert panels that examine the genetic epidemiologic evidence as well as supportive evidence from experimental model systems. These curations, as well as user interfaces, are constantly evolving, as data from multiple sources becomes available for consideration by expert panels.[52,53] The ClinGen website (https://clinicalgenome.org/) includes lists and links to the over 20 working groups and over 30 variant curation panels, links to multiple educational modules, and publicly available browser tools. Data continues to accumulate, and the number of expert panels that have been trained using ClinGen programs is also growing, with the aim to decrease heterogeneity of interpretation about significance of gene/variant/disease associations.[54,55] Clinicians should be aware that although data does continue to accumulate, recent reports indicate that some discordance among the experts can exist. In one study, 17 of 158 variants tested for consistency of classification had a discordance that could affect clinical recommendations (pathogenic or likely pathogenic vs. VUS, likely benign or benign).[56] In addition to these variations in classifications, sequencing and curation may not identify a variant, but the test may be negative because of technical limitations of testing, or because the affected gene–disease relationship has not been described. Also, some disorders, like those from epigenetic alterations like Prader–Willi or Beckwith–Wiedemann syndrome, or repeat disorders like congenital myotonic dystrophy, may not be diagnosed.[48]

Despite its limitations, NGS testing in infants and children with suspected but previously undiagnosed genetic disease suggests that utilization of deeper sequencing is likely to lead to diagnoses more often, and more rapidly, than previous incremental approaches.[57–59] In a report on 307 infants from three tertiary centers in Shanghai, China, WES or sequence panels identified pathogenic genetic etiologies in over 40% of the patients, including genetic etiologies in over 60% of the infants who died during the study's 180-day follow-up period. Of note and indicative of the importance of selection criteria for which babies to test with NGS, infants in the study cohort who exhibited integument, complex immune-related conditions, metabolism, and nervous system signs had higher chances of carrying variants in known disease-causing genes.[60]

The next step for clinical application of NGS is using either full WES or WGS with a rapid (days rather than weeks) turnaround time for results linking phenotype to identified sequence variation. A recent meta-analysis of 37 studies compared diagnostic rates of WES and WGS with chromosomal microarray in children and concluded that WGS/WES should be considered as a first-line genetic test, based on the greater diagnostic and clinical utility of both, when compared to CMA (<20% diagnostic yield). Interestingly, this analysis found that the diagnostic utility of WGS (41%) was similar to WES (36%). Additionally, the meta-analysis of studies, inclusive of over 20,000 children and published between 2011 and 2017, found that availability of parental samples for "trio" analysis of WES and WGS and hospital-based interpretation enhanced diagnostic utility.[58]

Initial studies in the NICU patient population have thus far reported successful outcomes, quoting WGS diagnostic

percentages of 42% to 57%, clinical management alterations (as a result of the diagnosis) in 30% to 72%, and change in outcomes experienced by 24% to 34% of patients in the studies who receive rapid WGS.[57,59,61,62]

The NIH-funded "Newborn Sequencing In Genomic medicine and public HealTh" NSIGHT1-randomized controlled trial (NCT02225522) aimed to enroll 1000 critically ill infants less than 4 months old with illnesses of unknown etiology to determine if rapid (26 hours) whole-genome sequencing (rWGS), followed by variant interpretation utilizing American College of Medical Genetics guidelines for pathogenic and likely pathogenic classifications, increased the proportion of infants who received a genetic diagnosis within 28 days compared to standard genetic tests including newborn screening (included in analysis for both groups), chromosomal testing (karyotyping, FISH), CMA, tests of methylation and CNVs, metabolic testing, and nonexpedited targeted genotyping or sequencing.[63] In this study, the next-generation (nextgen) sequencing approach utilized did not detect CNVs and structural variants. The study was terminated early, after 65 infants (64 in the NICU) were enrolled, because approximately 80% of the control infants received sequencing as a standard test as nonexpedited WGS became available as a standard diagnostic test during the study, and 5 of the control infants received "compassionate cross-over" to rWGS. For the primary outcome, 10 of 32 infants randomized to rWGS, and 3 of 33 infants randomized to standard testing were diagnosed within 28 days. Among infants enrolled in the first 25 postnatal days, 7 of 22 infants randomized to rWGS were diagnosed within their first 28 postnatal days while none of the infants randomized to standard testing had a diagnosis by postnatal day 28. Overall, 41% of the rWGS infants were diagnosed with a genetic disease, and 24% of the infants limited to standard testing ended the study with a genetic diagnosis. It is important to note that 5 (33%) of 15 diagnoses by standard tests would not have been detected by rWGS due to copy number or structural variants ($n = 4$) or a change in DNA methylation ($n = 1$). Also of note, the study protocol required confirmation of rWGS genotyping results by another method prior to clinical reporting except in cases where life-threatening progression was imminent. De novo rather than inherited variants accounted for more than half of the causative or likely pathogenic variants. Importantly, management changed for 10 of the 21 infants with diagnoses.[64]

The increasing availability of sequencing panels (Table 25.4) for certain phenotypes, and WES and even WGS during the study period changed the definition of "standard tests," which contributed to the early termination of the study. These factors, along with the growing list of validated genes and variants identified to be associated with or causative of disease which increases the potential clinical impact of utilizing sequencing in the NICU, led the study group to implement the second Newborn Sequencing in Genomic Medicine and Public Health study (NSIGHT2; NCT03211039), a randomized trial comparing rWGS to rapid whole-exome sequencing (rWES) in infants at a single tertiary center with diseases of unknown etiology admitted to pediatric or neonatal intensive care units.[64]

TABLE 25.4 Sequencing Panels for Neonatal Respiratory Disease

Test Name	Vendor	Genes Tested
Neonatal Respiratory Distress Panel	Prevention Genetics	5 genes: *ABCA3, FOXF1; NKX2-1; SFTPB; SFTPC*
Neonatal Respiratory Distress—Surfactant Dysfunction Panel	Blueprint Genetics	5 genes: *ABCA3; FOXF1; NKX2-1, SFTPB; SFTPC*
Neonatal Respiratory Distress Panel	Invitae	111 genes: *ABCA3 ACE AFF4 AGT AGTR1 AK7 ALB ARL6 ARMC4 BBIP1 BBS1 BBS10 BBS12 BBS2 BBS4 BBS5 BBS7 BBS9 C11orf70 C8orf37 CCDC103 CCDC114 CCDC151 CCDC39 CCDC40 CCDC65 CCNO CD40 CD40LG CEP164 CEP19 CEP290 CFAP298 CFTR COPA CSF2RA CSF2RB CXCR4 DKC1 DNAAF1 DNAAF2 DNAAF3 DNAAF4 DNAAF5 DNAH1 DNAH11 DNAH5 DNAH8 DNAH9 DNAI1 DNAI2 DNAJB13 DNAL1 DRC1 ELANE FBN3 FLNA FOXF1 GAS8 GATA2 HSD11B2 IFT172 IFT27 IFT74 IL1RN INPPL1 ITGA3 KIF7 LRRC56 LRRC6 LZTFL1 MARS MCIDAS MKKS MKS1 MTHFR MTM1 NDST1 NKX2-1 NME8 NOTCH2 OFD1 PARN PIEZO2 PIH1D3 REN RPGR RSPH1 RSPH3 RSPH4A RSPH9 RTEL1 SARS2 SCLT1 SDCCAG8 SFTPB SFTPC SLC27A4 SLC34A2 SLC7A7 SPAG1 TERC TERT TINF2 TMEM165 TMEM173 TRAPPC3 TRIM32 TTC8 WDPCP ZMYND10*
Surfactant Dysfunction Panel	GeneDx	5 genes: *ABCA3, CSF2RA, CSF2RB, SFTPB, SFTPC*
PulmZoom	Johns Hopkins DNA Diagnostic Laboratory	106 genes: Mucociliary disorders: *ARMC4, CFAP298, CFAP300, CCDC103, CCDC114, CCDC151, CCDC39, CCDC40, CCDC65, CCNO, CFTR, DNAAF1, DNAAF2, DNAAF3, DNAAF4, DNAAF5, DNAH1, DNAH9, DNAH11, DNAH5, DNAH8, DNAI1, DNAI2, DNAJB13, DNAL1, DRC1, GAS8, HYDIN, LRRC56, LRRC6, MCIDAS, NME8, OFD1, PIH1D3, RPGR, RSPH1, RSPH3, RSPH4A, RSPH9, SCNN1A, SCNN1B, SCNN1G, SPAG1, SPZ1, TTC25, ZMYND10* Interstitial lung disease: *ABCA3, AP3B1, COPA, CSF2RA, CSF2RB, DKC1, ELMOD2, FLCN, FLNA, FOXF1, GATA2, GBA, HPS1, HPS4, IDUA, MARS, NAF1, NF1, NKX2-1, NPC2, OAS1, PARN, RTEL1, SFTPA1, SFTPA2, SFTPB, SFTPC, SLC34A2, SLC7A7, SMPD1, STAT3, TERC, TERT, TINF2, TMEM173, TSC1, TSC2, ZCCHC8* Pulmonary vascular disorders: *CVRL1, BMPR1B, BMPR2, CA12, CAV1, COL1A1, COL3A1, EIF2AK4, ENG, FBN1, FOXF1, GDF2, KCNA5, KCNK3, RASA1, SMAD9*

The NSIGHT2 study aimed to compare diagnosis of genetic disease obtained by rWGS or rWES, and to compare analysis of only the proband's sample with analysis using familial trios. The study team did not randomize enrolled infants who were identified as "gravely ill" but provided them with ultra-rapid whole-genome sequencing (urWGS). Genetic diseases were diagnosed by identification of pathogenic or likely pathogenic variants in genes known to cause diseases with similar presentations to those observed in study infants. For NSIGHT2, infants <4 months of age, with time from admission or time from development of a feature suggestive of a genetic condition of <96 hours were eligible. Exclusion criteria included infection or sepsis with normal response to therapy, prematurity alone, isolated unconjugated hyperbilirubinemia, hypoxic-ischemic encephalopathy with a precipitating event, a previously confirmed genetic diagnosis that explained the condition, isolated transient neonatal tachypnea, and nonviable neonates with modified code status. In this study, different from NSIGHT1, initial genomic interpretation was done on the proband alone. If no diagnosis was made in singleton analysis, the infant was reanalyzed as a trio. In this study reflecting technology advances since NSIGHT1, molecular methodologies for WGS and WES could detect CNVs and indels. Analysis, interpretation, and reporting required an average of 6 hours of expert effort. If rWGS or rWES established a provisional diagnosis for which a specific treatment was available to prevent morbidity or mortality, this was immediately conveyed to the clinical team. Of note, and always an issue when considering WGS or WES in the clinical realm, secondary findings such as variants linked with adult-onset disease risk were not reported, but medically actionable incidental findings were reported if families provided consent to receiving this information.[65]

Consistent with the goal of studying WGS and WES as a first-tier test in infants in the first 4 postnatal months—a group estimated to have a relatively low population pretest probability for a genetic diagnosis—almost half of the infants admitted to ICUs during the study period were identified as eligible. Two hundred thirteen were ultimately enrolled (37%). Ninety-four infants were randomized to receive rWGS and 95 infants to receive rWES. Overall, 24% of these infants received a genetic diagnosis. Diagnostic performance (19% vs. 20%) and time to result (11.0 vs. 11.2 days) were quite similar for rWGS and rWES. Among the 24 critically ill infants who had urWGS, 11 (46%) received a diagnosis and median time to result was 4.6 days. No infant received a false positive result. One rWGS infant's diagnosis would have been missed had he been randomized to the rWES group. More than half of the identified variants were sequence variants, and the others were CNVs or structural variants, or small insertions or deletions. More than half of the variants were de novo. Diagnostic yield was improved for only 1 of 147 infants by analyzing the trio in addition to the proband; however, for infants in the urWGS group, trios were genotyped to eliminate the time needed for confirmatory sequencing of the identified variants in the proband plus parents which was done for infants in the rWES and rWGS groups.[65] In addition to the diagnostic endpoints, participating clinicians perceived high clinical utility and low likelihood of harm with first-tier rapid genomic sequencing of infants in ICUs with diseases of unknown etiology. The rapid sequencing and delivery of results was perceived as beneficial irrespective of whether results were positive or negative.[66]

In addition to the groundbreaking single center NSIGHT studies, two multicenter studies of the application of NGS have been focused in the NICU: (1) the NICU-Seq study (NCT03290469) and (2) the GEMINI study (NCT03890679).

NICU-Seq (*NICUSeq: A Trial to Evaluate the Clinical Utility of Human Whole Genome Sequencing [WGS] Compared to Standard of Care [SOC] in Acute Care Neonates and Infants)* completed enrollment in 2020. Investigators at five centers enrolled infants to receive either SOC testing as determined by the site clinical team, or clinical whole genome sequencing (cWGS). Upon enrollment, each proband was randomly assigned to the 15-day cWGS group or the SOC group. A time-delayed study design was used to ensure that all participants received access to WGS. The experimental cWGS group received results of the cWGS at 15 days while undergoing standard care, and the SOC group received cWGS results at 60 days while undergoing SOC. Eligible patients were up to 120 days of age, with a suspected genetic disease based on objective clinical findings. At least one biological parent was required for participation. Exclusion criteria included an established genetic diagnosis, high clinical suspicion for aneuploidy, or full explanation of the patient's phenotype by complications of prematurity. Three hundred and fifty-four subjects, 176 infants in the early arm and 178 in the delayed arm, were enrolled over 19 months. The mean participant age at enrollment was 15 days. More than 80% of patients were in the NICU at time of enrollment. The rate of infants with molecular diagnosis by 60 days was higher among those in the 15-day cWGS group compared with SOC testing (31% vs. 15 %). At 60 days, twice as many infants in the early group versus the delayed group received a change of management (COM, the study's primary outcome), 21.1% versus 10.3%. By 90 days, after the SOC group's cWGS results were known, 28% of the SOC infants had COM. No differences in hospital length of stay or survival were observed. Usual-care testing during the observation period varied substantially by site, ranging from karyotyping to WGS, with negative microarray testing making up the majority of test results. Overall, approximately two thirds of the infants with a genetic diagnosis had a COM, approximately one-fourth of the entire study cohort. One infant in the early group was diagnosed by WGS with Wiskott-Aldrich syndrome (OMIM 301000) and received a corrective bone marrow transplant. Another infant, enrolled in the delayed group at 11 postnatal days with epilepsy, was ultimately diagnosed by WGS with an unsuspected *KCNQ2* (OMIM 602235) variation. Her ongoing metabolic work-up was stopped, and ineffective administration of pyridoxine was discontinued. WGS revealed a wide range of causal variant types, including terminal and interstitial chromosomal copy number variants, complex compound heterozygous variants, mitochondrial variants, and SMN1 copy loss leading to spinal muscular atrophy. The study team concluded that their findings demonstrate that WGS leads to focused, and therefore improved, patient care and should be considered as a primary tool in the assessment of critically ill infants with a suspected genetic disease.[67]

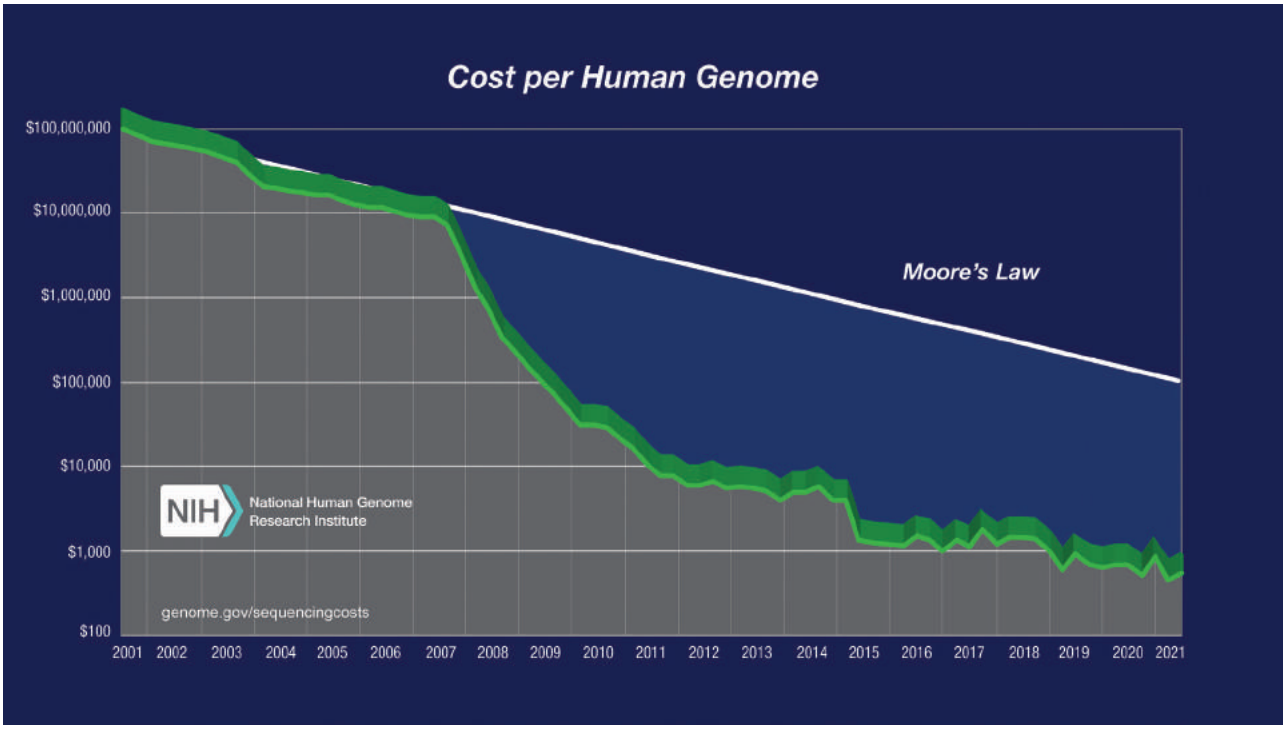
The GEMINI study *(Genomic Medicine for Ill Neonates and Infants*) aims to compare the clinical and economic utility of performing rapid whole genomic sequencing versus a targeted genomic sequencing panel (NewbornDx, which sequences 1722 actionable genes targeting disorders presenting in infancy) on 400 high-risk neonates and infants at 6 hospitals who are suspected of having a genetic disorder. The infants will be up to 1 year of age, with signs and symptoms of a genetic disorder. Exclusion criteria include already having a known genetic diagnosis, having a major congenital anomaly associated with a chromosomal anomaly

detected on prenatal testing, presence of a congenital infection or being nonviable because of extreme prematurity. Infants will undergo NewbornDx and rWGS (proband) testing. The biological parent(s), when available, will undergo NewbornDx gene panel testing at the same time as the infant. For rWGS, the infant will undergo testing first. If a specific diagnosis consistent with the phenotype is not made with rWGS proband analysis alone, the parent(s) will undergo rWGS. Both tests are designed to have sequence and analysis of results to identify variants that could contribute to disease in 14 or fewer days. Infants are classified as urgent if they (1) require mechanical ventilation, (2) exhibit severe neurological complications, (3) are hemodynamically unstable, or (4) are categorized as such at the request of the site's principal investigator. Urgent cases undergo ultra-rapid sequencing and analysis with a preliminary report generated within 72 hours of specimen arrival. Sequencing results undergo *in silico* analysis in comparing individual infants' results against known normals, and then the hundreds of variants are screened against known disease gene databases for likelihood of contribution to disease by panels of experts. Analysis includes assessment of inheritance pattern, frequency of variant, variant consequence, and reports in public databases. Variants are classified as pathogenic (P), likely pathogenic (LP), or VUS.

In the recently published interim report of the first 113 enrolled patients, diagnostic and/or VUSs were returned for 51 patients (45%). Results were concordant between WGS and the selected high-risk panel in 83 patients (73%). Significant alterations to care were made for 29 infants after results were obtained. Lessons learned included the limitations of the targeted sequencing to identify aneuploidy and other structural variants, and identification of variants in 8 genes identified by WGS but not assessed on the targeted sequencing panel. Three parents and other family members were newly diagnosed with a genetic condition based on the infant's diagnosis. Notably, while the rate of identifying likely causative genetic variants approaches 50%, even with WGS, more than 50% of the infants did not have a genetic diagnosis. One possible reason is that the rapid sequencing and analytics used are limited to identifying variation in genes and regions already known to be related to disease. As databases and analytic techniques become more robust or as an infant develops additional signs and symptoms to add to the information, genetic disorders may still be identified. Lastly, some infants may not have a genetic cause. Teratogens, environmental exposures, and potential epigenetic modifications could contribute to disease. While sequencing technology has advanced, the ability to understand and interpret these results and how they relate to or influence complex processes in newborns is limited and continues to evolve.[68]

Incidental Findings

While evidence supporting next-generation sequencing approaches is growing and costs of sequencing and time to result drop, cost alone will not be the only consideration in this application of sequencing (https://www.genome.gov/about-genomics/fact-sheets/DNA-Sequencing-Costs-Data) (Fig. 25.3). The ethical issues of incidental findings arising from sequencing must be addressed as these approaches are applied in the NICU. WES and WGS can disclose a range of risks applicable much later in life. Guidelines for these incidental findings in the context of neonatal testing are evolving and debate around the issue continues, as evidenced by the use of consent for incidental findings in NSIGHT2. The American College of Medical Genetics has



• **Fig. 25.3** Cost per Human Genome Sequence, Changes Over Time. To illustrate the nature of the reductions in deoxyribonucleic acid sequencing costs, the graph also shows hypothetical data reflecting Moore's law, which describes a long-term trend in the computer hardware industry that involves the doubling of "compute power" every 2 years (see: Moore's law [wikipedia.org]). Technology improvements that "keep up" with Moore's Law are widely regarded to be doing exceedingly well, making it useful for comparison. The drop-off in 2008 occurs with sequencing labs switching to nextgen rapid sequencing techniques. (https://www.genome.gov/about-genomics/fact-sheets/DNA-Sequencing-Costs-Data.)

addressed this issue and now provides guidance, updated annually, on the recommended genes (now 73) for reporting results when extensive sequencing is done.[69,70]

Genomics of Common Complex Diseases Associated With Prematurity

While NGS-based testing continues to inform clinicians and families about the genes and variants that contribute to complex, severe phenotypes in NICU patients, identifying genes and variants associated with the more common complex disorders of prematurity such as retinopathy of prematurity (ROP), intraventricular hemorrhage (IVH), necrotizing enterocolitis (NEC), and bronchopulmonary dysplasia (BPD) remains a challenge.

Morbidities of prematurity are strongly associated with mortality and long-term neurodevelopmental disabilities.[71–73] Identifying genetic variations related to these diseases would inform development of prevention and treatment approaches based on the individual patient's genotype. Dosing and treatment strategies based on genotype exist in several diseases usually associated with adult onset, including cancer, with various tumor genotypes guiding therapeutic decisions.[74] In addition, when management trials targeting these morbidities are undertaken, considering genetic variation as a potential confounder on the effect of specific management strategies may be important, as in the interactions of *CYP2C19* genotypes in combination with clopidogrel and aspirin in adults.[75,76]

Before going to the trouble of testing genetic variants for associations with disease, heritability, a quantitative measure of the extent to which genetic factors account for the phenotypic variance, must be assessed in the target population. For newborns, the process has relied heavily on testing disease prevalence among monozygotic versus dizygotic twins, as outlined by Bhandari and Gruen.[77] In studies with mono- and dizygotic twins, significant heritability has been identified for ROP, NEC, sepsis, IVH, and BPD.[78–83]

With the establishment of an inherited basis for these common complex neonatal diseases, researchers began to test associations between "single nucleotide polymorphisms" (SNPs) and disease. Having the minor (less common) allele can sometimes increase or decrease the functionality of the gene product. Early work on neonatal diseases focused on assessing associations between SNPs in genes for TNF alpha or IL-6, proteins associated with immune response or inflammation, and diseases such as BPD or late-onset sepsis, often with disparate results for the same SNP.[83,84] Tests for associations with a handful of SNPs in relatively small cohorts have dominated early reports, and some have identified associations with p values <0.05; however, with 3 billion nucleotides comprising the genome, over 30 million known SNPs, and thousands of variants in the genome of any one individual, and relatively small sample sizes, replication has proven difficult, and the concept of the candidate gene analysis, or, more specifically, candidate SNP analysis, approach was called into question unless study cohorts included thousands of cases and controls.[85,86]

With early evidence encouraging a more agnostic approach, broader arrays of genes and variants have been developed to test common variations in multiple candidate genes across the genome, short of whole exome or whole genome sequencing. When an allele for a SNP has a known genotype, the SNPs in linkage disequilibrium can have specific genotypes that frequently occur together. With expanding knowledge of the genome, many SNPs that are in linkage disequilibrium have been identified. This allows for "imputation" of multiple "tagSNPs," in which actual genotyping of one SNP allows prediction of the genotypes of 5 to 10 neighboring SNPs.[87]

In a genome-wide association study (GWAS) hundreds of thousands, even millions, of SNPs are genotyped and tagSNPs imputed, and then each SNP can be individually tested for association in well-phenotyped cohorts. Statistical approaches have been adapted to the millions of tested associations using GWAS. The standard has been to determine "genome wide significance" when the P values identified with the comparison are less than 5×10^{-8}.[87,88] In order to realistically test hypotheses using GWAS, large cohorts with thousands of subjects with the disease phenotype are ideal. As an example, in a case control study testing APOE gene variants in Alzheimer disease patients, variants were found to be associated with Alzheimer with a P value of 2.52×10^{-53}.[89] NICU patients included in GWAS studies have numbered in the hundreds and rarely thousands, far fewer than in the adult cohorts.[90–95]

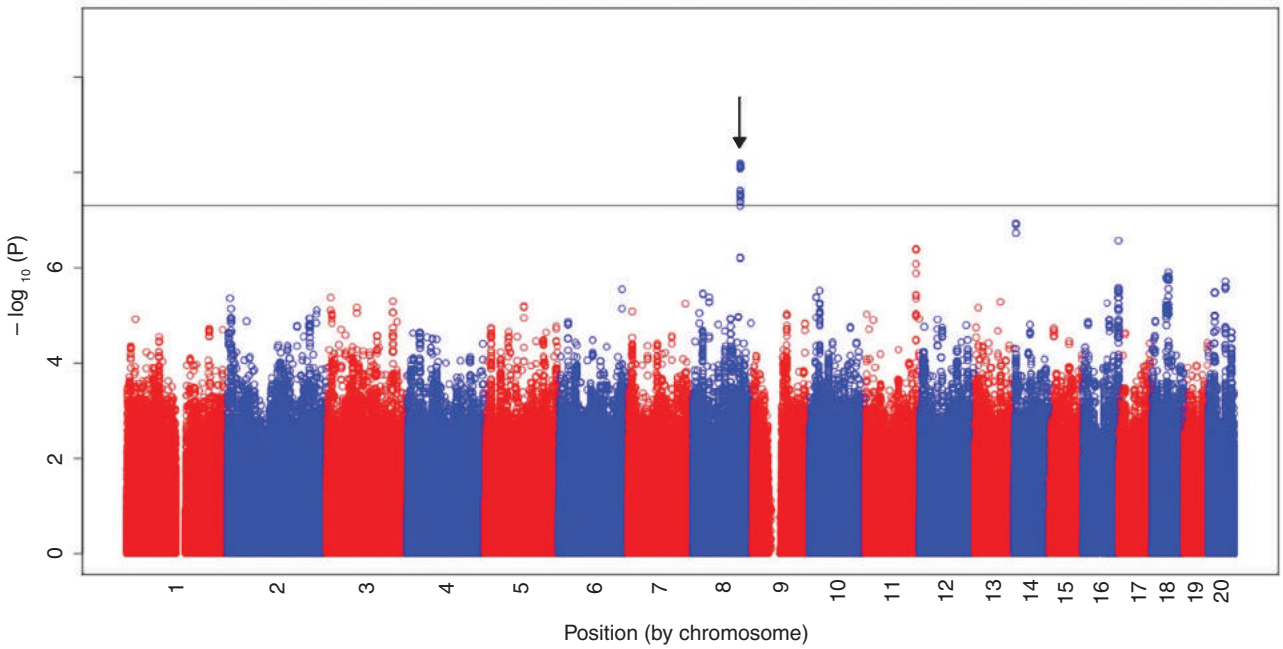
An additional challenge for genomic analysis among extremely preterm cohorts are the differences in minor allele frequency among study cohorts of differing ancestry. While studies using samples from relatively homogenous nations, such as Iceland, may produce fairly homogenous, replicable results, results from cohorts of mixed ancestry, which is more common in the United States, present challenges to analyzing associations between variants that differ in prevalence in cohorts of different ancestry.[96,97]

Retinopathy of Prematurity

Multiple attempts have been made to identify associations that are plausibly associated with the pathophysiologic mechanisms of ROP, including VEGF and VEGF receptors, but the candidate gene/SNP studies have not identified variants with genome wide significance. One report, which tested a panel of over 1000 variants in over 100 genes related to inflammation and organ development, identified two intronic variants in the brain-derived neurotrophic factor (BDNF) gene in patients with severe ROP (versus nonsevere or no ROP) with a P value of less than 5×10^{-7}.[98] Lower serum levels of BDNF have been associated with a higher likelihood of ROP in premature infants. This adds some plausibility to an ROP association with BDNF variants, or an association with another gene product/component of a pathway important for neurovascular development that includes BDNF.[99–101] While promising, impact of variants on BDNF levels has not been described. More recently, epigenetic analysis has identified placental CpG methylation of 12 different genes associated with development of prethreshold ROP. Interestingly, the genes with methylation changes associated with ROP included BDNF.[102] No GWAS has been done to examine ROP to date.

Necrotizing Enterocolitis

In a cohort study using a GWAS approach, minor allele(s) in a cluster of SNPs spanning a 43-kb region of chromosome 8 (8q23.3) conferred an odds ratio of 4.72 (95% confidence interval [CI]: 2.51 to 8.88) for elevated risk of NEC, with multiple SNPs associated with P values $<10^{-8}$ (Fig. 25.4). Like many candidate gene association studies done in the extremely preterm population, this analysis was limited by a small sample size ($n = 751$, only 30 with surgical NEC), from multiple sites in the United States, with significant ancestry admixture. Interestingly, the increased risk was similar for all three genetic ancestries represented in this population.[103] The investigators were not able to validate the associations



• **Fig. 25.4** Manhattan Plot of Single Nucleotide Polymorphisms (SNPs) That Exhibited Association With Surgical Necrotizing Enterocolitis (NEC) Versus Controls. Manhattan Plot of SNPs that exhibited association with surgical NEC versus controls. Data shown are –log P values on the Y axis along versus chromosome locations on the X axis. Arrow points to the NECRISK region on chromosome 8.104.

of the SNPs in the chromosome 8 region with NEC in a separate cohort ($n = 1018$, 26 with surgical NEC) of premature infants enrolled in a study using GWAS to identify variants associated with severe IVH.[93] In in silico analysis, the NEC-associated region of chromosome 8 appears to be evolutionarily conserved, but without any previously identified genes. Pathway analysis, testing variants in multiple pathway-linked genes for associations with NEC, identified associations with over 50 pathways, including pathways involved with immune response, growth regulation, and G-protein signaling. Interestingly, the *LTB4R* gene close to the chromosome 14 region with strong association with surgical NEC encodes an eicosanoid receptor. Pathway analysis revealed eicosanoid receptor signaling as the second most prominent pathway affected by NEC-associated SNPs. LTB4 plays a significant role in a toll-like receptor 4 and cyclooxygenase-2 mediated mechanism of intestinal ischemia/reperfusion injury.[103]

Intraventricular Hemorrhage

Several candidate gene studies have been conducted in relatively small cohorts testing associations between SNPs in inflammation, complement, and coagulation pathways.[104,105] The Gene Targets for Intraventricular Hemorrhage study group conducted a candidate gene study of SNPs in 7 genes, in 224 preterm infants with grade III-IV IVH, and 389 matched controls. Only SNPs in the Methylenetetrahydrofolate Reductase (MTHFR) gene gave even equivocal results of an association with severe IVH.[106] In the GWAS analysis, the group tested over 600,000 SNPs in 458 neonates with severe IVH and 866 infants without IVH, from U.S. and Scandinavian cohorts. No individual SNP reached genome-wide significance. A 10-SNP haplotype ranging from the intergenic region of *GM140* and *CACNA1E* to the intron region of *CACNA1E* had a P value of 7.16×10^{-10}. CACNA1E (calcium channel, voltage-dependent, R type, alpha 1E subunit) is mutated in a Mendelian form of hemiplegic migraine, a known vascular phenotype, as well as various epilepsies (Online Mendelian Inheritance in Man, OMIM. Johns Hopkins University, Baltimore, MD. MIM Number: 618285: last edited: 11/18/2020). None of the prior candidate individual SNPs reached genome-wide significance. Like many similar endeavors, the summary of this GWAS for severe IVH concluded, "Because common variants have small-to-moderate effects, a large-scale neonatal genomic medicine network must be developed with the infrastructural capacity to host an accessible database of sequence variants, their phenotypic associations and environmental risk factors."[93]

Bronchopulmonary Dysplasia

Severe ROP, NEC, and IVH each have phenotypes that are defined by imaging or direct visualization. However, BPD is usually defined by some level of respiratory support, making the anatomic and physiologic details of the phenotype that might be influenced by genetic variations challenging.[107,108] Five groups have used an agnostic GWAS approach to try to identify variants or groups of variants in genes and pathways associated with epidemiologically defined BPD and met with limited success.[90–92,94,95] Hadchouel identified SNPs in the *SPOCK2* gene associated with BPD in their discovery cohorts (of African and Caucasian ancestry) and a replication cohort.[90] Wang et al. studied over 2000 very low birthweight infants born in California, and did not identify genomic loci or pathways associated with moderate to severe BPD with genome-wide significance.[91] Ambalavanan et al. studied 751 infants with birthweights ranging between 401 and 1000 g (428 diagnosed with BPD or who died) and found no SNPs that achieved genome-wide significance. Pathways of lung development and repair and novel molecules and pathways (adenosine deaminase, targets of microRNA or miR-219) were associated with BPD. Pathways associated with mild BPD were different from the pathways associated with severe BPD. In addition, the variants/pathways associated with BPD varied by ancestry.[92,109]

In a Finnish case control study ($n = 60$ infants with moderate-to-severe BPD; $n = 114$ control infants), an SNP flanking the C-reactive protein gene had the strongest association with BPD ($P = 3.4 \times 10^{-6}$), and in multivariable logistic regression this SNP was associated with BPD in two replication cohorts (one Finnish and one comprised of European and African individuals).[94,95] In the most recent GWAS study, which tested 9 million genotyped and imputed variants from 387 preterm infants who participated in the Trial of Late Surfactant (TolSURF) study for associations with BPD, no individual SNPs were associated with BPD with genome wide significance. Similar to the study by Ambalavanan, this group reported that genetic ancestry was associated with survival without BPD. The associations between genetic ancestry and BPD suggest that patterns of genetic variation in infants with or without BPD are likely to differ by continental origin.[95] Ultimately, the five GWAS studies failed to identify a specific gene or gene variant to be specifically and convincingly associated with BPD; however, the studies, individually and collectively, are limited by small sample sizes and high levels of phenotype variation.

Investigators have also applied WES to BPD, with hopes that much more extensive sequencing in patients with extreme phenotypes could identify rare variants associated with disease.[110–112] Carrera et al. studied 26 infants with severe BPD. Identified variants were classified as likely to have high, moderate, or low impact based on predicted protein effects, and whether or not they were novel variants in genes with occurrence in more than one subject. As expected, each subject had approximately 200,000 identified variants, with about 10% of all variants identified from all subjects as possibly having moderate or high impact. In each sample, approximately 100 variants were identified that were hypothesized by structural predictive analysis to have an impact on protein structure. Two subjects had novel missense mutations in *ABCA3*, a gene whose product is responsible for transporting phospholipids to lamellar bodies in type II alveolar cells. Rare and novel variants in *NOS2* and toll-receptor genes and C-reactive protein were also identified.[110] Li et al. used newborn screening samples for exome sequencing of 50 twin pairs, including 51 infants with BPD. The accumulated variants in 258 genes in the subjects with BPD had a significantly higher haploinsufficiency score (haploinsufficiency can be defined as the situation when one copy of a gene is either deleted or has a loss-of-function variant, and dosage of the gene product is reduced enough to affect function).[111,113] Variants clustered in genes in pathways involved in embryonic epithelial development, collagen organization, and Wnt-signaling were increased among the infants with BPD compared with non-BPD infants. This group also looked at tissue expression in human tissue from patients with BPD, and in lung tissue from the hyperoxic mouse model of BPD, and detected increased expression in the exome-identified BPD candidate genes in hyperoxia exposed animals.[111] Most recently, Hamvas et al. reported WES results from 146 subjects (85 with BPD and 61 unaffected) enrolled in the Prematurity and Respiratory Outcomes Program (PROP). Three hundred forty-five genes with extremely rare, nonsynonymous variants (variants that would lead to a change in the amino acid sequence of the gene product) were identified only in the BPD-affected subjects. This study, the largest to date, replicated 28 genes with extremely rare variants in patients with BPD that were previously associated with BPD in the California cohort reported by Li et al.[111,112]

The GWAS and WES studies to date are also limited by their small sample size and challenges to phenotyping, as well as their ability to identify rare variants in noncoding regions that may be of importance to the pathogenesis of BPD, or other important morbidities of prematurity; however, the WES approach has identified associations with genes containing rare variants and candidate pathways, and the WGS approach has yet to be tested. As described in the NGS studies in the NICU, wider application of these extensive sequencing methodologies to more infants are likely to uncover genetic contributors to disease in individual infants.[114]

Conclusion

The application of genomics and genetics to clinical neonatology is evolving rapidly as prices drop and the speed of sequencing, identifying, and analyzing variants for their association with a growing number of gene-disease databases accelerates.[46] Diagnoses are being made at a much more rapid pace, and clinical care must adapt quickly to the new knowledge. That said, we have the "good problem" of how best to apply this growing knowledge. As an example, a NICU clinician seeking to understand why a premature infant has worse than expected respiratory disease could consider a number of approaches to test for variants in a number of genes that contribute to respiratory disease.[114,115] One choice would be to order a gene panel to avoid the current cost of WGS or WES. But which panel to choose? A quick Google search using the search terms "respiratory disease neonate genetic panel" identifies four different products, from four companies. One additional panel, a well-known test for respiratory-related problems, by the group at Johns Hopkins DNA Diagnostic lab is also included as an option. The testing panels cover a range of 5 to 125 genes. All utilize sequencing of the exons of the panel's genes and areas close to the exons that are likely regulatory regions. Three genes, ABCA3, SFTPB, and SFTPC, are included in all five panels. Two genes, FOXF1 and NKX2-1, are included in four panels, and two genes, CSF2RA and CSF2RB, are included in two panels.

Clearly, we are in an extremely rapid learning phase of how best to apply genomics in the NICU! Clinicians should be aware of the details and the range of the genetic tests that can be ordered, their strengths and weaknesses, and should strongly consider partnering with colleagues in medical genetics to best interpret results and to discuss their ramifications with families.

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26

Prenatal Diagnosis and Counseling

EDITH Y. CHENG AND J. CRAIG JACKSON

KEY POINTS

- All pregnant women should have the option to undergo prenatal screening/diagnosis for genetic conditions and/or birth defects.
- Specific indications for genetic counseling and prenatal diagnosis testing include a history of chromosome abnormality, Mendelian genetic disorder, or metabolic disorder; increased risk for neural tube defect; abnormal maternal serum screening test; abnormal cell free DNA result; or a fetal anomaly suspected/diagnosed on ultrasound.
- Successful prenatal diagnosis requires a known condition associated with a structural abnormality visible in the fetus, a biochemical abnormality in amniotic fluid/amniocytes, or a known molecular mutation.
- Preimplantation genetic screening/diagnosis selects out embryos with a genetic condition or aneuploidy and transfers either chromosomally normal embryos or embryos without the at-risk genetic condition.
- Prenatal counseling is especially challenging because decisions with lifelong impact are often required on behalf of the unborn patient, and when the interests of the fetus and parents are in conflict.
- It is essential to respond empathetically to the inevitable strong emotion resulting from the unwanted news of a fetal abnormality and to align with the parents' goals and values before proposing a treatment plan.

Background

In the late 1970s, only two testing programs comprised the paradigms in prenatal screening/diagnosis: (1) elevated maternal serum alpha fetoprotein (MSAFP) to screen for open neural tube defects (NTDs) and (2) maternal age over 35 years at delivery to screen for Down syndrome (DS). Neither program used imaging routinely and only second trimester amniocentesis for fetal karyotyping was available. Today, more than 50 years later, genomic discoveries and advances in fetal imaging technology present women with many prenatal screening and/or diagnostic options for the evaluation of their fetus for birth defects and/or genomic abnormalities and, in some cases, provide an opportunity for in utero treatment. Early diagnosis of birth defects broadens the scope of management options and allows preparation for delivery and postnatal support, all of which have improved viability and outcome of serious birth defects that in previous decades would be termed prenatally as "lethal anomalies." The care of the mother–fetus dyad is now multidisciplinary through partnerships between maternal–fetal medicine, neonatology, pediatric surgery, and pediatric subspecialists. The goal of this chapter is to discuss the breadth of prenatal diagnosis to illustrate the complexity of the technology and choices; recognize their benefits, accuracy, and limitations; and understand their impact in the care of pregnancies with genetic disorders or fetal anomalies.

Principles of Prenatal Screening and Diagnosis

Screening is the systematic application of a test to identify individuals at high risk for an asymptomatic, well-defined serious medical condition with an established incidence, for which identification would lead to prevention and/or treatment. The screening test should be cost effective, simple and safe, readily available and accessible, and should have a well-defined performance. An accurate diagnostic test should be available to confirm or refute the screen positive result. In prenatal screening programs, there should be timely transfer of test results, counseling, respect for the ethical and cultural values and decisions of patients, and full discussion of all options if the suspected condition is confirmed.

Diagnosis of a suspected condition in an at-risk fetus requires a known diagnosis for which the condition is associated with a detectable abnormality either within the fetus and/or the fetal tissues. This could be in the form of a structural abnormality readily seen on ultrasound (that appears at the appropriate developmental stage of the system affected), a cytogenetic abnormality, a biochemical abnormality in the amniotic fluid or in cultured amniocytes, a genomic duplication or deficiency identified on microarray analysis, or a known genetic mutation associated with the condition. For example, a mother with autosomal dominant achondroplasia has a 50% chance of having an affected child. Knowing that in an affected fetus the long bones do not demonstrate growth deceleration until after 24 weeks' gestation, a normal growth ultrasound at 20 weeks' gestation would not be reassuring, but a normal growth ultrasound at 30 weeks' gestation would essentially exclude the diagnosis in her fetus. Conversely, if she had a known mutation for achondroplasia, genetic testing of chorionic villi retrieved at 13 weeks' gestation or amniocytes retrieved by amniocentesis at 16 weeks would inform the status of the fetus before the condition is visible on prenatal imaging; or, preimplantation testing for the mutation in embryos with transfer of an unaffected embryo would eliminate the risk of an affected fetus.

Invasive Prenatal Diagnostic Procedures

Midtrimester Genetic Amniocentesis

The term *amniocentesis* refers to the procedure of removing amniotic fluid under ultrasound guidance from the uterus and is performed for many reasons. For prenatal diagnosis, it is usually performed between 15 and 20 weeks' gestation. The amniotic fluid contains desquamated cells from fetal skin, bladder, and the gastrointestinal tract, which serve as sources for cytogenetic and enzymatic/biochemical studies. DNA can also be extracted from these cultured amniocytes for genomic studies. Proteins such as alpha fetoprotein (AFP) and acetylcholinesterase are measured to confirm an open NTD suspected on ultrasound.

The benefits of second trimester amniocentesis include its large international clinical experience of over 40 years; the standardization of culture and cytogenomic techniques which decrease the culture failure rate to 0.1%; and its diagnostic accuracy, broad availability, and relative safety.[1] Based predominantly on data obtained in the 1980s and 1990s when second trimester amniocentesis was widely performed, the incidence for minor complications such as cramping and leakage of fluid immediately after the procedure was collectively about 1%, while the incidence of significant complications such as chorioamnionitis and/or miscarriage was 0.25% to 0.5%.[2]

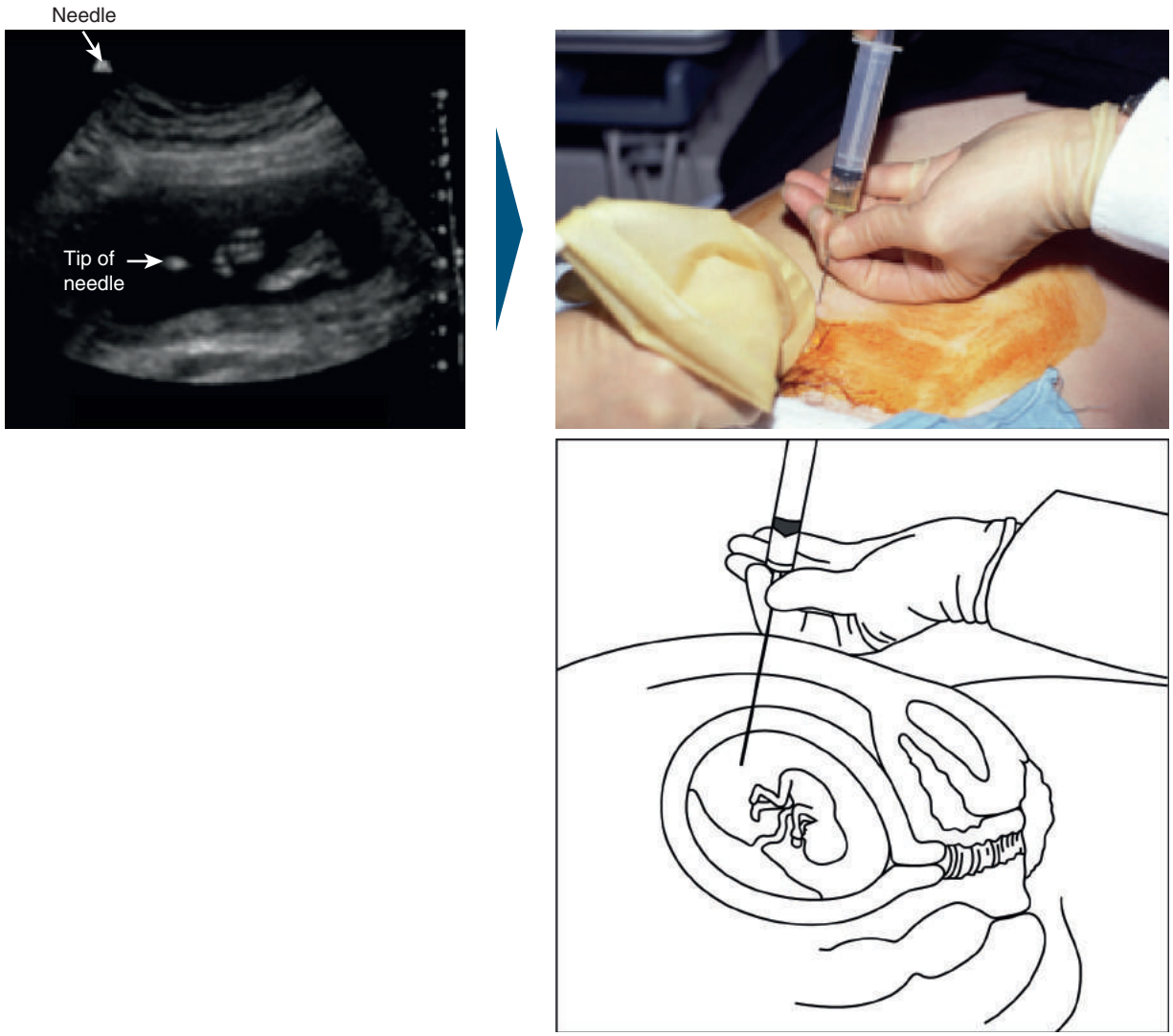
The relative safety of midtrimester amniocentesis when completed by experienced providers has been confirmed by many studies. The multicenter First Trimester and Second Trimester Evaluation Risk (FASTER) trial in 2004 observed a procedural loss rate after second trimester amniocentesis (and before 24 weeks' gestation) of 0.06% or 1/1600.[3] Prior studies reporting high pregnancy loss rate likely reflect the nuances that contribute to the safety of any procedure that requires technical expertise. Ultrasound guidance, use of a smaller 22-gauge needle, and a large volume of patients at a referral institution allowed practitioners to maintain their technical skills. Each institution should calculate its own complication rate. In 2008, Odibo and colleagues reported on a single center's 16-year experience and identified a fetal loss rate of 0.13% or 1/769. A 2015 meta-analysis of miscarriage after amniocentesis in more than 42,000 women who had the procedure—compared with 138,000 who did not—estimated the procedural loss rate to be approximately 0.11% or 1/900.[4,5] These data support that midtrimester amniocentesis is safe when the procedure is performed by experienced providers in large volume referral centers. Thus the current estimated procedural risk discussed with patients is approximately 0.1% to 0.3%. To date, even with maternal serum screening and cfDNA as options for screening, midtrimester amniocentesis remains the procedure indicated for prenatal diagnosis of fetuses with ultrasound anomalies and/or screening results suspicious for fetal cytogenomic abnormalities.

Early amniocentesis, performed between 11 and 14 weeks' gestation, was briefly explored in the late 1990s. The only large prospective study, the Canadian Early and Mid-Trimester Amniocentesis Trial, randomized 4334 women to early amniocentesis versus midtrimester amniocentesis and observed a higher pregnancy loss rate, more rupture of membranes, more culture failures, and greater procedural difficulty in the early amniocentesis group.[6,7] An unanticipated observation was a 1.3% incidence of club feet when early amniocentesis was performed between 11 and 13 weeks' gestation, compared with 0.1% after midtrimester amniocentesis.

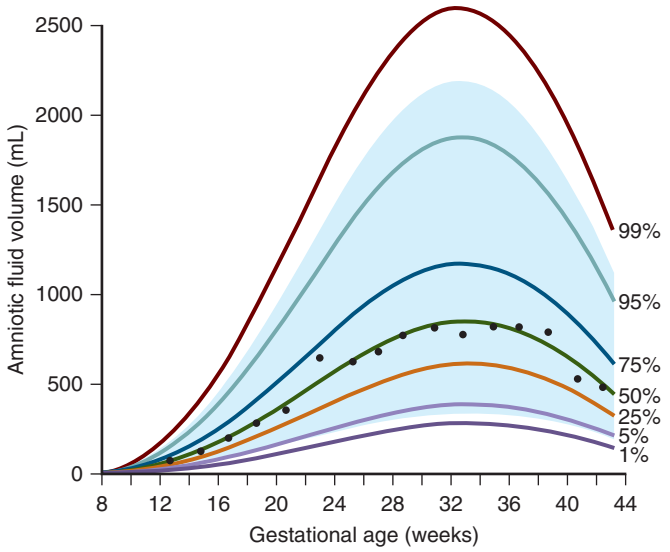
The timing of second trimester amniocentesis is based on a number of factors. Midtrimester amniocentesis is usually performed after 15 to 16 weeks of pregnancy because the uterus prior to this gestational age is still within the maternal pelvis and not easily accessible (Fig. 26.1). At midtrimester, the volume of amniotic fluid is about 150 to 300 mL, with the fetal kidneys beginning to produce urine (Fig. 26.2). There is uncertainty about the rate of fetal urine production in early pregnancy, but at 25 weeks' gestation, the fetal urine output is estimated to be about 110 mL/kg/24 h. For fetal karyotyping and genomic studies, approximately 20 to 40 ml of amniotic fluid is necessary to obtain an adequate number of amniocytes for culture in order to provide results with appropriate accuracy and confidence. While midtrimester amniocentesis has not been associated with permanent structural or functional consequences to the exposed fetuses, the association of early amniocentesis with a 10-fold increase in club feet demonstrates the importance of an adequate amount of amniotic fluid at this developmental stage for normal orthopedic development of the fetus. Midtrimester amniocentesis performed later, at 18 to 22 weeks' gestation, is technically easier, and there is less concern for removing 40 ml of amniotic fluid. However, cases requiring complex molecular testing may involve weeks of analysis, thus extending completion of testing late in pregnancy and potentially limiting management options.

Chorionic Villus Sampling

Chorionic villus sampling (CVS) involves the aspiration of the chorion frondosum either transabdominally or transcervically between 10 and 13 weeks' gestation (Fig. 26.3). Trophoblasts and mesenchymal core cells of the chorionic villi provide actively growing cells for karyotype and genomic analyses and biochemical/enzymatic studies. In contrast to second trimester amniocentesis, mosaicism (the finding of two or more cell lines with a different chromosome constitution—usually trisomy) occurs in about 1% to 2% of cases.[8] Because the cell types represent both extraembryonic and embryonic tissue, resolution of a mosaic CVS result requires identification of the source of the cytogenomic abnormality by completing an amniocentesis and, in some cases, fetal blood sampling for further clarification. As a result of CVS, the developmental processes of *confined placental mosaicism, trisomic rescue*, and *uniparental disomy* were discovered.[9] Depending on the placental cell lineage from which the chromosome abnormality was derived, confined placental mosaicism could result in (1) generalized mosaicism affecting both the placenta and fetus; (2) mosaicism in the placenta only and a diploid/chromosomally normal fetus; (3) chromosome abnormality confined to the placenta with a chromosomally normal fetus; or (4) chromosomally normal placenta and a mosaic fetus. Trisomic rescue refers to the process by which the zygote began as a trisomic conceptus and, through postzygotic loss of the extra chromosome, became diploid while the placenta remained mosaic or completely abnormal. The clinical consequence of trisomic rescue is chromosome dependent because some genes require the presence of both maternal and paternal copies to express a normal phenotype. In the case of chromosome 15, for example, if CVS demonstrated mosaicism for



• **Fig. 26.1** Midtrimester genetic amniocentesis under ultrasound guidance.

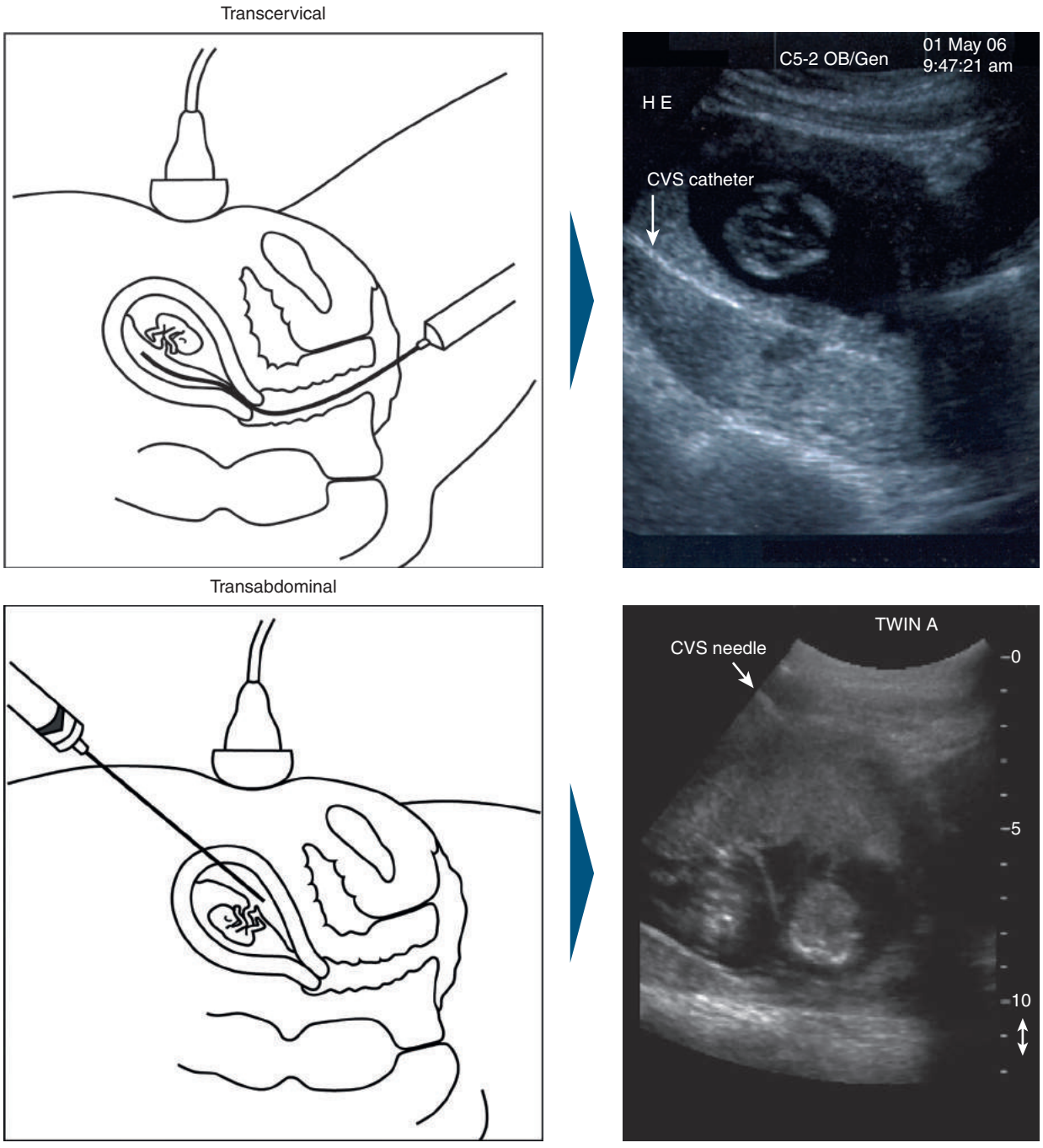


• **Fig. 26.2** Volume of amniotic fluid across fetal gestation. (Adapted from Bruce RA, Wolf EF. Normal amniotic fluid volume changes throughout pregnancy. *Am J Obstet Gynecol.* 1989;161:382–388.)

trisomy 15 and genetic amniocentesis demonstrated that the fetus was diploid for chromosome 15, studies to confirm that biparental inheritance must be completed to predict a normal fetal phenotype. However, if the fetus contained two maternal copies of chromosome 15, it would be predicted to have Prader–Willi syndrome, or Angelman syndrome if it had two paternal copies of chromosome 15.

CVS is associated with a pregnancy loss rate of about 1/500.[5] It requires operator expertise and continuous ultrasound guidance. Maternal cell contamination studies are completed to discriminate between female fetal results and contamination from maternal cells. CVS allows for early diagnosis at a time when the privacy of the pregnancy can still be maintained and should be considered if complex diagnostic strategies (requiring time) are anticipated. Because CVS is dependent on operator expertise within a small gestational age window, it is not readily accessible to all patients.

Characteristics of midtrimester amniocentesis and CVS are compared in Table 26.1.



• **Fig. 26.3** Transcervical and transabdominal chorionic villus sampling under ultrasound guidance. *CVS*, Chorionic villus sampling.

Percutaneous Umbilical Cord Blood Sampling

Fetal cordocentesis or percutaneous umbilical cord blood sampling (PUBS) was introduced in 1985.[10] The ease of DNA-based testing for genetic conditions has essentially replaced PUBS as a diagnostic tool in prenatal diagnosis. Today, the sampling of fetal blood is most commonly used for the diagnosis of fetal anemia or thrombocytopenia. However, there remains rare chromosomal abnormalities identified from CVS or midtrimester amniocentesis in which PUBS is necessary to clarify the true fetal chromosome abnormality. As a therapeutic tool, it is used for *in utero* transfusion of blood or platelets and, rarely, for administration of antiarrhythmic medications for the treatment of fetal tachyarrhythmias. The procedure is completed under continuous ultrasound guidance with a 22-gauge spinal needle placed into the umbilical vein and can be performed beginning as early as 18 weeks' gestation and subsequently throughout the remainder of the pregnancy. Before 18 weeks' gestation, the fetal umbilical vein may be too small, although our group has completed a successful transfusion of a 16-week hydropic fetus due to Kell isoimmunization; in this type of urgent situation, direct transfusion into the fetal heart is also possible. Exsanguination (if the cord is lacerated), periumbilical vein hematoma in Wharton jelly, preterm rupture of membranes, preterm labor, or placental abruption

TABLE 26.1 Characteristics of Midtrimester Amniocentesis and Chorionic Villus Sampling for Prenatal Diagnosis

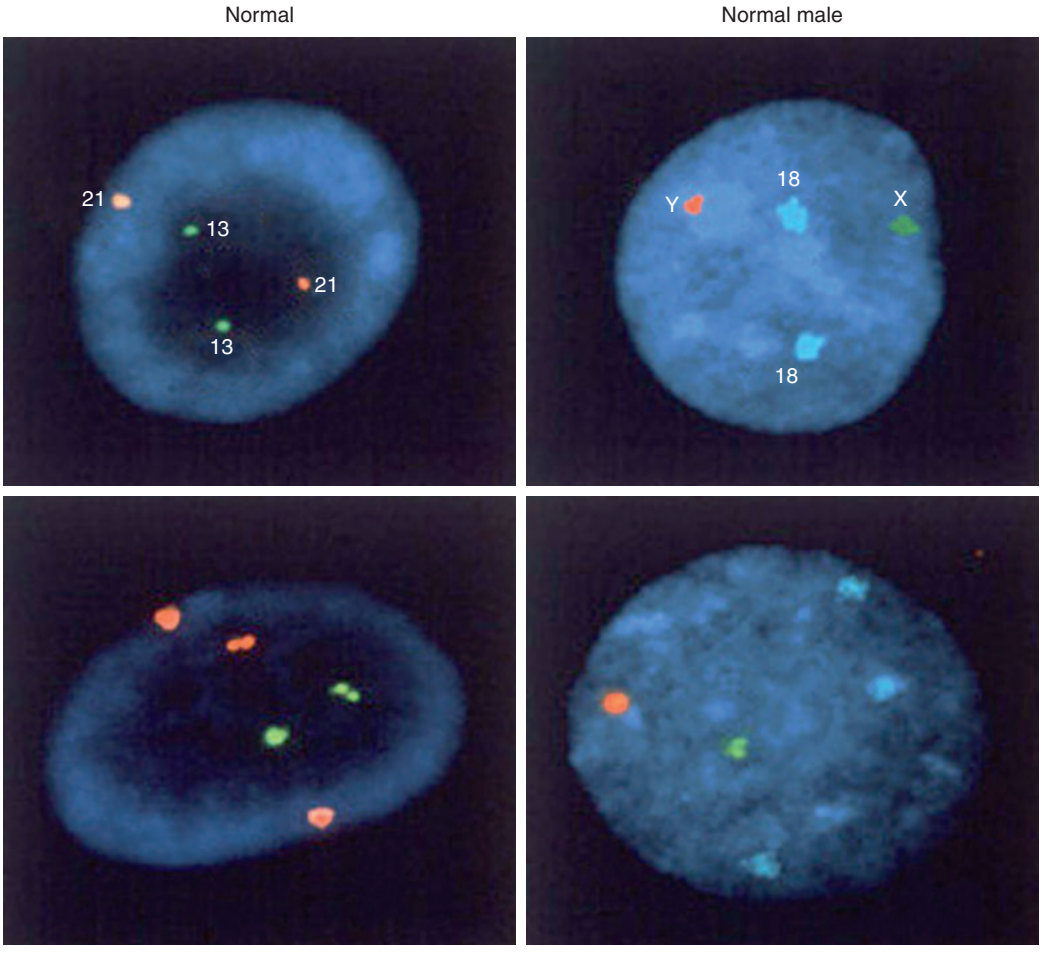
	CVS	Amniocentesis
Gestational age at procedure	10–13 weeks	15–20 weeks
Miscarriage rate	1/500	1/500–1/800
Culture mosaicism	1%–2%	0.1%
Turnaround time	7–10 days	7–14 days
Diagnostic accuracy	>99%	>99%
IFISH	+	+
Karyotype	+	+
Microarray	+	+
Neural tube defect screening*	–	+

*Women who undergo CVS will need to have midtrimester maternal serum screening for maternal serum alpha fetoprotein.
CVS, Chorionic villus sampling; *IFISH*, interphase fluorescent in situ hybridization.

are some of the complications of PUBS. The procedure requires operator expertise and the risk of the procedure is about 1%.

Genetic Testing of the Fetus

Fetal karyotyping still has a role in prenatal diagnosis and can be completed on chorionic villi, amniocytes, and fetal blood. Interphase fluorescent in situ hybridization (IFISH) with small chromosome-specific DNA probes (10 to 300 kb) is used for rapid confirmation of suspected aneuploidy in uncultured amniocytes (Fig. 26.4). Historically, IFISH has been used for identification of extra chromosome material and specific submicroscopic gene deletions associated with genetic disorders. Today, this cytogenetic tool has been largely replaced by microarray analyses that expose genomic deficiencies and duplications that are smaller than those previously identified by IFISH. IFISH to exclude trisomy 21, 18, or 13 on cord blood at delivery may be helpful when rapid (24-hour) turnaround may be used to assist in decision making, such as whether to offer life-sustaining neonatal surgery. In cases in which a genetic condition is not associated with a known mutation, biochemical and enzymatic/functional studies of cultured amniocytes may be the only means for prenatal diagnosis for the condition in question.



• **Fig. 26.4** Interphase fluorescent in situ hybridization on uncultured amniocytes. The hybridization process takes approximately 24 hours after which the signals are counted for diagnosis.

Microarray Technology

Chromosomal microarray (CMA) technologies are bridges between cytogenetics and molecular genetics. They have greater resolution than traditional cytogenetics and are platforms designed to measure DNA regions for gains or losses across the entire genome simultaneously. The utility of microarray technology has been thoroughly evaluated in the postnatal and adult population, and now replaces the standard banded karyotype that geneticists relied on since the 1970s. CMA is now the first-line diagnostic test for individuals with autism spectrum disorder and unexplained birth defects and/or cognitive delay.[11] Compared with standard karyotyping, CMA detects a causative genomic imbalance in an additional 10% to 15% of these patients. In 2013, The American College of Obstetricians and Gynecologists and the American College of Medical Genetics independently supported the use of CMA analysis in prenatal diagnosis which is reaffirmed in the most recent ACOG/SMFM practice bulletin.[12–14] In a large multicentered trial supported by the Eunice Kennedy Shriver National Institute of Child Health and Human Development, CMA was found to perform just as well as standard karyotyping in the diagnosis of common aneuploidies in fetal samples (from CVS or amniocentesis) from women undergoing prenatal diagnosis.[15] In pregnancies with normal ultrasounds that had been referred for standard indications, such as advanced maternal age or positive screening for DS, CMA yielded clinically relevant information in an additional 1.7% of pregnancies. However, in pregnancies with abnormal ultrasounds and a normal karyotype, CMA identified clinically significant genomic changes in an additional 6% of pregnancies. The value of microarray analysis is also seen in the genetic evaluation of stillbirths. Reddy and colleagues found that microarray analysis yielded more relevant genetic information than karyotyping in term stillbirths with or without anomalies, even after correcting for aneuploidy.[16]

Although there is no consensus regarding the type of array platforms used in prenatal diagnosis, *high-density single nucleotide polymorphism (SNP) array* is now the platform of choice, as they allow for greater in-depth coverage of the genome to reveal smaller duplications and deficiencies. In addition, SNP arrays have the advantage of detecting long continuous stretches of DNA homozygosity (identical DNA sequences between a pair of chromosomes). These findings could reveal uniparental disomy, as seen in rare genetic conditions such as Angelman syndrome, as part of the work-up for confined placental mosaicism or could uncover consanguinity.

Because the mechanisms causing genetic conditions are diverse and complex, no microarray platform will be completely diagnostic. Genetic conditions not associated with a relative change in DNA sequences will not be identified. Depending on the platform used, low level mosaicism and genomic regions not represented by the platform may not be detected. Other mechanisms of genetic disease such as point mutations, methylation disorders, and mitochondrial disorders cannot be uncovered using microarray technology. In addition, as the resolution of the technology improves, secondary and incidental findings, both with and without known clinical implications, will arise as the genome is searched. Copy number variants not previously reported in established databases are referred to as *variations of unknown significance*. For these reasons, women should have pretest and posttest genetic counseling to discuss expectations and limitations of microarray testing in their pregnancy.

Whole Exome Sequencing

Whole exome sequencing (WES) focuses on targeted sequencing of the protein coding regions of the genomic DNA and shows promise as a new tool in gene discovery for complex diseases and for facilitating the accurate diagnosis of individuals with unsolved Mendelian conditions. Reports of increased diagnostic yield in children with unexplained but presumed underlying genetic syndrome after normal karyotype and CMA suggest that WES could be applied to the prenatal population of fetuses with unexplained complex malformations or stillbirths with anomalies.[17–21] One recent prospective cohort study of 234 pregnancies demonstrated an additional 10% yield for pathogenic variants in fetuses with abnormal ultrasounds and normal CMA.[22] A second study of 127 cases of unexplained Non-Immune Hydrops Fetalis identified a diagnostic genetic variant in 29% of cases.[23] More recent attention has been directed to rapid exome sequencing (rES) with turnaround times of 24 to 36 hours, which could provide valuable information for providers in the postnatal management of neonates with serious and/or life-threating conditions.[24] In spite of these exciting developments and increasing utilization of WES, this technology (at the time of this writing) remains a second-tier diagnostic test.

Noninvasive Prenatal Screening

Maternal Serum Screening

Second trimester maternal serum screening for abnormal fetal conditions in the 1970s preceded ultrasound and focused on identifying fetal open NTD through elevated MSAFP. An elevated MSAFP level of 2.5 multiples of the median (MoM) or greater generally signals a pregnancy that should undergo further evaluation. Because AFP is a circulating protein in the fetus, any disruption in the integrity of the fetal body, such as an open NTD, gastroschisis, or omphalocele and some dermatologic disorders, will be associated with elevations of AFP in maternal serum and amniotic fluid. Other considerations for elevated MSAFP are incorrect dating of the pregnancy, multiple gestation, impending fetal demise, and history of vaginal bleeding.

In 1984, screening for DS and trisomy 18, because of low MSAFP, was added.[25] Other analytes—human chorionic gonadotropin (hCG), unconjugated estriol (UE3), and inhibin-A (INH)—were subsequently added to improve the detection rate for DS. Analyte levels are reported in MoM to standardize the gestational age dependency of the interpretation of the levels.

In the first trimester, three markers—fetal nuchal thickness (NT) measured by ultrasound, maternal serum pregnancy-associated plasma protein-A (PAPP-A), and hCG—can be used to calculate a patient's risk for DS. The combination of all the components of the first and second trimester screening elements, known as the Integrated Screen, provides the highest detection rate for DS when a 5% screen positive rate is used.[26] However, many options for screening are available depending on the gestational age at entry to prenatal care, access to first trimester ultrasound for NT evaluation, and patient desire for information at different stages of her pregnancy.[14] The benefit of second trimester maternal serum screening is that it provides risks for three fetal conditions: DS, trisomy 18, and open NTD.

Cell-Free DNA (Noninvasive Prenatal Screening—NIPS)

cfDNA analysis in maternal plasma is used in noninvasive prenatal screening (NIPS) with greatest focus on prenatal detection of aneuploidy for chromosomes 13, 18, 21, and X. In pregnancy, approximately 3% to 20% of the total cfDNA in maternal plasma is derived from the pregnancy.[27,28] Although many refer to this as "free fetal DNA," the DNA is in fact derived *primarily from the placenta*, which serves as a surrogate for the fetus and is cleared from the maternal circulation within hours after delivery.[28,29] The molecular principle behind NIPS is the measurement of relative amounts of circulating free placental DNA compared with the amount of circulating free maternal DNA. In other words, a pregnancy at 14 weeks affected with DS will show an excess of DNA fragments for chromosome 21, assuming that the mother does not herself have DS or is not mosaic for DS. *cfDNA* is present throughout pregnancy, and unlike maternal serum screening, which is gestational age dependent, *cfDNA* can be used for aneuploidy screening into later gestational ages.

As in any screening program, the positive predictive value (PPV) of a test is dependent on the population prevalence of the disease. The performance of NIPS is highest in women age 40 and older, with a PPV of 91% to 99% for DS and lowest at 38% to 80% in women age 20.[15] The test performance for sex chromosome aneuploidy is low, with a PPV of only 39%, because the sex chromosome aneuploidy may reflect the mother rather than the fetus.[29] Sex determination of the fetus can be misleading if the mother is a transplant organ recipient.[30] False-positive and false-negative results may be related to fetal mosaicism, vanishing twin, confined placental mosaicism, unsuspected maternal chromosome abnormality, or unsuspected maternal malignancy.[31] For these reasons, the American College of Obstetricians and Gynecologists and the Society for Maternal–Fetal Medicine strongly recommend that NIPS should "be an informed patient choice after pretest counseling and should not be part of routine prenatal laboratory assessment." They further recommend that "a patient with a positive test result should be referred for genetic counseling and should be offered invasive prenatal diagnosis for confirmation of test results."[20]

Other considerations in the performance of cfDNA screening include its dependency on gestational age and "fetal fraction." Low fetal fraction (defined as less than 4% fetal DNA in maternal serum) is inversely associated with maternal body mass index. Early gestational age (less than 9–10 weeks) and concurrent maternal treatment with low molecular weight heparin have been associated with inadequate DNA, leading to "test failures" or "no call report."[32] The clinical significance of "test failure" cases is now becoming evident and continues to evolve. Low-risk women with no reportable results on cfDNA testing were found to have an aneuploidy rate of 2.7% (1/38) compared to low-risk women with adequate cfDNA fraction (1/236, 0.4%).[32] Another important consideration about current NIPS is that it offers reliable information about the three most common aneuploidies. In a recent review of 220 chromosome abnormalities identified prenatally through amniocentesis between 2009 and 2014, cfDNA testing did not detect about half of the clinically significant chromosome abnormalities that were found either by karyotyping or microarray when amniocentesis was performed.[33] More importantly, 79% of the abnormalities were from pregnancies with abnormal serum screening and/or abnormal ultrasound findings. These findings underscore the fact that current cfDNA technology is still a screening tool and does not yet have the breadth and depth to offer the scope of genomic information about the fetus with multiple complex anomalies that could be obtained through CVS or genetic amniocentesis. Although some companies are offering expanded testing for selected microdeletion syndromes, these tests have not been validated clinically and should not be chosen for diagnosis of a fetus with multiple anomalies; direct testing by CVS or amniocentesis remains the gold standard at this time.

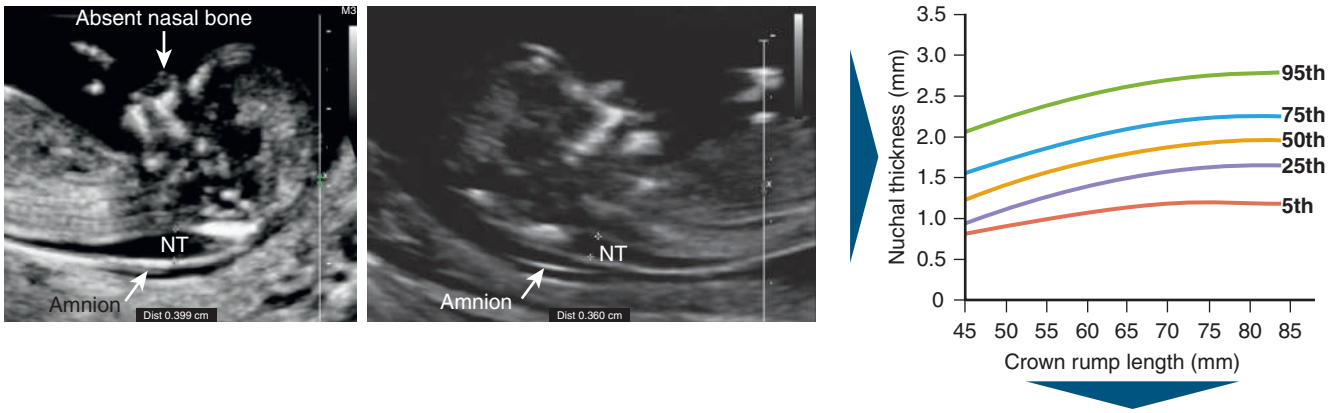
NIPS for twin gestations for aneuploidy screening is currently available.[34] However, the test does not identify the at-risk twin (unless there are ultrasound markers) or confirm the concordance of the fetal sexes, that is, the finding of Y chromosome material can indicate that both twins are male, or, at least one of the twins is male, unless there is ultrasound diagnosis of the fetal sexes. A positive result, even if there are ultrasound markers, should be confirmed by CVS or amniocentesis. Fetal demise and vanishing twin can produce inaccurate results. Therefore pregnancies that have undergone early fetal reduction should not use cfDNA as a screening tool for the remaining fetus(es).

Prenatal Fetal Imaging

First trimester nuchal translucency (NT) or *thickness* refers to the measurement of the normal subcutaneous fluid-filled space at the back of the fetal neck between 10 and 14 weeks' gestation. In normal fetuses, the maximal thickness increases with increasing gestational age as defined by the crown rump length (CRL). An increased NT is associated with DS, and is used in conjunction with maternal age and first and second trimester maternal serum analytes to provide the highest detection rate for DS.[14] Other chromosome abnormalities, fetal anomalies, and poor pregnancy outcomes are also observed with increasing NT (Fig. 26.5).

NT measurement is obtained by transabdominal imaging of the pregnancy, and its success as a screening tool depends on the accurate procurement of images for measurement, which is dependent on the size and position of the fetus, maternal habitus, and operator performance. Operator performance is audited for quality following formal training and certification of competency. The highest success rates in obtaining NT measurements are when the CRL is between 45 and 84 mm, corresponding to 11 weeks 3 days and 14 weeks 2 days of gestation, respectively.[35] The additional benefit of completing the ultrasound in this gestational age window is the opportunity to confirm pregnancy viability and identify other fetal anomalies such as anencephaly, abdominal wall defects, and possibly congenital heart defects.

Other ultrasound findings in the first trimester fetus that may be associated with DS include *absent* or *hypoplastic nasal bone, abnormal ductus venosus blood flow*, and *tricuspid regurgitation*. Initially thought promising, their performance may not be applicable to a general low risk population since their performance was judged on high-risk fetuses and the ultrasound acquisition required experienced sonographers. The scope of information regarding *second trimester ultrasonography for aneuploidy screening and diagnosis of fetal anomalies* is too broad and complex to cover in this chapter. In the United States, prenatal diagnosis units follow the practice guidelines developed in conjunction with the American College of Radiology, the American College of Obstetricians and Gynecologists, and the Society of Radiologists in Ultrasound.[36] The following discussion summarizes some key concepts in prenatal diagnosis and second trimester imaging of the fetus.



Nuchal thickness	Chromosomal defects	Normal karyotype		
		Fetal death	Major fetal abnormalities	Alive and well
<95th %ile	0.2%	1.3%	1.0%	97%
95th–99th %iles	3.7%	1.3%	2.5%	93%
3.5–4.4 mm	21.1%	2.7%	10.0%	70%
4.5–5.4 mm	33.3%	3.4%	18.5%	50%
5.5–6.4 mm	50.5%	10.1%	24.2%	30%
>6.5 mm	64.5%	19.0%	46.2%	15%

• **Fig. 26.5** Interpretation of nuchal thickness and relationship to gestational age and risk for fetal outcomes. *NT, Nuchal thickness*. (Adapted from Souka AP, Von Kaisenberg CS, Hyett JA, Sonek JD, Nicolaides KH. Increased nuchal thickness with normal karyotype. *Am J Obstet Gynecol.* 2005;192:1005–1021.)

In addition to an increased first trimester NT, DS fetuses can exhibit other abnormalities in the second trimester that would adjust the patient's *a priori* risk. Table 26.2 provides an overview of the common ultrasound markers and their likelihood ratios. Note that some of these markers signal other pathologic processes. Therefore it is important to consider the entire breadth of diagnoses for some of these markers. Calculation of DS adjusted risk, based on markers, can be performed at http://perinatology.com/calculators2.htm (Age-Adjusted Ultrasound Risk Assessment).

Accurate identification of *fetal NTD* can be readily accomplished in the second trimester ultrasound. This is especially important for maximizing management options for the pregnancy in light of the availability of in utero fetal surgery for repair of the defect.[37] The "lemon" sign representing scalloping of the frontal bones and the "banana" sign representing obliteration of the cisterna magna with distortion of the cerebellum due to herniation of the hindbrain (Chiari malformation) are the two most sensitive and specific findings for an open NTD. Observation of these cranial defects should initiate a detailed survey of the fetal spine. Mild ventriculomegaly in the second trimester is observed in only 70% of cases. The location and extent of the defect are important for anticipation of postnatal bowel and bladder function, ambulation, neurocognitive development, and consideration for *in utero* fetal surgery. Amniocentesis is recommended to confirm an open defect by demonstrating elevated amniotic fluid AFP levels and presence of acetylcholinesterase and to exclude fetal genomic abnormalities, which are found in 10% of fetuses with an open NTD.[38]

TABLE 26.2 Ultrasound Markers Associated With Aneuploidy and Down Syndrome

Marker	Likelihood Ratio
Cystic hygroma (first trimester)	>50% aneuploid, Turner syndrome
Isolated echogenic intracardiac focus (second trimester)	1.4–1.8 for DS
Renal pelviectasis ≥4 mm (20 weeks' gestation)	1.5–1.6 for DS
Echogenic bowel*	5.5–6.7 for DS
Ventriculomegaly (10–15 mm)	25 for DS
Isolated choroid plexus cyst(s) (≤20 weeks' gestation)	No association
Short femur length (<2.5%ile for gestational age)	1.1–2.2 for DS

*Grade 3 = density same as bone.
DS, Down syndrome.

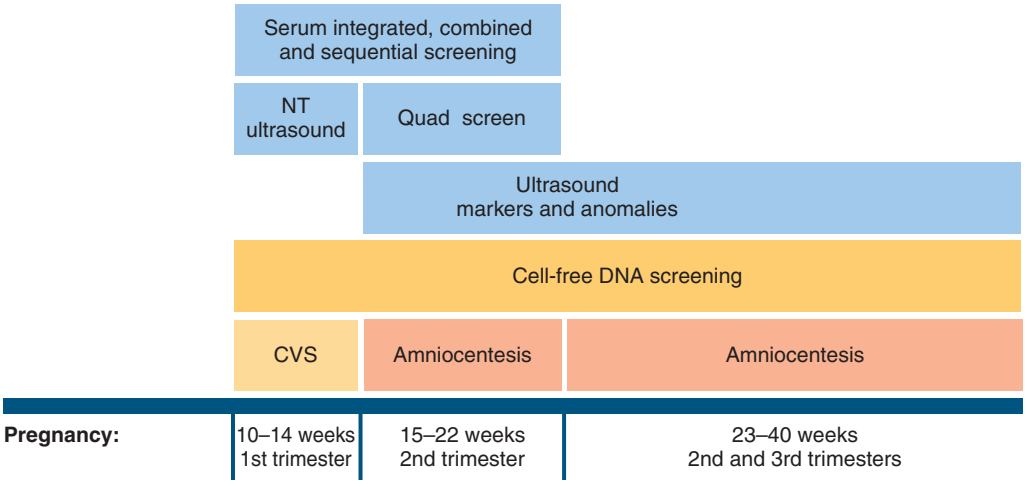
Eligibility for fetal surgery includes a chromosomally normal fetus diagnosed between 19 weeks 0 days and 25 weeks 6 days with an isolated myelomeningocele between T1 and S1, with evidence of hindbrain herniation in a nonobese (body mass index <35) healthy woman with no history or risk factor for preterm delivery. Among children who underwent in utero surgery for repair of spina bifida between 19 and 25 weeks'

gestation under the Management of Myelomeningocele Study (MOMs trial), there was a 50% reduction in the need for a ventricular shunt, less Chiari malformation, and improved motor skills, with twice as many children walking independently at 30 months compared with the postnatal surgery group.[39] First and second trimester ultrasounds readily diagnose *anencephaly, fetal gastroschisis*, and *fetal omphalocele*. The gestational age at which nuchal thickness (NT) screening is performed is especially efficient in identifying these conditions. Early diagnosis of fetal anencephaly maximizes management options and decreases maternal complications. Large gastroschisis is readily identified during the evaluation of the NT. Fetal omphaloceles may be ambiguous in the first trimester because of the physiologic herniation of the midgut into the base of the umbilical cord before 12 weeks' gestation. After 12 weeks, the gut returns to the abdominal cavity; persistence of a midline abdominal wall saccular defect with the umbilical cord arising from the apex of the sac and abdominal contents in the sac is diagnostic of an omphalocele. In contrast, gastroschisis is typically paraumbilical to the right of the cord insertion with free floating eviscerated bowel in the amniotic fluid. It is important to differentiate between these two anatomic defects; gastroschisis is rarely associated with structural or chromosome abnormalities. However, this condition is associated with a high rate of intrauterine growth restriction, oligohydramnios, and intrauterine fetal demise; prenatal care pathways for surveillance may be helpful in optimizing the fetus for postnatal interventions. In contrast, omphalocele is strongly associated with other pathologic processes, including other structural anomalies and genomic or syndromic conditions. Amniocentesis is recommended to exclude an underlying genomic imbalance that could impact postnatal interventions and prognosis. Fetuses with gastroschisis tolerate labor and vaginal delivery well, while fetuses with large omphaloceles are delivered by cesarean section because of the risk of rupture during vaginal delivery.

Prenatal skeletal dysplasia should be suspected when the long bone measurements are at or less than the 5th percentile or more than three standard deviations below the mean for gestational age.[40] Concurrent abnormalities such as fractures (osteogenesis imperfecta types II–IV), shape of the skull (thanatophoric dwarf), or decreased mineralization (hypophosphatasia) may direct the diagnosis. However, the diagnosis of most nonlethal congenital skeletal dysplasias will not be made until after birth or even into adulthood. Thus prenatal diagnosis of skeletal dysplasia raises the possibility of lethal pulmonary hypoplasia, which directs the prenatal and postnatal management of the pregnancy.

To date, two-dimensional ultrasound remains the standard imaging modality for prenatal screening and diagnosis. Three-dimensional ultrasound is helpful in visualizing any external structural abnormalities such as cleft lip/palate and spina bifida. The use of fetal magnetic resonance imaging (MRI) is increasing and is most helpful in evaluating fetal intracranial abnormalities suspected on ultrasound. Fetal MRI is noninvasive, does not involve ionizing radiation, and has no known short- or long-term effects on fetus or mother. The main drawback is fetal motion, but the development of fast MRI sequences has significantly reduced motion artifact. A recent review[41] provides a practical approach to fetal brain MRI performance and interpretation. Both fetal and postnatal brain MRI may play important roles in the diagnosis and management of patients with CNS anomalies, and together can be complementary in treatment planning.[42] Fetal MRI may be valuable in the prenatal diagnosis of fetal neck, chest, and abdominal malformations.[43] It is useful in estimating the degree of pulmonary hypoplasia in fetuses with congenital diaphragmatic hernia and predicting their need for ECMO and likelihood of survival.[44]

In summary, noninvasive prenatal screening has expanded to cover the entire course of pregnancy, allowing directed diagnosis of several common chromosomal conditions without invasive testing (Fig. 26.6). In the United States, ACOG recommends that all women, regardless of maternal age or history, should be offered prenatal screening (serum screening with or without NT screening, cfDNA, 2nd trimester ultrasound, 2nd trimester serum screening, CVS, or amniocentesis).[14] The next frontier in genomic medicine and prenatal diagnosis is clinical application of noninvasive sequencing of the entire prenatal genome for prenatal diagnosis which is beyond the scope of this chapter. This is a rapidly expanding area of development in diagnostic platforms, improving precision, and turn around time with the intent for early diagnosis to optimize choices and possible prenatal treatment; these advances will undoubtedly add to our understanding of genomic regulation of normal and abnormal developmental processes, but like cfDNA, will uncover information for which we may not yet have experience for interpretation.[45–49]



• **Fig. 26.6** Prenatal screening and testing options during pregnancy. *CVS*, Chorionic villus sampling; *NT*, nuchal thickness.

Preimplantation Genetic Diagnosis/ Screening

Preimplantation Genetics

Preimplantation genetic testing (PGT) provides genetic and/or aneuploidy information about the embryo, requires in vitro fertilization (IVF) technology, and can be considered a form of prenatal diagnosis. PGT-A refers to preimplantation testing for aneuploidy to identify euploid embryos for transfer. PGT-M refers to preimplantation genetic testing of embryos for single gene mendelian disorders, and PGT-SR refers to testing of embryos at risk for chromosomal structural rearrangements. Knowledge of the genetic mechanisms and risks for fetal anomalies as a function of the IVF and PGT processes is important as these women undergo prenatal screening with ultrasound, maternal serum screening, or NIPS. For the neonatologist, this background information may influence, for example, the suspicion that a newborn may have Beckwith–Wiedemann syndrome, since this syndrome is associated with pregnancies achieved through IVF technologies. Women of advanced maternal age who achieve pregnancies using their own eggs often undergo PGT-A of the embryos for aneuploidy. Confirmatory testing by CVS or amniocentesis is recommended, but few women elect to undergo an invasive procedure that risks a much-desired pregnancy. NIPS offers these women an intermediate solution for "confirmation" of a euploid fetus. However, knowledge of the number of embryos transferred and concordance with the number of viable fetuses seen on ultrasound are important if NIPS is used; an embryonic demise of a transferred twin could result in discordant NIPS results. Unique to IVF pregnancies, regardless of whether or not embryos have undergone PGT, is the concern for genetic conditions and/or birth defects as a result of imprinting errors or other mechanisms that may disrupt the normal developmental processes of the early embryo. Maternal serum screening for aneuploidy is not as robust in twin gestations as it is in singleton pregnancies. NIPS for twin gestation has now been accepted. ACOG recommends appropriate choice of patients and counseling before proceeding.[14] Definitive prenatal diagnosis of twins discordant for birth defects still requires CVS or amniocentesis.

Building a Prenatal Diagnosis Center

Whatever its size and scope, a prenatal diagnosis program can provide pregnant people and their partners with accurate information about fetal diagnosis and prognosis within the limits of their center's capacities and their team's expertise. The care team can be transparent about those limits and refer to other centers as appropriate. Only large prenatal centers should attempt fetal intervention, given the extraordinary resources of people and capital required[50] and the complexity of counseling and consent.[51]

The People

1. **Maternal Fetal Medicine:** Experienced and skilled MFM physicians determine whether an affected fetus may benefit from fetal intervention, extra fetal monitoring, or special services at or soon after delivery. They work closely with pediatric specialists who are experts in caring for patients with congenital defects. They are skilled in communicating difficult or serious news (see section on Prenatal Counseling) and provide information tailored to what the parents want and need to hear. They and their teammates can sensitively explore whether the parents wish to end the pregnancy if a life-threatening birth defect is detected, and then help arrange or refer as appropriate. In partnership with the referring provider, they ensure high-quality obstetrical care through the end of the pregnancy.
2. **Nursing:** One or more nurses or nurse practitioners assist and empower prenatal patients during the often long and complex journey until delivery. They are knowledgeable about the typical timing and urgency of visits, and which tests and specialists need to be involved and when. Ideally, they serve as a navigator throughout each clinic visit, participating in the preclinic huddle, the counseling, and the wrap-up for next steps. They can also make available teaching materials and videos of parents sharing their experiences with their children with similar fetal diagnosis. For an example of a video for parents, see https://www.seattlechildrens.org/conditions/gastroschisis/.
3. **Support staff:** One or more staff members can assist with the many nonclinical tasks such as scheduling complex clinic visits with multiple procedures and specialists, communicating with referring providers, maintaining a database, etc.
4. **Genetic counselor:** Given the ever-changing field of genetic testing and the complexity of prenatal counseling, it is very helpful to have a genetic counselor embedded in the prenatal clinic.
5. **Social worker:** Given the diverse life experiences and resources of prenatal clinic patients, and the complex housing and transportation needs of those who live remotely from the clinic, a social worker or other mental health professional, knowledgeable about the physical and psychological needs of pregnant women and their families, is essential. When appropriate, they can connect the patient with a parent who has gone through a similar experience or to an online support group.
6. **Neonatologists:** It is essential that the MFM have a close working relationship with neonatologists or neonatal advanced practice providers who can help develop postnatal plans for the baby and sometimes assist the family in making difficult choices (see section The Role of a Neonatologist in Prenatal Diagnosis Clinic).
7. **Pediatric and surgical specialists:** Based on volume, the pediatric specialists most likely to be needed, either in person or virtually, include fetal cardiologists, general surgeons, neurodevelopmental experts, nephrologists, urologists, skeletal dysplasia experts, cardiac surgeons, cardiac intensivists, otolaryngologists, and members of the single-ventricle cardiology team. Radiologists with experience in prenatal diagnosis by ultrasound and MRI are invaluable in many situations.
8. **Sonographers:** Fetal ultrasound and echocardiography require advanced training and skills. They work closely with MFM and prenatal cardiologists, respectively.
9. **Palliative care:** Whenever the postnatal journey will likely be difficult or long, prenatal consultation with palliative care provides an extra layer of support. This is true regardless of the newborn's prognosis for survival but is especially important if the parents are considering immediate comfort care after delivery for a life-threatening fetal condition.

The Infrastructure

The basic facilities required for a prenatal diagnosis center include rooms for ultrasound, echocardiography, and counseling. Ideally there will also be equipment for virtual interdisciplinary visits with

multiple specialists simultaneously. Procedures like amniocentesis, termination of pregnancy, and fetal intervention require a hospital partner with labor and delivery as well as NICU capabilities, and advanced genetic testing requires partnerships with hospital and private laboratories. A description of the requirements of a fetal intervention program is beyond the scope of this chapter.

In addition to close connection with a high-risk delivery hospital, prenatal diagnosis programs benefit from having one or more of the following:

1. Database for quality improvement and for reporting to outside agencies
2. Quality improvement programs that are connected to their home departments of obstetrics, neonatology, cardiology, surgery, and neurodevelopment, and their long-term follow-up programs
3. Research programs related to prenatal care and fetal outcomes
4. Telemedicine support for virtual counseling
5. Training and continuing education programs
6. Regular interdisciplinary conferences for management of challenging prenatal cases; perinatal genetics, imaging, and pathology conferences; mortality and morbidity review, and neonatal outcomes of recent deliveries.

A Practical Guide to Prenatal Counseling

Prepare for the encounter: Ideally each member of the prenatal clinic team will have advanced communication skills due to the challenges of delivering difficult or serious news to prenatal patients.[52] Many of these patients and their partners will be grieving over the loss of their dreams for a healthy baby and the normal life experiences of pregnancy and childbirth. For many, this grief will be compounded by guilt, anxiety over diagnostic and prognostic uncertainty, and/or ambivalence about the outcome. The requirement for a personalized antenatal counseling approach is well described by Haward et al.[53] and is highly recommended for those doing this challenging work.

Because of the complex and multidisciplinary nature of the issues, more than one provider will often be involved in counseling. For these reasons, a precounseling team meeting to discuss obstetrical and maternal issues, delivery planning, neonatal support, and psychosocial readiness of the patient and partner is critical to everyone's understanding of different perspectives. The team should review the most up-to-date information about the fetus and the parents' psychosocial readiness to hear the information, discuss the goals for the encounter, and agree on the headline—a one- or two-sentence summary of the problem that includes both information and meaning, such as *"we've discovered that your baby has small, underdeveloped lungs and we're worried that she may not live to go home from the hospital."* The team should also coordinate their counseling so that the encounter is seamless, with each specialty provider adding content that collectively will give the patient the whole picture of the pregnancy, labor and delivery, and postnatal care of the baby. See Box 26.1 for summary of the key steps.

Connection: Practices that foster connecting to patients include (1) preparing with intention, (2) listening intently and completely, (3) agreeing on what matters most, (4) connecting with the patient's story, and (5) exploring emotional cues.[54–56] Creating an inviting neutral environment is important in relationship development. Because clinic staff and nurses often have had preconsultation encounters with the patient, for example, scheduling appointments, instructions, and anticipatory explanations about the nature of the consults, they play a critical role in introducing and connecting the subspecialty team members to the patient. In our program, the nurse assigned to the patient will escort the patient into different rooms for ultrasound and echocardiography, and then participate in the counseling session with specialists. When beginning the session, each participant should introduce themselves and their role, and then ask the patient and partner *"What do you like to be called? What would you like us to know about you? How can we be most helpful to you today?"* This sends the message that the team is engaged in the entirety of their situation and not just the medical issues; it is important that the patient and partner understand that the prenatal team is respectful of the impact of the diagnosis on the baby, parents, and extended family.

The VitalTalk REMAP acronym, widely used by palliative care providers and communication experts,[57,58] is useful for structuring the counseling, especially if unwelcome and unexpected news will be shared and a life-altering decision must be made.

Reframe: Most patients referred to a prenatal diagnosis center have already been given news from their obstetrical provider that there is something seriously wrong with the pregnancy or baby. Many have already searched the internet and talked to family and friends which may lead to misunderstanding or incorrect assumptions. Thus, it is important to begin by assessing what the patient and partner know or think they know about their situation, and then reframe the discussion around what is known and what it means. Ask *"So that I know where to begin, could you tell me what you know so far about your situation?"* If they already understand the key information, skip to the Emotion step below. If not, ask permission with *"Would it be okay if we talk about what we've discovered so far and what is concerning to us?"* If yes, ask *"Is there anyone else you'd like to be here with you, or on the phone or videocall?"* Provide the headline as developed in the huddle, and then wait for a response which is usually one of emotion. If there is no visible emotional reaction, ask *"we just gave you some hard news—how are you doing with it?"*

Emotion: Effective headlines almost always lead to intense emotions like disappointment, sadness, anger, and fear. It is counterproductive to give more information without first allowing the patient and partner to process their emotional reaction to the headline. The VitalTalk NURSE acronym (www.vitaltalk.org) provides ways to respond empathetically, depending on the situation: Naming, Understanding, Respecting, Supporting, and Exploring statements. Examples of NURSE statements include, respectively, *"I can see that you're disappointed," "This news must be*

• BOX 26.1 Key Steps in Counseling

Prepare for the encounter: huddle, create headline with information and meaning.

Connect

REMAP

Reframe	Assess their understanding of the problem. Provide the headline and wait for emotional response.
Emotion	Respond empathetically with NURSE statements: Naming, Understanding, Respecting, Supporting, and Exploring.
Map out values	Determine what is important, concerns, tradeoffs.
Align	Show your work and confirm accuracy.
Plan	Ask permission to make a recommendation. Propose a plan. Check-in. Reassure.

*such a shock," "You've done everything you could do for your baby," "We'll be here for you," and "Could you tell me more about what you mean when you say***?"* Until emotions have been acknowledged and validated as normal with one or more of these statements, defer answering questions that appear to be requests for information but are actually emotional cues that need to be addressed before proceeding. When the fetal defects are complicated, the cumulative information from each subspecialist can be overwhelming. Pause after each NURSE statement to gauge its impact and check in periodically: *"We just said a lot about the heart defect—how are you doing with this information? Let us know when you are ready to talk about***."*

Map out values: Before reviewing options, it is critical to gather information about the parents' values and goals. Determine what is important by asking *"Knowing this news, what is important to you?" and "What do you think might be important to your baby?"* Ask *"what worries or concerns do you have*?" When appropriate, ask about tradeoffs: *"What abilities are so critical to your baby's life that you can't imagine him or her living without them?"* If they respond that they are hoping for a miracle, respond with "*we are hoping for a miracle with you."* After pausing, ask *"Would it be okay to talk about what would be important to you if the miracle we're hoping for doesn't happen?"* When they are ready for information, let the parents take the lead in asking questions, and then respond specifically, concisely, and compassionately—pausing as they absorb the news. Ask whether they are the kind of person who wants to hear all the details and statistics, or someone who wants just the big picture. Avoid cognitive overload. Stop to listen. Respond empathetically when new emotions arise. Allow room for silence—it is often followed by important reflections or insightful questions.

Align values with treatment options: Show your work and confirm its accuracy: "It sounds like*** is important to you, and that you're worried about***. Did I get that right? Is there anything that I missed?"

Plan: Ask permission: "Would it be okay if I made a recommendation based on the things you just told me?" If yes, propose a plan: "From what you've shared with me, and given where things are medically, I recommend***." Check-in: "How does that sound?" Reassure: "No matter what happens, we will be here to support you through each step of this journey." And then follow through on your commitment.

The Role of a Neonatologist in Prenatal Diagnosis Clinic

Depending on the size and complexity of the prenatal diagnosis program and the availability of other pediatric specialists, the role of the neonatologist[59] or neonatal advanced practice provider may include one or more of the following:

1. **What to expect at delivery:** Prospective parents who are expected to have anything other than a normal delivery and a neonatal course should meet with someone who can accurately describe the most likely events after birth and the spectrum of other possible outcomes. This may be provided by the prenatal clinic nurse or nurse practitioner if they have recent delivery room and NICU experience, or by the prenatal medical specialists like cardiologists and general surgeons. However, most prenatal clinic patients appreciate the opportunity to meet during the final weeks before delivery with a NICU provider who can represent the NICU team, review in detail the plans for their baby immediately after birth, answer questions, and when appropriate offer continuity of care of their baby after delivery. The parents may benefit from reassurance that the NICU team present at delivery will be knowledgeable about the birth plan, prepared to address every contingency after birth, and flexible in modifying the plan if required based on the parents' values and goals. Here are some suggestions for beginning this conversation:
   - "Hi, I'm***. I'm a neonatologist who works with the newborn intensive care team who will care for your baby after birth. I'm here today to review the plans for your baby and answer your questions—is that okay? Is there anything else you'd like to cover? So that I know where to begin, could you tell me what you already know about the plans for your baby after birth?"
   - After listening carefully without any interruption or corrections, ask "Would you like me to describe the most likely events for your baby after delivery? If so, do you want details, or the big picture?"
   - To help the NICU team understand the parents' preferences, especially since there may be unexpected problems after delivery, ask "What is most important to you after delivery of your baby?", "What else is important?", "What are you worried will happen after your baby is born?", and "What else are you worried about?".
2. **Interdisciplinary counseling with pediatric specialists:** When the NICU stay is likely to be complicated and prolonged, parents may find it helpful to meet early in pregnancy with the relevant specialists and a neonatologist at the same time because of their different areas of expertise and experience. It will be reassuring to the parents to see that the members of their team are all on the same page and that they communicate well with one another.
3. **Refine the details of the birth plan:** Although the general outline of the plan is often developed by the MFM and pediatric specialists, the neonatologist is especially qualified to give advice on location of delivery due to knowledge of the services the newborn may need and the abilities of NICUs in the region to provide them safely and effectively. Also, in collaboration with the other pediatric specialists, the neonatologist should have input into proposing the timing of transfer of the baby from delivery room to NICU or to another hospital for definitive treatment. This is because the specialists may underestimate the importance of keeping the mother and baby together during the first hour after birth for bonding and successful breast feeding if that can be done safely, or understand the level of monitoring available in different NICUs. When fetal imaging suggests that the baby's survival may depend on immediate surgical or catheter-directed intervention (e.g., obstructed total anomalous pulmonary venous return) and the delivery hospital does not provide those services, the neonatologist can help arrange for the transport team to be present at the delivery. Patients with surgical problems like congenital diaphragmatic hernia without severe pulmonary hypoplasia on prenatal imaging should generally be admitted to the delivery hospital's NICU for confirmation of good endotracheal tube placement if placed, insertion of umbilical catheters, and adjustment of respiratory support before transfer when stable to a surgical NICU. Newborns with known ductal-dependent heart defects can often have their transfer from the delivery hospital to a cardiac ICU deferred for several hours if started on alprostadil in the first 1 to 2 hours after birth and then carefully monitored

in the NICU while facilitating maternal bonding and breast feeding. Babies too premature or small for cardiac intervention may benefit from staying days or weeks in the delivery hospital's NICU, on alprostadil if ductal dependent, until larger and mature enough for intervention.

4. **Develop birth plan when survival is uncertain:** When survival without severe impairment is unlikely and yet the parents say that they want full intervention for their unborn baby, the neonatologist can represent the NICU team and its treatment philosophy during prenatal counseling. The critical elements of this counseling are described in the Practical Guide to Prenatal Counseling section of this chapter. Among the prenatal team members, the neonatologist may be best positioned to explain the degree of diagnostic and prognostic uncertainty, review the options of immediate comfort care versus transferring the baby elsewhere for intervention, and compassionately discussing the tradeoffs between efforts to prolong life and to prevent suffering. Input from experts in perinatal palliative care can be of great benefit before and after delivery in these situations.
5. **Undecided about whether to continue the pregnancy:** The MFM who has knowledge of methods of ending pregnancy and local legal restrictions is essential to these difficult conversations, but the parents' decision may hinge on the likelihood of survival and quality of life. When the fetus has a condition like hypoplastic left heart syndrome, the cardiologist is best equipped to answer the parents' questions. However, for conditions at risk for life-threatening respiratory problems (e.g., extreme prematurity, prolonged oligohydramnios, diaphragmatic hernia, skeletal dysplasia), the neonatologist can provide valuable information to the parents regarding the degree of uncertainty from prenatal testing and the range of possible outcomes.

Summary

This exciting period of discoveries and advancements in prenatal diagnosis offers women a broad choice of studies to investigate the health of the fetus. For couples at high risk for a known condition, the option of PGT or DNA-based prenatal diagnosis offers them hope and a chance for a healthy baby. For fetuses with life-threatening structural anomalies, such as hypoplastic left heart syndrome, prenatal diagnosis offers preparation for delivery at a high-risk center where neonatal support and subspecialty intervention offer the neonate a good chance for survival. In these efforts, we have educated our patients and ourselves, for in the last 40 years we have had a window to peek into the developmental processes of the human embryo through imaging and genomics. Now, we have the potential to understand the action of developmental genes over time in both normal and abnormal conditions, along the fetal–pediatric–adult continuum. And in these opportunities to improve survival of serious and complex conditions, parents are faced with difficult decisions. Centers that have the capabilities to offer intervention will need to be prepared to provide the complex counseling needed to support families through their journey.

Suggested Readings

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27

The Dysmorphic Infant

K. TAYLOR WILD, SARAH E. SHEPPARD, AND ELAINE H. ZACKAI

KEY POINTS

- A genetic diagnosis can direct medical care (treatment, screening for other anomalies or issues that will arise in the future), provide information about prognosis, and give a recurrence risk to families.
- A genetics evaluation should be considered for a patient in the setting of multiple anatomic anomalies, known maternal exposure to a teratogen, a history of familial disorders, increased carrier frequency or ethnic risk, consanguinity, or multiple pregnancy losses.
- The essential components of a genetic evaluation include the medical history, family history, dysmorphology examination, literature review, and diagnostic testing.
- Exome sequencing (ES) allows for a genetic diagnosis in about one-third of patients.[1]
- In cases in which there is no clear diagnosis, prognosis and treatment should be determined according to the organ systems involved and the extent of their impairment.

TABLE 27.1 Genetic Disorders in Pediatric Hospital Admissions

Genetic Disorders	Montreal (1973)	Seattle (1978)	Cleveland (2004)
Chromosome, single gene (%)	7.3	4.5	11
Polygenic (%)	29	49	60
Nongenetic disorders (%)	64	47	29
Total number of admissions	12,801	4115	5747

Genetic disorders have a major impact on public health, as indicated by several large epidemiologic studies (Table 27.1).[2–5] Genetic factors may also contribute to more than two-thirds of the conditions prompting admission to a children's hospital.[4] Early identification of the genetic nature of a given condition may then help to appropriately focus resources for providing better care to these individuals. It is therefore critical to implement a systematic approach to evaluating a newborn with dysmorphic features or congenital anomalies. This chapter outlines a general approach.

When and Why to Consider a Genetic Evaluation

A genetic evaluation can provide many benefits besides providing an underlying diagnosis. Management changes may occur in over half of patients.[1] Management changes may include screening for additional congenital anomalies, screening for hypertrophic cardiomyopathy or cancer, or changes to an inhibitor (in vascular anomalies). Importantly, genetic diagnosis can also give families a recurrence risk and give them the option for preimplantation genetic testing or other prenatal diagnostic options.

When should a genetics evaluation be considered? The following clinical situations prompt a further genetic evaluation and counseling by a specialist:

- Multiple anatomic anomalies
- History of maternal exposure to teratogens
- Familial disorders
- Increased carrier frequency or ethnic risk
- Consanguinity
- Multiple pregnancy losses

If a congenital anomaly is identified in the presenting patient or proband, especially if the defect is associated with other anatomic anomalies, short stature, or developmental delays, the features of a specific genetic syndrome may be present. A known history of maternal exposure to a potential teratogen would also be an indication for consultation. Conditions appearing to be familial, a family history of hereditary disorders involving malformation of a major organ, or major physical differences such as unusual body proportions, short stature, or irregular skin pigmentation would warrant genetic investigation. Intellectual disability, blindness, hearing loss, or neurologic deterioration in multiple family members suggests a genetic etiology. Likewise, a genetics evaluation is indicated for strong family history of cancer or a defined ethnic risk for specific disorders, such as the higher carrier frequency for Tay–Sachs disease in individuals with Ashkenazi Jewish heritage. The occurrence of multiple pregnancy losses would also raise the suspicion of a genetically influenced cause and indicate the need for further investigation and counseling.

Patterns of Anomalies

When performing the evaluation, it is important for the physician to consider if the anomaly is primary or secondary and how the multiple congenital anomalies may be associated with each other. The occurrence of malformations can fit into one of several

• BOX 27.1 Underlying Mechanisms of Malformation

Syndrome: Pathogenetically related pattern of anomalies
Sequence: Pattern of anomalies derived from a presumed or known previous anomaly or mechanical disturbance
Association: Nonrandom occurrence of multiple anomalies
Field Defect: Disturbance of a developmental field leading to a pattern of anomalies

• BOX 27.2 Processes Leading to Altered Form or Structure

Deformation: Abnormal form resulting from mechanical forces
Disruption: Morphologic defect caused by interference with a previously normal developmental process
Dysplasia: Altered morphology because of abnormal organization of cells into a given tissue

categories (Box 27.1). A *syndrome* is a "collection of anomalies involving more than one developmental region or organ system."[6] The word itself means a "running together" or "pattern of multiple anomalies thought to be pathogenically related." Therefore, a given congenital anomaly may be an isolated defect in an otherwise normal individual or part of a multiple malformation syndrome. In some individuals, a *sequence* may occur when the primary malformation itself can determine additional defects through an interrelated cascade of physical and functional processes. A classic example is the Pierre Robin sequence (PRS), consisting of a small, recessed jaw, midline U-shaped cleft palate, and relatively large and protruding tongue. The primary anomaly is the small jaw, which does not allow adequate room for the tongue and displaces it superiorly. The displaced tongue prevents closure of the palatine shelves, causing the cleft palate.

In addition, a cluster of several malformations that are not developmentally related can occur in a nonrandom fashion called an *association* that may appear without characteristic dysmorphic features. One such statistically nonrandom association of defects consists of *v*ertebral defects, *a*nal atresia, *c*ardiac defects, *t*racheoesophageal fistula, *e*sophageal atresia, *r*enal dysplasia, and *l*imb anomalies (VACTERL). It should be noted that not all features need to be present and that the extent of involvement of each system is widely variable. Although there have been various candidate genes and chromosomal regions described in some patients with VACTERL association, there is no common genetic etiology known at this time,[7] likely due to the heterogenous presentation of these patients. However, there are many overlapping genetic syndromes that need to be excluded before concluding that a patient has VACTERL association. Associations often manifest as sporadic rather than familial occurrences. Because they are not clearly related by a common etiology or pathogenesis, associations are not considered syndromes and do not technically constitute a diagnosis. Instead, they are a recognition of a statistically significant association of features. It is important to remember that many of these same anomalies can occur as features of chromosomal aneuploidy or other syndromes. Chromosomal aneuploidy refers to the presence of an abnormal number of chromosomes in a cell.

Syndromic malformations tend to occur in more than one developmental field. A *field defect* or complex is a set of primary malformations in a developmental field that originates from a single or primary abnormality in embryonic development (see Box 27.1).

When generating a differential diagnosis of malformations that might occur together, the evaluator must also consider structures that may appear abnormally formed but in fact are structures that underwent normal development and then received some insult that distorted their true form (Box 27.2). For example, a *deformation* describes the abnormal form, shape, or position of a part of the body that was caused by mechanical forces. Examples are clubfoot, hip dislocation, and craniofacial asymmetry; they can result from intrinsic (embryonic) or extrinsic (intrauterine) mechanical forces that alter the shape or position of an organ or part that had already undergone normal differentiation. Deformations are estimated to occur in 2% of births, and such factors as fetal crowding from the presence of multiple fetuses and uterine malformations, as well as oligohydramnios, and a face presentation during delivery can cause them.

TABLE 27.2 Examples of Morphologic Differences

Malformation	Cardiac septal defects Cleft lip
Deformation	Clubfoot
Disruption	Amniotic bands
Dysplasia	*Localized*: hemangioma *Generalized* (skeletal): achondroplasia

Along similar lines, a *disruption* describes a "morphologic defect of an organ, part of an organ, or larger region of the body resulting from the extrinsic breakdown of, or an interference with, an originally normal developmental process."[6] The classic example of a disruption is entanglement of the fetus in amniotic bands. Amniotic bands are ribbons of amnion that have ruptured *in utero* and cause disruptions of normal developmental processes in the fetus, either through physical blockage or interruption of the blood supply or by entangling and tearing of developing structures. This effect is seen most often with digits and limbs, and remnants of the bands, or constriction marks, can frequently be seen at birth. If the fetus should swallow a band, a cleft palate might result; this etiology is a very different etiology from that of cleft palate occurring as a primary malformation. Recurrence risk counseling of the parent would be very different in these two scenarios.

Dysplasias occur when there is "an abnormal organization of cells into tissue(s) and its morphologic results."[6] Dysplasia tends to be tissue-specific rather than organ-specific (e.g., skeletal dysplasia) and can be localized or generalized.

In summary, structural or morphologic changes identified at birth can occur during intrauterine development as a result of malformations, deformations, disruptions, or dysplasias. However, approximately 90% of deformations undergo spontaneous correction. Malformations and disruptions often require surgical intervention when possible. Dysplasias are typically not correctable, and the affected individual experiences the clinical effects of the underlying cell or tissue abnormality for life (Table 27.2).

Genetics Evaluation

The clinical geneticist incorporates the following five essential tools in the evaluation of a child suspected of having a primary genetic disorder:

- History: prenatal, birth, and medical
- Pedigree analysis and family history
- Specialized clinical evaluations: Physical examination and adjunct studies
- Literature review
- Specialized laboratory tests (e.g., karyotype, chromosomal microarray, sequencing)

History

Prenatal

A complete gestational history should be generated, including details of conception, prenatal testing, exposures, and pregnancy course. The maternal age at conception should be documented as the risk of chromosomal anomalies due to nondisjunction rises with maternal age. Paternal age should also be documented as there is increasing evidence that the risk of autism, certain types of developmental disorders, and forms of craniosynostosis, including Apert syndrome and Muenke syndrome, increases with paternal age.[8–10] Any use of assisted reproductive technology to aid in conception should also be noted as there is an increased risk of imprinting disorders such as Beckwith–Wiedemann syndrome (BWS), Prader–Willi syndrome, and Angelman syndrome in children born with the assistance of in vitro fertilization and intracytoplasmic sperm injection.[11] Results of prenatal testing may include carrier testing, maternal serum and/or noninvasive prenatal screening, ultrasonography and other imaging techniques such as fetal echocardiogram or magnetic resonance imaging (MRI) if applicable, and diagnostic genetic testing through chorionic villus sampling or amniocentesis (Box 27.3).

Noninvasive prenatal screening (NIPS) is a screening test for chromosomal aneuploidies that is obtained from cell-free DNA from the fetus that circulates in the maternal serum. It can be drawn from a peripheral blood draw as early as 10 to 11 weeks of gestation. NIPS was initially developed to screen for trisomy 13, 18, and 21 and has very high sensitivity, specificity, and positive predictive values for these disorders.[12–14] For trisomy 21, NIPS has a higher sensitivity, a lower false-positive rate, and a higher positive predictive value than standard screening with nuchal translucency measurement and biochemical analytes.[14] NIPS was subsequently expanded to screen for sex chromosome aneuploidy, but has a poorer detection rate and a higher false-positive rate for the sex chromosome aneuploidies, particularly for monosomy X.[15] Some companies have even expanded NIPS further to include five common microdeletion syndromes including 1p36, 4p (Wolf–Hirschhorn syndrome), 5p (cri-du-chat syndrome), 15q11-13 (Prader–Willi or Angelman syndrome), or 22q11.2 deletion syndrome. However, while NIPS will screen for these microdeletion disorders, the positive predictive value is low at 9.2%.[16] Furthermore, ACOG does not recommend NIPS screening for microdeletion syndromes and this is not performed at most centers.[17] It is important to note that NIPS is only a screening test and should be confirmed with diagnostic testing.

It is important to identify prenatal exposures. Prenatal exposures may include infection, medications, maternal habits such as alcohol and drug use, maternal chronic illnesses such as maternal diabetes or obesity, travel, and other *teratogens*. Teratogens are environmental agents that may cause structural and functional diseases in an exposed fetus. Each teratogen may have a characteristic expression pattern, with a specific range of associated structural anomalies and dysmorphic features. Specific effects and the extent of those effects depend on the time of exposure, duration, and dosage, as well as interactions with maternal and genetic susceptibility factors. In general, more severe effects are typically correlated with exposure early in the pregnancy and with more extensive (i.e., higher dose) exposure. The list of well-documented human teratogens is short and includes such substances as alcohol, thalidomide, warfarin, trimethadione, valproate, isotretinoin, angiotensin converting enzyme inhibitors, chemotherapeutics, lithium, amiodarone, azole fungicides, high-dose methotrexate, and hydantoin.[18] If history of an exposure is documented, an effort should be made to identify the developmental time and level of exposure. This information is critical, because the counseling and calculation of recurrence risk for a given malformation are vastly different if environmental exposures are involved.

Another important component of the gestational history is obtaining information on fetal activity, size, position, and amniotic fluid. Often, the mother's subjective impressions can be further confirmed by examining obstetric records of the prenatal period. A history of hypotonia may be further supplemented by reports of poor fetal movements and breech presentation and a history of seizures may be supplemented by frequent fetal hiccups. Oligohydramnios (decreased amniotic fluid volume) can be associated with either a fluid leak or a genitourinary abnormality, whereas polyhydramnios (excess amniotic fluid volume) can be seen in fetuses with neuromuscular disease or gastrointestinal malformations.

• BOX 27.3 Elements of Prenatal History for the Dysmorphic Newborn

Maternal Health
Age
Disease: diabetes, hypertension, obesity, seizure disorder

Mode of Conception
Natural
Assisted reproductive technologies
Fertility medications
In vitro fertilization
Intracytoplasmic sperm injection
Gamete intrafallopian transfer
Artificial insemination

Exposures
Medications
Alcohol
Environmental agents
Travel
Infections (gestational age at exposure)

Prenatal Testing
Ultrasonography (gestational age performed)
Advanced fetal imaging (fetal echocardiogram or MRI)
Maternal serum screening
Noninvasive prenatal screening (NIPS)
Chorionic villus sampling or amniocentesis

Birth

Perinatal information including gestational age, fetal position at delivery, the length of labor, type of delivery, and any evidence of fetal distress, such as passage of meconium, are all relevant data (Box 27.4). Apgar scores, the need for resuscitation, birth parameters (weight, length, and head circumference), any malformations seen at birth, and all abnormal test results should be noted.

Medical

A full review of the medical issues of the child should include the baby's general health, newborn screen results, any laboratory or imaging results, identification of any chronic medical issues, detailed feeding history, and need for hospitalization or surgeries. Evaluation of growth, review of systems, detailed developmental assessment, and notation of unusual behaviors can also provide important clues to a diagnosis.

Pedigree Analysis and Family History

A critical part of any genetic evaluation is the family history (Box 27.5); this is best accomplished by creating a three-generation pedigree, which is a schematic diagram depicting familial relationships using standard accepted symbols (Fig. 27.1). This formal record can also be used to summarize positive responses elicited during the interview. Special attention should be paid to ethnic origins of both sides of the family, consanguinity, and any first-degree relatives with similar malformations to those of the patient being evaluated, also known as the *index case, proband*, or *propositus*. An extended family history should be used to identify relatives with congenital anomalies, developmental abnormalities, physical differences, or sudden death. Often photographs can provide clear objective evidence of a descriptive history. If any family members have had genetic testing, it is important to obtain the reports.

Reproductive histories, especially of the parents, should be elicited. Specifically, questions should be asked about infertility, miscarriages, and stillbirths. The occurrence of more than two first-trimester miscarriages increases the probability of finding a balanced translocation in one parent.[19,20] A balanced translocation is a rearrangement of genetic material such that two chromosomes have an equal exchange without loss or gain of material. There are typically no associated clinical features with such a rearrangement. However, when chromosomes align to recombine for meiosis in the sperm or egg, this exchange produces a risk of unequal distribution and an unbalanced translocation in the resulting fetus. In this case, there would be aneuploidy for part of a chromosome. It has been estimated that 25% of stillbirths exhibit single or multiple malformations, and in at least half of these cases there is a genetic etiology for the malformations. Couples with two or more pregnancy losses should undergo routine chromosome analysis or karyotyping to evaluate for a balanced translocation. A chromosomal microarray should be performed on fetal tissue (i.e., amniotic fluid, placenta, or products of conception) in the evaluation of intrauterine fetal death or stillbirth.[21] Compared to a karyotype, a chromosomal microarray yields a higher rate of results, and it can identify maternal cell contamination, which is important in reducing false-negative results; however a microarray will not detect a balanced translocation. In summary, in a patient with multiple congenital anomalies and parents with history of infertility or multiple pregnancy losses, the clinician should be suspicious for a balanced translocation in one of the parents manifesting as an unbalanced translocation in the proband.

Obtaining a formal family history is helpful in discovering information that is often critical to making a diagnosis. Positive responses may help to discern a Mendelian pattern of inheritance for a given genetic disorder. For example, a disease affecting every

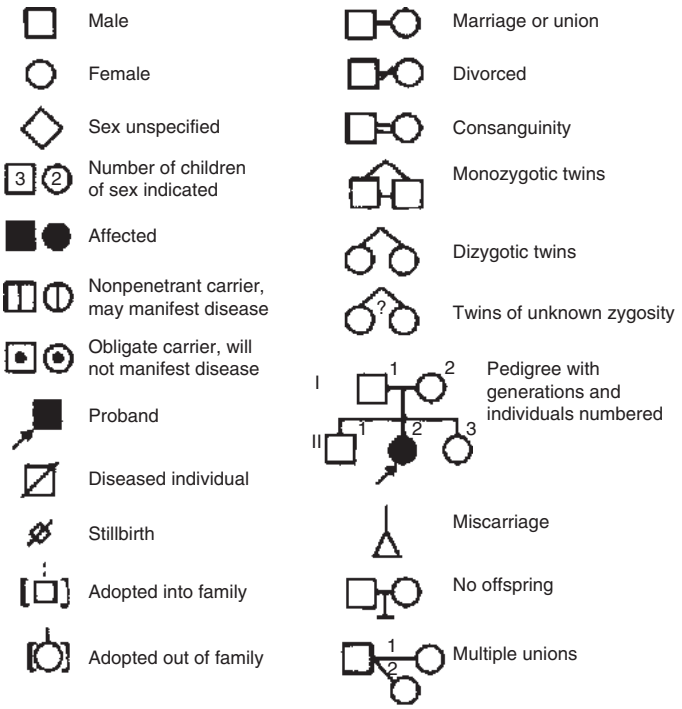
• BOX 27.4 Elements of Perinatal and Birth History for the Dysmorphic Newborn

- Fetal activity
- Delivery
- Type (e.g., indication for cesarean section)
- Gestational age
- Fetal presentation
- Apgar scores, history of distress, or resuscitation
- Growth parameters
- Malformations noted

• BOX 27.5 Elements of Pedigree Analysis and Family History for the Dysmorphic Newborn

Identification of relatives with:

- Congenital anomalies (especially those similar to proband)
- Intellectual disability
- Photographs (objective evidence)
- Genetic testing
- Parental reproductive history
- Pregnancy losses (gestational ages), including intrauterine fetal demise
- Infertility
- Medical histories of primary relatives
- Ethnic origin
- Consanguinity



• **Fig. 27.1** Symbols commonly used for pedigree notation.

generation, with both males and females involved, such as Marfan syndrome, displays an autosomal dominant pattern of inheritance. A pattern of X-linked recessive disease, such as hemophilia, instead shows affected males related through unaffected or minimally affected females; transmission in this pattern should not occur from father to son.

Specialized Clinical Evaluations

Physical Examination for Dysmorphology

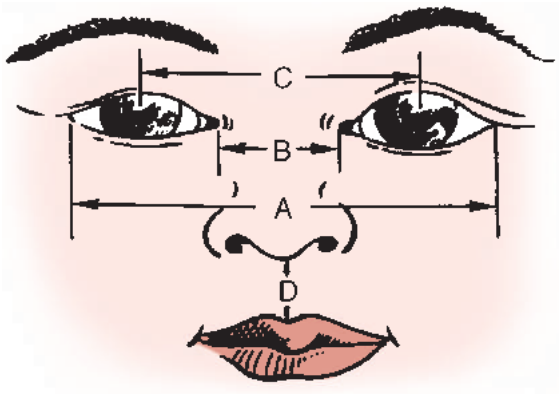
A congenital malformation can be described as a "morphologic defect of an organ, part of an organ, or larger region of the body resulting from an intrinsically abnormal developmental process."[22] The term *dysmorphology* was introduced by Dr. David Smith in the 1960s to describe the study of human congenital malformations.[6,23] This study of "abnormal form" emphasizes a focus on structural errors in development with an attempt to identify the underlying genetic etiology and pathogenesis of the disorder.

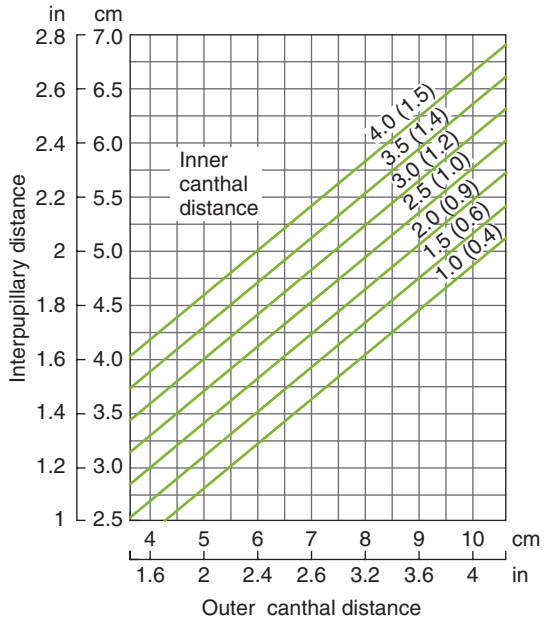
In a landmark study, Feingold and Bossert examined more than 2000 children to define normal values for a number of physical features.[24] These standards were devised as screening tools to objectively identify children with differences possibly attributable to a genetic disorder. Important measurements include head circumference, inner and outer canthal distances, interpupillary distances (IPDs), ear length, ear placement, internipple distances, chest circumference, and hand and foot lengths. In some instances, limb length and limb girth are needed. Other graphs and measurements using age-appropriate standards can be found in compendia such as the *Handbook of Physical Measurements*.[25]

The assessment should begin with newborn growth parameters that can reflect the degree of any prenatal insult. Measurements such as length, weight (usually reflecting nutrition), and head circumference should be plotted on newborn graphs. Gestational age-appropriate graphs should be used for premature neonates. It is often helpful to express values that are outside the normal range as 50th percentile for a different gestational age or in the number of standard deviations above or below the mean. For example, a full-term baby with microcephaly may have a head circumference of less than the fifth percentile for 38 weeks. This degree of microcephaly could be stated more clearly if described as $Z = -2.5$ or 50th percentile for 32 weeks' gestational age.

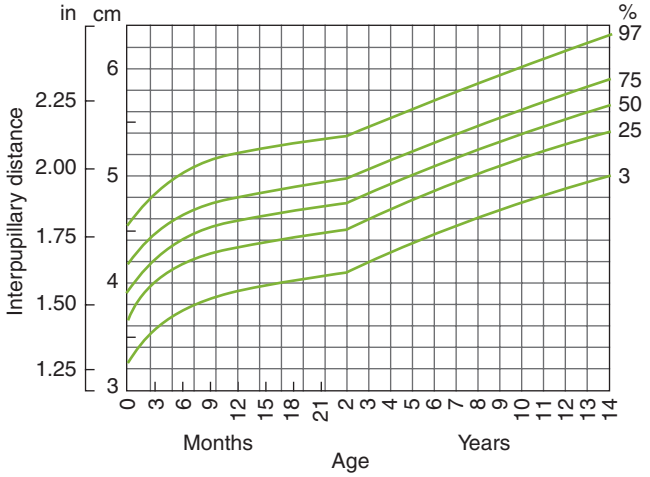
A complete physical examination should include assessment of patient anatomy for features varying from usual or normal standards. This assessment can often provide clues to embryologic mechanisms. The data obtained should then be interpreted using comprehensive standard tables that are available for these purposes. Special attention to familial or ethnic variation should be considered. There is a lack of phenotypic images available in globally diverse populations. As clinical findings in individuals with genetic syndromes can differ across different population groups, atlases will need to be created that are more representative of the global population.[26–28] The Atlas of Human Malformation Syndromes is one such resource.[27]

The shape and size of the head and fontanelles should be noted as well as the cranial sutures, with assessment for evidence of craniosynostosis or an underlying brain malformation. Any scalp defects should also be documented. The shape of the forehead, appearance of the eyebrows (such as synophrys), and the texture and distribution of hair should be noted. The spacing of the eyes, or canthal measurements (Fig. 27.2), the IPD (Fig. 27.3; see also Fig. 27.2), palpebral fissure lengths (Fig. 27.4), presence or absence of colobomata and epicanthal folds, and determination





• **Fig. 27.2** Canthal Measurements. Various eye measurements are depicted *(top)*. *A*, outer canthal distance; *B*, inner canthal distance; *C*, interpupillary distance (*IPD*), which is difficult to measure directly. The IPD can be determined using the graph at the *bottom* or with the Pryor formula: IPD = (A – B) 2 + B. (From Feingold M, Bossert WH. Normal values for selected physical parameters: an aid to syndrome delineation. *Birth Defects Orig Artic Ser*. 1974;10:1–16.)[24]



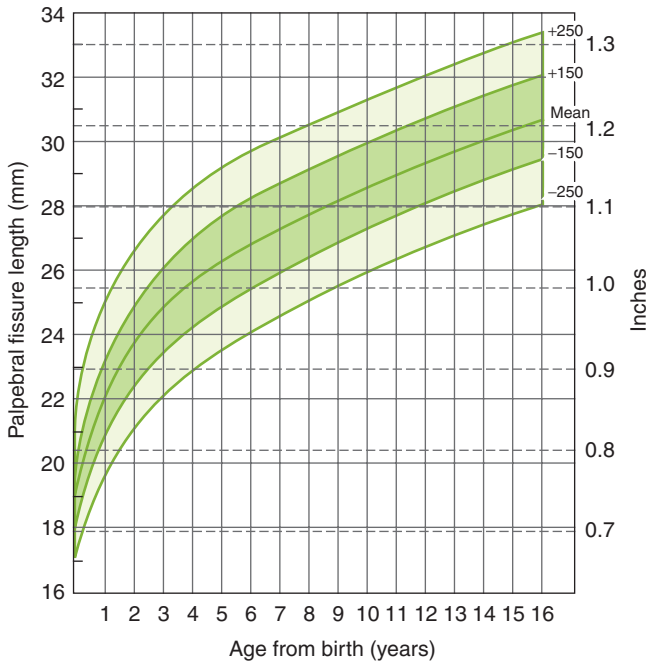
• **Fig. 27.3** A nomogram for interpupillary distance at different ages for both sexes. (From Feingold M, Bossert WH. Normal values for selected physical parameters: an aid to syndrome delineation. *Birth Defects Orig Artic Ser*. 1974;10:1–16.)[24]

of whether the palpebral fissures are turned upward or downward are components of the morphological examination of the eyes. Examination of the ears should include a search for preauricular and postauricular pits and tags, and assessment of the placement (Fig. 27.5), length (Fig. 27.6), and folding of the ear is important. Ear development occurs in a temporal frame similar to that of the kidneys during embryogenesis, and external ear anomalies can be associated with renal anomalies. Evaluation of the nose should cover the shape of the nasal bridge, nasal tip, the alae nasi, presence of anteverted nares, the length of the columella, and patency of the choanae. The mouth and throat are examined for the philtrum, presence of a cleft lip or palate; the shape of the palate and uvula is noted, and the presence of unusual features, such as tongue deformities, lip pits, multiple frenula, and natal teeth, is recorded. A small retrognathic or receding chin, which can be a part of several syndromes or an isolated finding, should be noted. The neck is inspected for excess nuchal folds or skin, evidence of webbing, and pits or branchial clefts. Any bony abnormalities in the neck should prompt an evaluation of the cervical vertebrae to confirm cervical and airway stability.

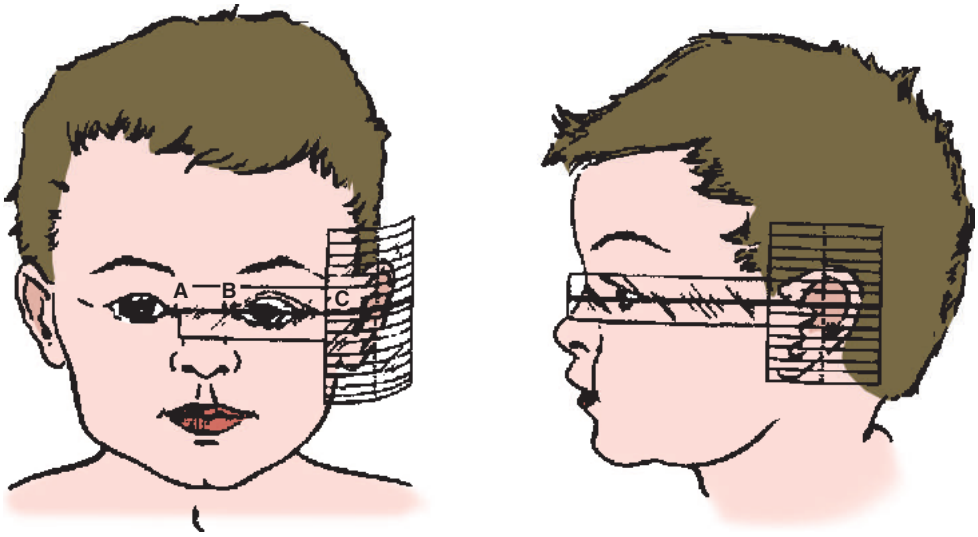
Evaluation of the chest and thorax involves lung auscultation and cardiac examination. Abnormal findings should prompt a consultation with a pediatric cardiologist and appropriate echocardiographic or invasive studies as needed. External measurements include determining the internipple distance and its ratio with respect to the chest circumference (Fig. 27.7). The presence of inverted or supernumerary nipples should be noted. The abdominal examination is focused on determining whether organomegaly is present, a finding that may be associated with an inborn error of metabolism. The umbilicus should also be examined, with any hernias and the number of vessels present in the newborn cord being noted. A two-vessel cord, in which only a single artery is present, can be associated with cardiac, renal, and other anomalies.[29] The genitourinary examination concentrates on determining whether anomalies such as hypospadias, chordee, cryptorchidism, other abnormalities of the scrotum, microphallus, and ambiguous genitalia are present. These external anomalies may be associated with internal anomalies involving the upper urinary tract as well. The anus is examined for evidence of tags, its placement, and its patency.

The back should be assessed, especially for the shape of the spine and any associated defects, such as myelomeningocele. These defects prompt further radiologic evaluation to assess for potential functional limitations. In addition, a sacral dimple or hair tuft at the base of the spine should be noted, because either could signify developmental abnormalities in the underlying neural tissue.

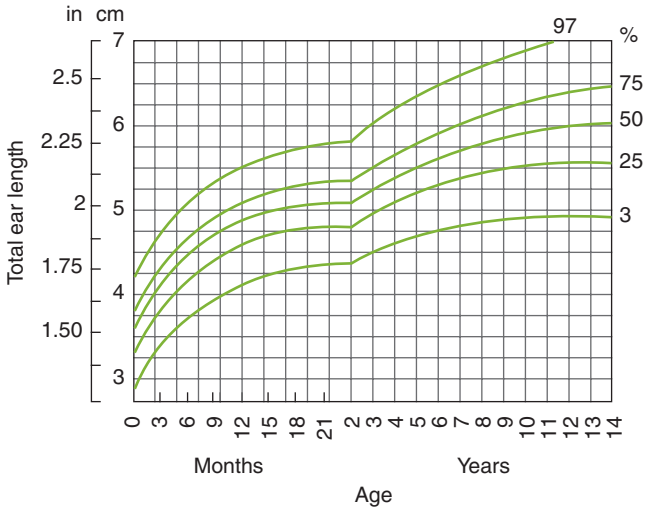
Minor anomalies are often manifested in the extremities. Gross differences in the hands and feet include polydactyly (more than five digits), syndactyly (fusion of the digits), and clinodactyly (incurving of the digits). It should be noted whether the extra



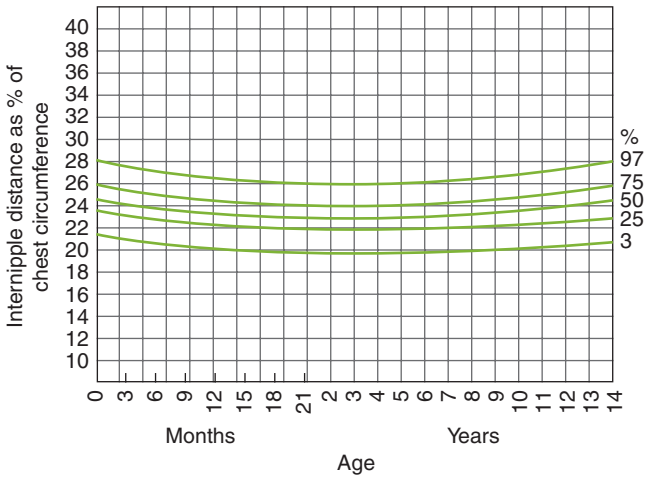
• **Fig. 27.4** A graph of palpebral fissure length from birth to age 16 years for both sexes. (From Gripp KW, Slavotinek AM, Hall JG, Allanson JE. *Handbook of Physical Measurements*. 3rd ed. Oxford UK: Oxford University Press; 2013.)[25]



• **Fig. 27.5** Ear Placement. Using the medial canthi *A* and *B* as landmarks, one draws a central horizontal line and extends it to a point *C* on the side of the face. Ears attached below this line are considered low set. (From Feingold M, Bossert WH. Normal values for selected physical parameters: an aid to syndrome delineation. *Birth Defects Orig Artic Ser*. 1974;10:1–16.)[24]



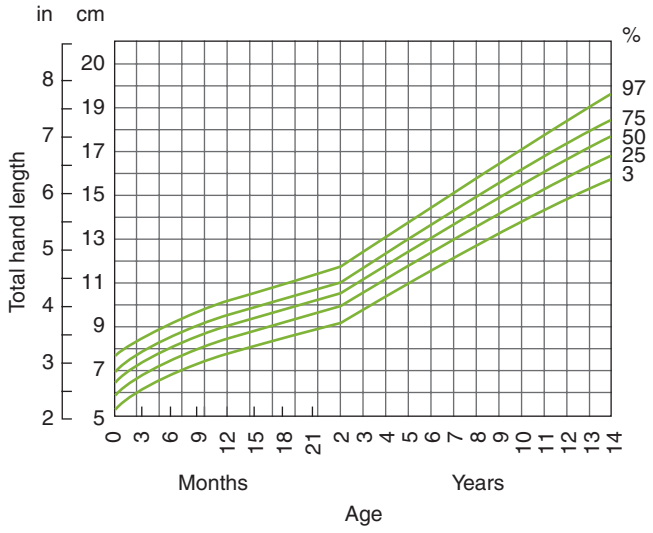
• **Fig. 27.6** Graph showing various percentiles for ear length plotted against age. (From Feingold M, Bossert WH. Normal values for selected physical parameters: an aid to syndrome delineation. *Birth Defects Orig Artic Ser.* 1974;10:1–16.)[24]



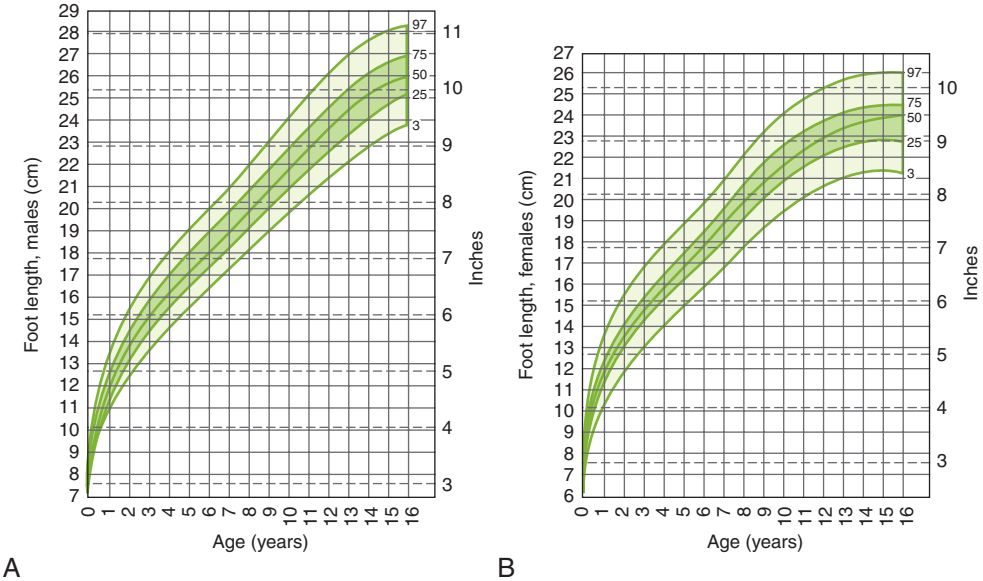
• **Fig. 27.7** The internipple distance as a percentage of the chest circumference plotted against age for both sexes. (From Feingold M, Bossert WH. Normal values for selected physical parameters: an aid to syndrome delineation. *Birth Defects Orig Artic Ser*. 1974;10:1–16.)[24]

digits are located in a preaxial (first digit) or postaxial (fifth digit) position. Hand and foot lengths, which should be expressed as a percentile measured on age-appropriate graphs (Figs. 27.8 and 27.9), are important to document. Often these data can provide important clues to a unifying syndrome.

Dermal ridge patterns, or dermatoglyphics, are formed on the palms and soles early in embryonic life, and they vary considerably among individuals. This variation can be inherited and can be influenced by disturbances to the development of the peripheral limb buds. Environmental exposures and chromosomal aberrations can greatly affect the formation of these structures and are reflected by the dermatoglyphic pattern of an individual. Each of the distal phalanges has one of three basic dermal ridge patterns: arches, whorls, or loops (Fig. 27.10). The predominance of a single pattern can be an associated feature of a genetic disorder. For example, the occurrence of arches on eight or more digits is a rare event but is frequently encountered in children with trisomy 18 (Table 27.3).



• **Fig. 27.8** The total hand length plotted against age for both sexes. (From Feingold M, Bossert WH. Normal values for selected physical parameters: an aid to syndrome delineation. *Birth Defects Orig Artic Ser.* 1974;10:1–16.)[24]



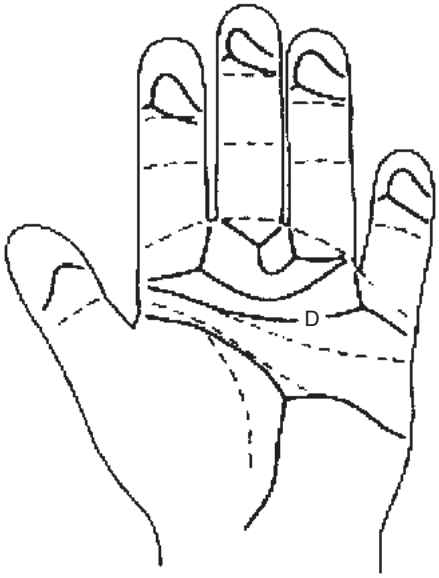
• **Fig. 27.9** Total foot lengths plotted against age for boys (A) and girls (B). (From Gripp KW, Slavotinek AM, Hall JG, Allanson JE. *Handbook of Physical Measurements*. 3rd ed. Oxford, UK: Oxford University Press; 2013.)[25]

• **Fig. 27.10** Basic fingerprint patterns (dermatoglyphics). (From Holt SB. *The Genetics of Dermal Ridges*. Springfield, IL: Charles C. Thomas; 1968.)[30]

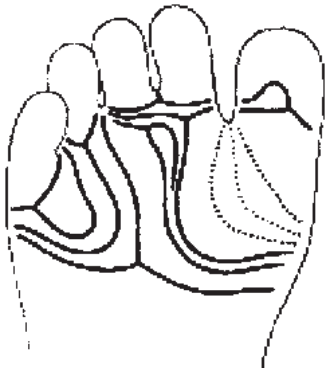
TABLE 27.3 Dermatoglyphic Patterns Associated With Specific Dysmorphic Disorders

Dermatoglyphic Pattern	Associated Disorders
Excess arches	Trisomy 13, trisomy 18, Klinefelter syndrome (47, XXY), deletion 5p (cri-du-chat syndrome), fetal phenytoin exposure
Excess ulnar loops	Trisomy 21, 22q11.2 deletion syndrome[37]
Excess whorls	Smith–Lemli–Opitz syndrome, Turner syndrome (45, X), 18q deletion

Deltas, or triradii, form at the convergence of three sets of ridges on the palm. This junction is where the hypothenar, thenar, and distal palmar patterns converge. There are typically no triradii in the hypothenar area of the palm, but when patterning is present or is large, a distal triradius arises, which is found in only 4% of normal Caucasian individuals, but in 85% of patients with trisomy 21 (Down syndrome).[30,31] A single transverse palmar crease is found in 4% of controls, but in more than half of patients with trisomy 21 (Fig. 27.11), and in even greater proportions in patients



• **Fig. 27.11** The patterns on the hand of a patient with Down syndrome depicting the palmar crease (*D*). (From Holt SB. *The Genetics of Dermal Ridges*. Springfield, IL: Charles C. Thomas; 1968.)[30]



• **Fig. 27.12** The distal sole of the foot of a patient with Down syndrome, depicting the characteristic "open field" hallucal pattern. (From Holt SB. *The Genetics of Dermal Ridges*. Springfield, IL: Charles C Thomas; 1968.)[30]

with other trisomies. The hallucal area of the foot, located at the base of the big toe, also has a dermal ridge pattern, usually a loop or whorl. A simple pattern or open field in this region is found in less than 1% of controls, but in more than 50% of patients with Down syndrome (Fig. 27.12).[30,31] This unusual dermal pattern is also associated with hypoplasia of the hallucal pad and a wide space, sometimes called a "sandal gap deformity," between the great and second toes in these patients.

An examination of the skin is also important, since phakomatoses or skin manifestations can herald the presence of an underlying disorder.[32] Examples are café-au-lait spots (associated with neurofibromatosis type I) and ash leaf spots (associated with tuberous sclerosis and detected with the use of a Wood lamp). Irregular pigmentation can be suggestive of chromosomal mosaicism, in which the different skin pigmentation patterns represent a different, mixed chromosomal composition.[33] Capillary malformations and other skin diseases are also noteworthy.

Finally, a careful neurologic examination with input from a specialist is often warranted in the child with multiple anomalies, because the neurologic status is often the most reliable prognostic indicator. Evaluation of tone, feeding, unusual movements, and

• BOX 27.6 Adjunct Studies in the Evaluation of the Dysmorphic Newborn

- Ultrasonography and/or MRI
- Echocardiogram
- Brain imaging (particularly MRI)
- Radiographs (skull, skeletal, etc.)
- Electroencephalography as indicated
- Electromyography as indicated
- Subspecialty consultation

the presence of seizure activity are critical pieces of diagnostic information.

Adjunct Studies

An exhaustive physical examination often reveals differences that require further evaluation for diagnostic, prognostic, and treatment purposes (Box 27.6). For instance, poor feeding or cyanosis may prompt an echocardiogram or recurrent emesis may lead to an abdominal ultrasound that may lead to detection of internal organ malformations. Differences in head shape suggest the need for skull radiographs, three-dimensional computed tomography, or MRI of the brain. A disproportionality of the limbs prompts a skeletal survey and bone age measurement.

It is prudent in children with anomalies involving multiple systems to obtain the input of relevant specialists. This step is often essential to medical decision making and for planning interventions. Abnormal neurologic findings should prompt a consultation with a trained specialist and interpretation of studies such as head ultrasonography, brain MRI, brainstem auditory evoked responses, and electroencephalogram for seizure activity. Muscle dysfunction might result in the ordering of electromyography, muscle ultrasound, or nerve conduction studies. Visual involvement requires a fundoscopic examination by an experienced pediatric ophthalmologist, and sometimes studies of visual evoked responses or electroretinograms are needed to predict visual prognosis. Often, there are well-characterized genetic disorders that have a specific pattern of abnormal findings in these highly specialized studies. Even when a unifying diagnosis is reached, there is often variation in the clinical phenotype, and determining the patient's prognosis on a system-by-system basis is typically the most appropriate and accurate way to proceed.

Literature Review

After the history and physical evaluation are complete, a cross-reference of two or more anomalies is useful to generate a differential diagnosis. When the rest of the neonate's physical and history findings are added, the possibilities can often be narrowed down to a few entities that may be amenable to diagnostic testing. If multiple anomalies are present, it is usually best to start with the least common. As Aase has stated, "The best clues are the rarest. The physical features that will be the most helpful on differential diagnosis are those infrequently seen either in isolation or as part of syndromes. Quite often, these are not the most obvious anomalies or even the ones that have the greatest significance for the patient's health."[6] Cross-referencing is usually best accomplished by using published compendia of malformation syndromes. These compendia have been supplemented by databases that are accessible online (i.e., GeneReviews, Online Mendelian Inheritance in Man, Ovid MEDLINE, PubMed, SimulConsult, Phenomizer, etc.). The availability of such tools allows the cross-referenced features to be compared easily with those of other described syndromes that may include similar malformations. This systematic review produces a differential diagnosis for the constellation of features described and identifies references to pertinent literature.

The recognition of patterns of genetic entities involves the comparison of the proband with the examiner's personal experience of known cases and a search of the literature. Multiple anomalies may be causally related, occur together in a statistically associated basis, or occur together merely by chance. Diagnosis of a genetic disorder relies heavily on the ability of the clinician to suspect, detect, and correctly interpret physical and developmental findings and to recognize specific patterns. Accurate diagnosis of a syndrome in a child is important to the identification of major complications and their treatment if possible. It is also crucial for long-term management of patients and for parental counseling about recurrence in future offspring.

Specialized Laboratory Tests

In sorting through the multiple possibilities listed, the geneticist uses another important tool—the availability of highly specialized cytogenetic and molecular genetic testing, including:

- Karyotype
- Fluorescence in situ hybridization
- Chromosomal microarray (see Chapter 28)
- Molecular analysis, such as sequencing (single gene, panel, exome, genome), methylation testing (e.g., for Beckwith–Wiedemann, Prader–Willi, or Angelman syndromes), or polymerase chain reaction-based techniques (e.g., for congenital myotonic dystrophy or fragile X syndrome).

The most common methods of genetic testing are summarized in Table 27.4. The standard karyotype, or analysis of stretched and stained chromosome preparations usually taken from a peripheral blood sample, can often confirm a suggested diagnosis or explain a set of major malformations not classically encountered together. Further description of specific chromosomal abnormalities is addressed in Chapter 28; it is sufficient to note that multiple malformation syndromes can result from large visible chromosome rearrangements that lead to deletion or addition of genetic material (aneuploidy). These rearrangements can involve an entire arm of a chromosome or can be submicroscopic, requiring further special testing. While a karyotype is still the only test to detect chromosomal rearrangements, more specialized molecular testing methods, such as a chromosomal microarray, have since become the standard of care, as an adjunct to or instead of karyotype analysis. In general, microarray-based methods are currently focused on detecting copy number changes (smaller deletions or duplications not detectable by a karyotype) and can be performed in a targeted or genome-wide fashion.[34] For individuals with features suggestive of a particular syndrome or with an anomaly that has known associated gene(s), individual gene sequencing or next-generation sequencing (NGS) of a panel of genes can be performed. For instance, in an individual with pleural effusions and pulmonic stenosis, a Noonan syndrome gene panel may be helpful, whereas in an individual with craniosynostosis, a craniosynostosis gene panel may be helpful. For individuals with multiple

TABLE 27.4 Common Methods of Genetic Testing

Test	Methodology	Clinical Indication
Karyotype	• Involves analysis of the entire chromosome complement under a microscope. • A solution of colchicine is used to arrest cells during cell division. A Giemsa dye is then applied to cells to make them visible under the microscope.	• Used to identify aneuploidy involving whole chromosomes, or large (>5–7 Mb) rearrangements such as duplications and deletions within chromosomes (i.e., trisomy 13, 18, or 21 or disorders of the sex chromosomes). • Still the only clinical diagnostic technique that can detect balanced translocations where chromosomal material from one chromosome is rearranged onto another.
Fluorescence in situ hybridization (FISH)	• Specific fluorescent-tagged DNA probes representing a single locus or part of a particular chromosomal region hybridize to that region of the genome.	• Clinicians must know and specifically order the proper FISH test for a given chromosomal locus. • Can be used to identify deletions such as 22q11.2 deletions (although FISH can miss small deletions).
Chromosomal microarray	• Uses synthesized oligonucleotide probes or single nucleotide polymorphism (SNP)-based probes densely arranged on a microarray chip to assess for submicroscopic chromosome rearrangements. • Automated processing. • Copy number variation is compared against a large panel of standards.	• Often the first-line study for cases of multiple congenital malformations. • Can detect copy number changes (deletions or duplications) in the 50–100 kb range. • Disadvantages: 1. Changes of unknown significance can be detected and require further investigation and often examination of parental samples to help interpret the proband's results. 2. Structural changes that do not result in copy number variation, such as a balanced translocation or inversion of part of a chromosome, will not be detected by this technique.
Direct gene sequencing or single gene or gene panels	• Alterations of a single base pair, or insertions or deletions of even a small number of nucleotides, can disrupt the gene and subsequently, its protein product. • Because these mutations can be as small as a single base pair, they cannot be detected by a karyotype or chromosomal microarray. • Direct sequencing of a target gene can detect a single base pair mutation or a small insertion or deletion. Larger deletions or duplications that involve either part or all of the gene are often more difficult to detect, being too small to detect on a chromosomal microarray, but too large to detect with standard sequencing. These genetic lesions often require PCR-based deletion/duplication testing targeted to a specific gene locus.	• With well-characterized phenotypes, a clinician can sequence a specific gene or genes associated with that phenotype. • Over the past decade or so, next-generation sequencing (NGS) has allowed multiple genes associated with a phenotype to be tested simultaneously in a gene panel.
Exome sequencing (ES)	• Sequences the protein-coding regions or exons of the human genome. This includes approximately 20,000 genes and accounts for about 2% of the total genome. • Following massively parallel sequencing, the individual's sequence is compared to reference DNA sequences, published control individuals, and the patient's family members. • Clinical analysis is required to reduce thousands of variants to those that may be related to the individual's phenotype. • Limited to the protein-coding regions of the genome.	• Useful for individuals with multiple congenital anomalies without a unifying diagnosis, or with a suspected, but unclear genetic diagnosis.
Genome sequencing (GS)	• Sequences the entire genome, including the noncoding regions (introns) and the coding regions (exons). • Like ES, GS requires significant clinical analysis to reduce thousands of variants to those that may be related to the individual's phenotype.	• Primarily done on a research basis. • Like ES, GS may be useful for individuals with multiple congenital anomalies without a unifying diagnosis, or with a suspected, but unclear genetic diagnosis. • May also be useful if a genetic diagnosis is strongly suspected, but ES was negative.
RNA sequencing (RNA-Seq)	• NGS is performed to detect and quantify RNA in a sample at a particular time. • RNA-Seq can evaluate the continuously changing cellular transcriptome.	• Can evaluate alternative gene spliced transcripts, posttranscriptional modifications, gene fusion, mutations/SNPs, and changes in gene expression over time.

Continued

TABLE 27.4 Common Methods of Genetic Testing—cont'd

Test	Methodology	Clinical Indication
Noninvasive prenatal screening (NIPS)	• Obtained from cell-free DNA in the maternal circulation that comes from the fetus. • Isolated cell-free DNA is then sequenced to assess for common aneuploidies. • Obtained from a peripheral blood draw as early as 10–11 weeks of gestation. • Is only a screening test.	• Developed to screen for trisomy 13, 18, and 21 during the first trimester of pregnancy. • Has high sensitivity, specificity, and positive predictive values for these disorders. • Now expanded to screen for sex chromosome aneuploidy, but has a poorer detection rate and a higher false-positive rate for the sex chromosome aneuploidies, particularly for monosomy X.

congenital anomalies without a unifying diagnosis, or with a suspected, but unclear genetic diagnosis, exome sequencing (ES) may be indicated. ES sequences all of the protein-coding regions or exons of the human genome. This includes approximately 20,000 genes, but accounts for only 2% of the genome. The advantages to ES are that it is a very comprehensive test and clinicians do not have to predetermine which genes to sequence. Disadvantages include that significant clinical analysis and expertise are required to reduce thousands of variants to those that may be related to an individual's phenotype, which makes the test expensive and time-intensive to complete. Finally, ES is limited to only the protein-coding regions of the genome. Thus, genome sequencing (GS), which sequences the entire genome, including the noncoding regions (introns) and the coding regions (exons), is now being performed, primarily on a research basis, as the most comprehensive genetic testing available. Like ES, GS requires significant clinical analysis and is both expensive and time-intensive to perform. RNA sequencing (RNA-Seq) is also emerging, primarily on a research basis, to evaluate the continuously changing cellular transcriptome. RNA-Seq allows for the evaluation of alternative gene spliced transcripts, posttranscriptional modifications, gene fusion, mutations/single nucleotide polymorphism (SNPs), and changes in gene expression over time.

For some disorders, other types of specialized testing methods may be indicated. For instance, if there is suspicion for a methylation disorder such as Beckwith–Wiedemann, Prader–Willi, Angelman, or Russell Silver syndromes, methylation testing is required; for trinucleotide repeat disorders like congenital myotonic dystrophy or Fragile X syndrome, polymerase chain reaction–based techniques may be required. It can be useful to inquire with a geneticist or genetic counselor for the availability of specific gene mutation testing that may be clinically available or performed on a research basis. GeneTests and the Genetic Testing Registry are Internet databases of laboratories worldwide that provide such services (https://www.genetests.org; https://www.ncbi.nlm.nih.gov/gtr/).

There is also an increasing awareness about the importance of the sample type used for genetic testing. Classically, genetic testing has been performed on either peripheral blood samples or on chorionic villi or amniotic fluid when testing is performed prenatally. However, with disorders that may have mosaicism, it is essential to sequence the affected tissue itself to obtain a diagnosis. This is because somatic mutations, which may occur in a single body cell, may not be detected in a peripheral blood sample, in contrast to germline mutations which occur in gametes and are thus present in every cell.

Diagnosis

There are cases in which, after a detailed examination, exhaustive literature search, and genetic testing, no unifying diagnosis is evident. Aase, a dysmorphologist, advises, "Don't panic! The absence of a diagnosis may be distressing to the diagnostician and the family, but it is much less dangerous than the possibility of assigning the wrong diagnosis with the risk of erroneous genetic and prognostic counseling and possibly hazardous treatment."[6] Therefore, in cases in which there is no clear diagnosis, prognosis and treatment should be determined according to the organ systems involved and the extent of their impairment. In addition, when the newborn has a severe, untreatable impairment or the patient's condition is critical, it may be prudent to offer and obtain consent for a full postmortem examination by an experienced pediatric pathologist. A skin sample, and sometimes blood, can be taken from the expired fetus (or newborn prior to death) for establishing a cell line or for extracting DNA for future testing. Information gained from such investigations may often become relevant for family members, including the parents, allowing one to provide accurate recurrence risk counseling and perhaps offer prenatal testing of a new pregnancy. Such information can often help to provide closure for the family as well.

Example Evaluations for Common Neonatal Anomalies

There are many common neonatal anomalies that should prompt an immediate genetics consultation. These include choanal atresia, cleft palate, congenital diaphragmatic hernia (CDH), congenital heart defects, craniosynostosis, micrognathia, omphalocele, any limb anomalies concerning for a skeletal dysplasia, and tracheoesophageal fistula, to name just a few. Many of these common neonatal anomalies have a broad genetic differential. For instance, in the evaluation of a cleft palate, it is important to determine whether the cleft palate is isolated or associated with other anomalies. A cleft palate may also occur as part of PRS, where the primary anomaly of micrognathia sets off a cascade of additional defects including glossoptosis, tongue-based airway obstruction, and in some cases, a secondary cleft palate. Among patients with PRS, about 30% are found to have Stickler syndrome.[35] Stickler syndrome is a clinical triad of PRS, hearing loss, and ocular abnormalities (high myopia, vitreoretinal degeneration, retinal detachment, cataracts). An eye exam is often very helpful in the diagnosis of Stickler syndrome, as myopia is a very unusual finding in infancy and in this context, if found, is strongly suggestive

of Stickler syndrome. A careful physical examination should be performed with close attention to the eyes, ears, and chin. If there is any hooding of the eyes, micrognathia, or ear anomalies, testing should be sent for 22q11.2 deletion syndrome. A chest x-ray should be obtained to evaluate for any rib gaps, which would be concerning for cerebrocostomandibular syndrome. Finally, the lips should be closely evaluated for any lip pits, which would be consistent with Van der Woude syndrome.

Similarly, in the case of an omphalocele, it is also important to look closely for other anomalies as 50% to 80% of babies will have an additional anomaly, including 50% of whom will have a congenital heart defect.[36] The syndromic differential for an omphalocele includes BWS, Carpenter, CHARGE (coloboma, heart defect, choanal atresia, retarded growth and development, ear anomalies), Goltz, Marshall–Smith, Meckel–Gruber, otopalato-digital type II, trisomy 13, and trisomy 18 syndromes. BWS occurs in ~25% of babies with small omphaloceles and includes an omphalocele (typically small), macroglossia, ear creases and/or pits, hemihypertrophy, and hypoglycemia. The presence of multiple associated anomalies should raise concern for trisomy 13 or 18. Omphalocele is also found as a component of the **O**mphalocele, **E**xstrophy of the bladder, **I**mperforate anus, **S**pinal defects complex. This complex has no known genetic etiology. Unlike omphalocele, gastroschisis is not commonly associated with other anomalies. It is typically secondary to a vascular accident and not an underlying genetic etiology.

As a final example, in the case of a CDH, a genetic etiology is currently identified in 30% of babies, although this number is growing. The genetic differential diagnosis for CDH can be divided into syndromic causes (meaning CDH in the context of other anomalies) and nonsyndromic causes. The most common syndromic causes of CDH are 22q11.2 deletion, Craniofrontal nasal, Cornelia de Lange, Donnai–Barrow, Fryns, Pallister–Killian, Simpson–Golabi–Behmel, Wolf–Hirschhorn, and trisomy 18 syndromes, as well as a growing list of other single gene disorders. There are also several nonsyndromic causes, including microdeletions at 15q26, 8p23.1, 8q23, or 1q41-42 that are important in terms of recurrence counseling. A careful physical exam should pay close attention to the growth parameters, chest circumference and nipples, hairline, genitalia, hands and feet, and fingernails. Large growth parameters raise suspicion for Simpson–Golabi–Behmel syndrome, a temporal sparing pattern of the hairline is concerning for Pallister–Killian syndrome, and widely spaced nipples and hypoplasia of the fingernails are concerning for Fryns syndrome. Multiple congenital anomalies including congenital heart disease, limb anomalies, or cleft palate, in addition to CDH, should raise suspicion for Cornelia de Lange syndrome or trisomy 18 syndrome.

Summary

Diseases with underlying genetic bases have significant effects on health care and its delivery. An appreciation of these entities, coupled with an organized, systematic evaluation, can help to define the nature of a given disorder and aid in the development of the optimal plan of treatment and care for the patient.

Suggested Online Resources

ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/).
Decipher—CNV with phenotype information (https://decipher.sanger.ac.uk/).
Elements of Morphology: (https://elementsofmorphology.nih.gov).
Gene Reviews (https://www.ncbi.nlm.nih.gov/books/NBK1116/).
"Help me understand genetics"—general introduction to fundamental topics related to human genetics (https://ghr.nlm.nih.gov/primer#).
OMIM—generally has the gene and/or condition; clinical synopsis is a summary (https://www.ncbi.nlm.nih.gov/omim).
Terminology for human malformations—helpful when learning your dysmorphology.
UCSC Genome Browser (https://genome.ucsc.edu).
UniProt protein database (http://www.uniprot.org/).
Unique—The Rare Chromosome Disorder Support Group (www.rarechromo.org).

Suggested Readings

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28

Chromosome Disorders

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KEY POINTS

- Early identification of an underlying genetic condition in a patient can aid in defining a treatment plan and help to identify resources for better care for patients and their families.
- In counseling the family of a newborn with a newly diagnosed chromosomal disorder, it is important to include the organ systems affected in the baby and the severity of each malformation when discussing prognosis. The variability of most phenotypes should be emphasized, with a care plan tailored to the needs of the individual patient.
- Although the prognosis in trisomy 13 and trisomy 18 is extremely poor, there is emerging evidence that in some cases, interventions can improve the survival and quality of life for the child and the family, and they should be discussed with the parents during a prenatal or postnatal visit.
- The use of chromosomal microarray testing allows the identification of smaller genomic deletions and duplications that are largely undetectable by standard karyotyping techniques, and it is recommended as a first-tier test in the genetic evaluation of a newborn with multiple congenital anomalies (excluding cases with high clinical suspicion for well-recognized whole chromosome disorders such as trisomies 13, 18, and 21 and Turner syndrome).
- Noninvasive prenatal screening (NIPS) is a relatively new technology that analyzes the cell-free fetal DNA fraction circulating in maternal peripheral blood. The fetal DNA is derived from cells forming the developing placenta and is accepted as an initial screen for select aneuploidy conditions. In cases where a high risk for an abnormality is identified, then diagnostic testing such as karyotyping or chromosomal microarray analysis of amniotic fluid or chorionic villus samples is recommended.

According to estimates from the Centers for Disease Control and Prevention (CDC), congenital malformations occur in approximately 3% of newborns in the United States. Congenital anomalies affecting major organ systems can result from chromosomal imbalances that affect the dosage or copy number of developmental genes that are located in the affected region. This chapter will focus on genetic disorders and syndromes with underlying chromosomal abnormalities that typically manifest themselves in the newborn period. In addition, it will discuss the shift in genetic evaluation and diagnosis in both prenatal and postnatal cases, to the use of microarray and next generation sequencing-based diagnostic techniques.

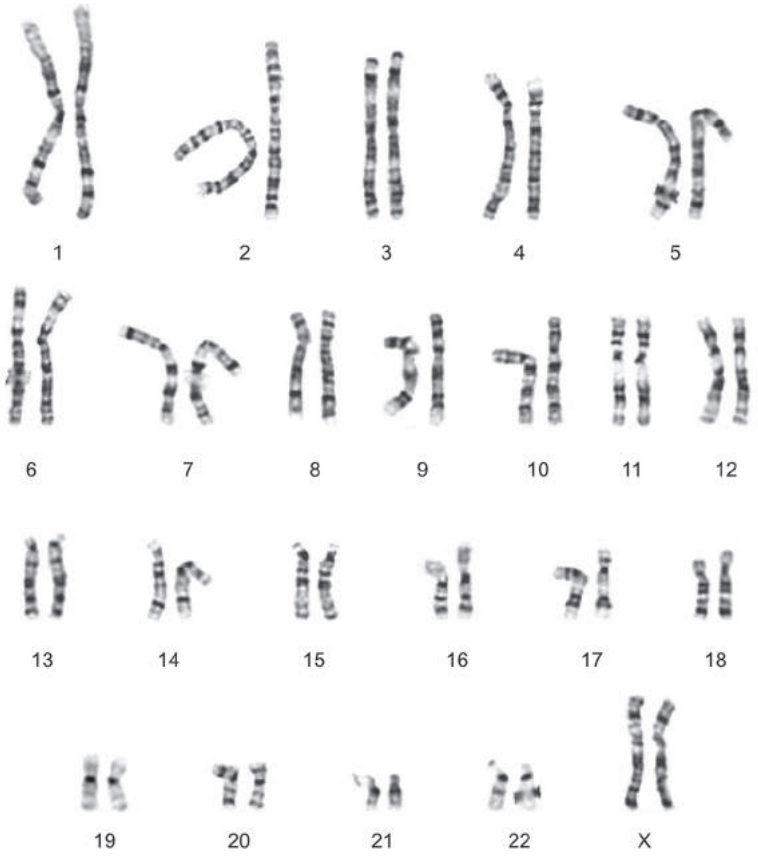
Human Karyotype

Chromosomes consist of single molecules of DNA whose structure is maintained by association with histones and other proteins. Chromosomes from dividing cells can be visualized under the light microscope as linear structures with two arms joined by a *centromere*. The short arm is designated the *p arm* and the long arm is designated the *q arm*. The ends or tips of the p and q arms are known as *telomeres*. The chromosome complement, or karyotype, in human cells normally consists of 46 chromosomes, with 22 pairs of autosomes (numbered in general from largest to smallest) and one set of sex chromosomes–two X chromosomes in genetic females (46,XX) and an X chromosome and a Y chromosome in genetic males (46,XY) (Fig. 28.1). After treatment with special dyes, each pair shows a distinctive size, centromeric position, and staining or banding pattern allowing it to be identified and classified. Human cells were first determined to have 46 chromosomes in 1956,[1,2] and the presence of recurrent numerical abnormalities in syndromes including Down, Turner, and Klinefelter syndrome were identified soon after.[3]

Karyotype analysis is performed in cells undergoing mitosis, or cell division, in which the chromosomes condense and can be stained and visualized. At this stage, each chromosome consists of two sister chromatids, which are the products of DNA replication. Karyotype analysis can be successfully performed on cell types that can be stimulated to divide and grow in culture, such as peripheral blood lymphocytes, skin fibroblasts, and amniocytes, or cell types that are normally undergoing rapid cell division, such as bone marrow or chorionic villi. Historically, several different staining methods have been described. However, G-banding (Giemsa staining) is most commonly used. At the highest resolution, G-banding can allow the detection of structural rearrangements as small as 5 to 10 million base pairs or 5 to 10 megabase (Mb) pairs, though the maximum attainable resolution varies by preparation and by specimen type.

Prior to fertilization, gamete formation, either spermatogenesis or oogenesis, is accomplished by a process known as *meiosis*. In the first part of meiosis (meiosis I), homologous chromosomes align as pairs and cross over, exchanging genetic material, also known as *recombination*. In this stage, named reduction division, the recombined pairs separate and the typical diploid content (46 chromosomes) of the cell is reduced by half to a haploid complement of 23 chromosomes. In the next stage, meiosis II, the sister chromatids of each chromosome separate, similar to mitosis.[4] The full diploid state of the cell (46 chromosomes) will be restored at the time of fertilization.

An imbalance of genetic material, or aneuploidy, occurs from a *net loss or gain* of genetic material during sperm or egg formation or less commonly, after fertilization during the initial divisions of the



• **Fig. 28.1** G-banded human female karyotype. The 46 chromosomes are arranged in 23 pairs, each with a specific banding pattern. (Courtesy UCLA Cytogenetics Laboratory.)

embryo. This missing or extra genetic material can be small pieces or parts of chromosomes (partial or segmental aneuploidy) or an entire chromosome itself. The classic recognizable aneuploidy syndromes involve trisomy (three copies of a full chromosome) such as those of chromosomes 13, 18, and 21, or monosomy (only a single copy) of a complete chromosome, such as the X chromosome. Aneuploidy can result from nondisjunction, a failure of normal chromosome separation. In such cases, a pair of homologs or sister chromatids does not separate in meiosis, and one daughter cell receives both copies of that pair, while the other cell receives none. This event can occur in either stage of gamete division, meiosis I or meiosis II. Most human meiotic nondisjunction arises during oocyte formation, specifically in maternal meiosis I. The occurrence of meiotic nondisjunction increases significantly with maternal age. Therefore, prenatal karyotyping from amniocentesis or chorionic villus sampling (CVS) is offered to women aged 35 years or older.[4]

Noninvasive prenatal screening (NIPS), also known as noninvasive prenatal testing (NIPT) or cell-free DNA (cfDNA) screening, is a relatively new technology that analyzes the cell-free fetal DNA fraction (derived from placental trophoblasts) circulating in maternal peripheral blood and can be offered starting at 9 to 10 weeks of gestation.[5,6] While NIPS was initially recommended for women at high risk for carrying a fetus with a chromosome abnormality, recent guidelines recommend that it should be offered to all pregnant women regardless of age or other risk factors.[7] The technical approaches and analyses vary by platform, but in general NIPS uses next-generation sequencing to assess the copy number of different chromosome regions in cfDNA. It is performed as an initial screen for common aneuploidy conditions, with some laboratories screening for rarer aneuploidies, as well as microdeletion/microduplication syndromes (see additional details below) and other segmental aneuploidies. An important metric to consider when counseling patients with abnormal NIPS results is the positive predictive value (PPV), which is the probability that a positive test result represents a *true positive*. While NIPS has high PPV for common aneuploidies such as trisomy 21, the PPV is significantly lower for rarer conditions such as microdeletions, and appropriate follow-up testing and genetic counseling are critical.[7–10] In addition, since the risk of trisomy increases with maternal age, the PPV of NIPS is age-dependent. For patients where NIPS shows a high risk of a chromosomal abnormality in the fetus, or with atypical/inconclusive results, diagnostic testing such as karyotyping or chromosomal microarray analysis of amniotic fluid or chorionic villus specimens is recommended.[7]

Nondisjunction can also occur later, in mitosis, with uneven division of genetic material during an early embryonic cell division. This can result in two cell lines: one trisomic lineage that is potentially viable and one monosomic line. If this event occurs after the first postzygotic division, cells with a normal chromosome complement may also coexist with cells containing an aneuploid complement, as a *mosaic* chromosome constitution. This is considered a possible mechanism for the occurrence of mosaic Down syndrome, where a percentage of the cells in the patient have three copies of chromosome 21, and the remainder of the cells have the expected two copies. Mosaicism is also seen with sex chromosome

aneuploidies, most notably mosaic Turner syndrome, where a subset of the cells examined show a 45,X complement and another population of cells from the patient may have a normal 46,XX or XY complement.

Partial aneuploidy may result from several mechanisms and may be inherited or can occur *de novo* seen only in the patient. For carriers of a balanced translocation, who have a rearrangement or exchange of material between chromosomes, the carrier parent has no net loss or gain of genetic material (balanced) and is usually phenotypically normal. However, segregation of these abnormal derivative chromosomes during meiosis can lead to offspring having an *unbalanced* rearrangement and its phenotypic consequences. Unbalanced rearrangements or translocations can also arise sporadically. This is often seen in recurrent deletion syndromes caused by the loss of genetic material from specific chromosomes (e.g., 1p–, 4p–, 5p–), with a resulting, often recognizable, phenotype.

Other chromosome microdeletions resulting in partial aneuploidy, have been mechanistically tied to aberrant recombination due to the presence of segmental duplications, or large "blocks" or segments of DNA that contain chromosome-specific repetitive sequences.[11] In these cases, the highly homologous repeats can mediate misalignment and nonallelic homologous recombination between two homologs. Segmental duplications are present in regions of the genome prone to rearrangements, such as the pericentromeric regions of 7q11, 15q11, 17q12, and 22q11, leading to the phenotypes seen in Williams–Beuren syndrome, Prader–Willi or Angelman syndrome, Charcot–Marie–Tooth disease or hereditary neuropathy with liability to pressure palsies, and the recurrent 22q11.2 deletion syndromes, respectively.[12]

Fluorescence *in situ* hybridization (FISH)

While deletions and duplications larger than 5 Mb may be observed by standard chromosome analysis, smaller rearrangements are generally not visible. These submicroscopic deletions and duplications are often referred to as copy number variants (CNVs). CNVs may be detected by fluorescence *in situ* hybridization or FISH, which utilizes fluorescently labeled DNA molecules ("probes") that bind to a specific region of interest in the genome, followed by visualization of the hybridization patterns using fluorescence microscopy.[13–15] FISH has many advantages including rapid turnaround time (same-day or overnight for certain specimen types), improved resolution compared to karyotype (can detect changes as small as 100 to 200 kb, depending on probe design), high sensitivity for mosaicism, and the ability to interrogate non-dividing tissues, which means FISH may be performed on a wide variety of specimen types. However, a limitation of FISH is that it is only informative for the specific regions being targeted and therefore requires prior knowledge of specific conditions to test for; even then, it may miss atypical rearrangements of these regions that are outside the region targeted by the FISH probes.

Chromosomal Microarray Analysis (CMA)

Another technology which has facilitated the identification of CNVs is chromosomal microarray analysis, which can identify submicroscopic losses and gains of chromosomal material (typically less than 5 Mb) that cannot be seen by standard karyotyping.[16–18] Chromosomal microarrays use DNA probes which are arrayed on a glass slide or other solid support, and patient DNA is fragmented and fluorescently labeled before being hybridized to the array (Fig. 28.2).[14,19] Microarray testing can be performed for either a targeted region, or more commonly, in a genome-wide fashion. One approach known as array comparative genomic hybridization (array CGH or aCGH) involves hybridizing both patient and control DNA, each labeled with different colored dyes, followed by comparison of the relative fluorescence intensities, identifying regions where the patient DNA shows a copy number loss or gain relative to the control. Another commonly used technique is single nucleotide polymorphism (SNP) chromosomal microarray, which includes probes targeting polymorphic regions in the human genome and involves an *in silico* comparison of fluorescence intensities to a set of control data. The resolution of an array depends on the density of probes; most genome-wide array platforms can detect copy number variants as small as 50 to 100 kb (kilobases). Targeted arrays with a high density of probes surrounding genes of interests may be able to detect smaller variants, such as those involving a single exon of a gene.

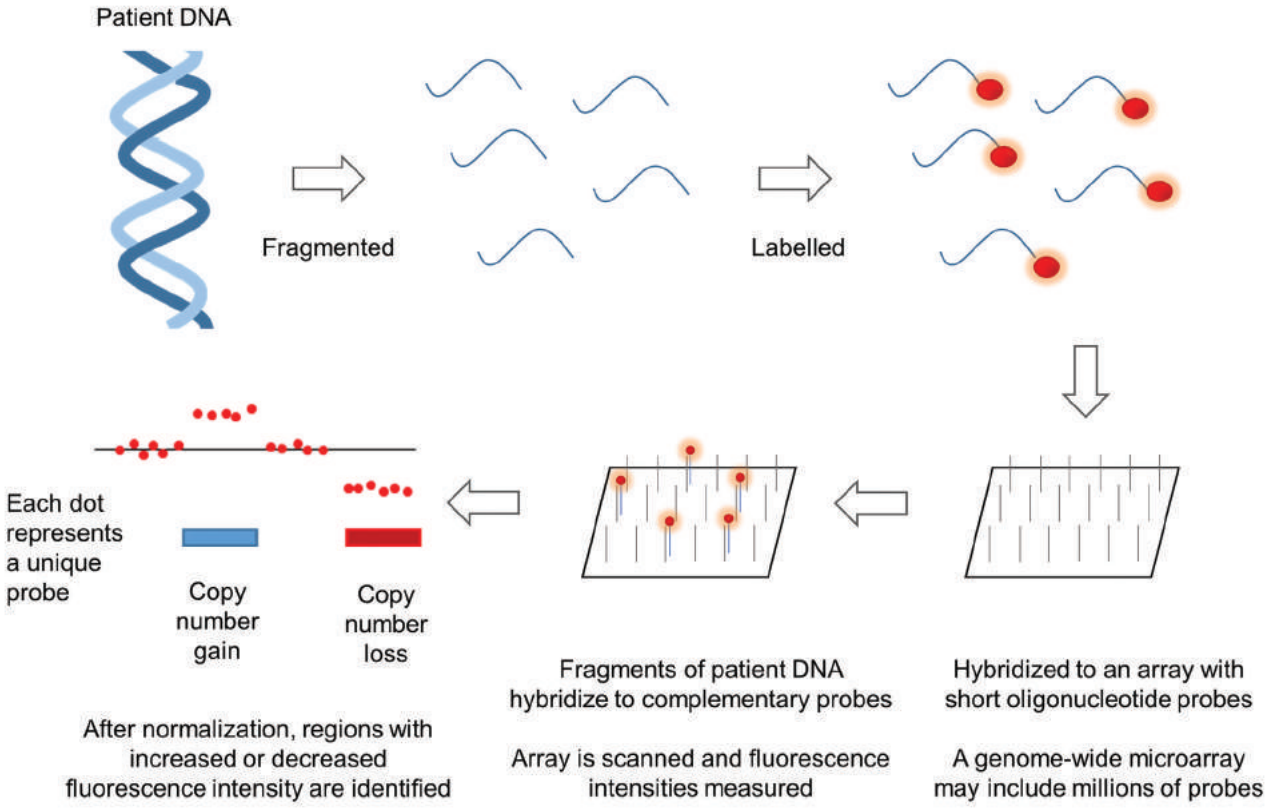
Microarray-based methods are focused on detecting copy number changes, while structural rearrangements that do not affect copy number such as balanced translocations or inversions are not detectable by this method. With a single test, microarrays can detect copy number variants in a genome-wide approach, revealing disorders usually identified by cytogenetic analysis and multiple individual FISH studies. The use of high-resolution microarrays in infants with multiple congenital anomalies has, in many cases, led to the identification of a specific genotype, with clinical evaluation then further defining the associated phenotype. In addition, SNP arrays include probes that can detect known polymorphisms present in the human genome, which may help identify regions of homozygosity, uniparental disomy (UPD), identity-by-descent, triploidy, mosaicism, chimerism, or maternal cell contamination.

In the postnatal setting, for patients with developmental delays/intellectual disability, autism spectrum disorder, or multiple congenital anomalies, CMA allows an increased diagnostic yield (approximately 15% to 20%) compared to G-banded karyotypes.[17] In the prenatal setting, in cases with ultrasound abnormalities and a normal karyotype, CMA provides additional information in 6% to 7% of cases.[20] Whereas microarray analysis can afford a robust and exceptional level of resolution from a diagnostic perspective, one major difficulty with interpretation of the results lies in assigning causality and clinical significance to the multiple alterations that are detected in each individual. Toward this end, the use of online databases (e.g., Database of Genomic Variants, http://dgv.tcag.ca/dgv/app/home) with information on normal variation in multiple ethnic populations and testing the infant's unaffected parents can help in discerning whether a copy number change is likely the cause of the patient's clinical features.

Trisomies

Down Syndrome (Trisomy 21)

Lejeune, Gautier, and Turpin (1959) demonstrated that trisomy of human chromosome 21 caused the constellation of findings recognized as Down syndrome (Fig. 28.3). This chromosome disorder was the first to be described and is the most common viable autosomal trisomy, occurring in approximately 1 in 700 live births in the United States.[21] The vast majority (>90%) occur secondary to meiotic nondisjunction, and a pronounced maternal age effect is encountered. Approximately 4% of cases are caused by a translocation that could be either *de novo* or inherited from a balanced translocation-carrier parent that subsequently becomes



• **Fig. 28.2** Example of a typical workflow for chromosomal microarray analysis. Patient DNA is fragmented and labeled, before being hybridized to an "array" of oligonucleotide probes on a solid support such as a glass slide. Following a period of hybridization and further processing, the arrays are scanned and the relative fluorescence intensity is measured. Normalization may be performed by concurrent hybridization of a control sample labeled with a different color (array CGH) or by a computational, *in silico* comparison to a set of controls (e.g., SNP microarray). *CGH*, comparative genomic hybridization; *SNP*, single nucleotide polymorphism.

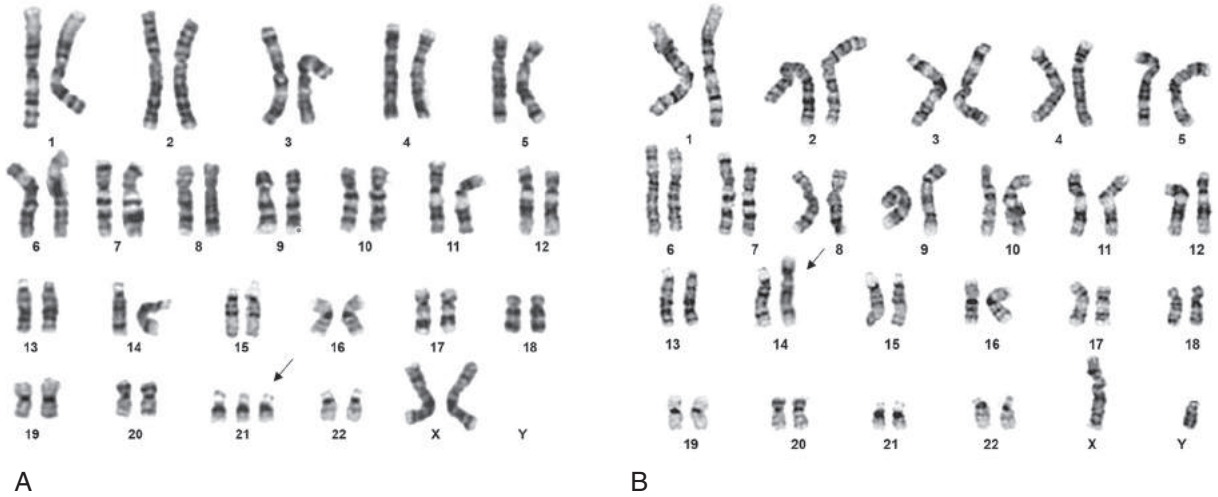
• **Fig. 28.3** Child with Down syndrome (trisomy 21). Some of the typical features such as epicanthal folds, flattened nasal bridge, posterior rotated ears, and fifth finger clinodactyly are demonstrated.

unbalanced causing trisomy in the fetus. Typically, the translocated chromosome 21 rearranges with another acrocentric chromosome, often chromosome 14, resulting in a Robertsonian translocation (Fig. 28.4). Mitotic nondisjunction, or mosaic Down syndrome, has been demonstrated in approximately 3% to 5% of cases as well, with variable features ranging from normal to a typical Down syndrome phenotype.

Clinical Features

It is the more common occurrence of Down syndrome in babies of older mothers that led to the recommendation for prenatal karyotyping for advanced maternal age (>35 years at the time of conception). Diagnostic samples are usually obtained by amniocentesis after 15 weeks' gestation or CVS at 10 to 12 weeks' gestation. Maternal serum analyte testing is recommended for prenatal screening purposes for all pregnant women, with results showing low alpha fetoprotein, low unconjugated estriol, and elevated total human chorionic gonadotropin levels. Noninvasive prenatal screening is also accepted as an initial study for fetal aneuploidy and is particularly robust for detecting trisomy 21.[7] Associated ultrasonographic findings for Down syndrome, including a cardiac defect, shortened long bones, underdeveloped fetal nasal bone, nuchal translucency or thickening, echogenic small bowel, and duodenal atresia ("double-bubble" sign), may be seen in 50% to 60% of fetuses.

Most patients with Down syndrome, if it is not diagnosed prenatally, are usually recognized at birth because of the



• **Fig. 28.4** Karyotypes that may be found in patients with Down syndrome or DS. (A) This case shows an extra copy of chromosome 21 (arrow), with 47 chromosomes total [47,XX,+21]. Standard trisomy is seen in ~95% of DS cases. (B) This case still shows three copies of chromosome 21, but the extra copy of 21 is present on chromosome 14 (arrow) in a (14;21) Robertsonian translocation, and there are 46 chromosomes total [46,XY,der(14;21)(q10;q10),+21]. Robertsonian translocations are observed in approximately 4% of DS. *(Courtesy UCLA Cytogenetics Laboratory.)*

well-established phenotypic features. The constellation of associated physical findings includes brachycephaly, the presence of a third or confluent fontanelle, upward-slanted palpebral fissures, epicanthal folds, Brushfield spots in the irises, flattened nasal root, small posteriorly rotated ears with overfolded superior helices, prominent tongue, short neck with excess nuchal skin, single palmar creases, brachydactyly, fifth-finger clinodactyly, exaggerated gap between the first and second toes, open field hallucal pattern, and hypotonia (see Fig. 28.3). Often the physical features conform to an easily distinguishable phenotype, but in some cases prematurity or ethnic variation can make a clinical diagnosis less straightforward. An immediate karyotype is indicated to confirm the diagnosis and its mechanism (e.g. trisomy or translocation) and to provide accurate recurrence risk counseling for the family. Malformations involving many organ systems have been described in Down syndrome, and whether the diagnosis is known prenatally or determined in the newborn period, several clinical investigations are warranted when this diagnosis is suggested.[22] The most common malformation is congenital heart disease (seen in over 50% of cases), which may require surgical intervention. Atrioventricular canal defects are often encountered (~40%), although ventricular septal defects (VSDs), atrial septal defects (ASDs), tetralogy of Fallot, and patent ductus arteriosus (PDA) are all described in the disorder. An echocardiogram is indicated in all cases, and medical and surgical interventions for cardiac lesions are routine. Gastrointestinal malformations, especially duodenal atresia (2% to 5%), in addition to Hirschsprung disease and less frequently encountered conditions, such as esophageal atresias, fistulas, and webs throughout the tract have been described. It is critical to carefully monitor the baby's feeding and bowel function before considering discharge from the nursery.

Although growth parameters can be within the normal range at birth, significantly decreased postnatal growth velocity is encountered in these patients. Separate growth curves have been devised for patients with Down syndrome because growth retardation involving height, weight, and head circumference has been well documented. However, the most recent health supervision guidelines for patients with Down syndrome recommend that patients be assessed on the basis of the World Health Organization or Centers for Disease Control and Prevention growth curves. An initial ophthalmologic evaluation is also indicated in the first few months of life and then annually, because strabismus, cataracts, myopia, and glaucoma have been shown to be more common in children with Down syndrome. In addition, hearing loss of heterogenous origin is present in approximately half of patients, with middle ear disease contributing to this problem.

Spinal cord compression caused by atlantoaxial subluxation from ligamentous laxity and subsequent neurologic sequelae can be a complication of the disorder. Radiographs are obtained in the early childhood years when there is concern for myopathic symptoms related to spinal cord compression (weakness, abnormal reflexes, incontinence, etc.). Physicians should be vigilant in evaluating the cervical spine, especially before procedures requiring positioning for anesthesia. Other associated disorders that merit screening are hypothyroidism in approximately 5% of patients, seen with the presence of thyroid autoantibodies. Initial evaluation occurs with newborn screening programs, followed by additional measurement of thyroid-stimulating hormone and free thyroxine levels at 6 months, 12 months, and then yearly thereafter. Hematopoietic abnormalities are common, and a complete blood count with differential should be performed at birth and later in infancy in accordance with published guidelines. Approximately 5% to 10% of newborns develop transient myeloproliferative disorder (TMD), which usually resolves spontaneously, but in 20% to 30% of cases these children will go on to develop myeloid leukemia of Down syndrome (ML-DS) by age 2 to 4 years.[23,24] Overall, the relative risk of leukemia is elevated in children with Down syndrome, who have a greater than 100-fold increased risk for myeloid leukemia (in particular, acute megakaryoblastic leukemia) and a 27-fold increased risk for acute lymphoblastic leukemia or ALL, in particular B-ALL.[24] Children with ML-DS show significantly better outcomes than non-DS children with AML, while children with DS-ALL have worse outcomes compared to non-DS cases, due to higher relapse rates, increased risk of infection, treatment-related

mortality, and induction failure.[24] Survival of patients with Down syndrome is shorter after a diagnosis of acute lymphoblastic leukemia than in diploid patients.[25] There is also an increased risk of iron-deficiency anemia, with recommended screening to include annual hemoglobin level measurement starting at 12 months of age then annually thereafter. If the hemoglobin level is low, then a complete blood count with iron studies should be performed.

Patients with Down syndrome demonstrate a wide range of developmental abilities, with highly variable personalities and behavioral phenotypes as well.[26] Central hypotonia with concomitant motor delay is most pronounced in the first 3 years of life, as are language delays. Therefore, immediate and intensive early intervention and developmental therapy are critical for maximizing the developmental outcome. A range of intellectual ability has been described, with conflicting data on genetic and environmental modifiers of outcome.[25] Seizure disorders occur in 5% to 10% of patients, often manifesting themselves in infancy.

The most common causes of death in patients with Down syndrome are related to congenital heart disease, infection (e.g., pneumonia) that is thought to be associated with defects in T-cell maturation and function, and malignancy.[27] Once medical and surgical interventions for the correction of associated congenital malformations are complete and successful, the long-term survival rate is good. However, less than half of patients survive to 60 years, and less than 15% survive past 68 years—and neurodegenerative disease with features of Alzheimer disease is encountered in many patients older than 40 years. Men with Down syndrome are almost always infertile, whereas small numbers of affected women have reproduced.[25]

In counseling the family of a newborn in whom Down syndrome has been diagnosed, it is important to include the organ systems affected in the baby and the severity of each malformation when one is defining a prognosis. Above all, the wide variability of the phenotype should be emphasized, with a care plan tailored to the needs of the individual patient.

Genetic Counseling

In cases with non-translocation Down syndrome (including mosaic cases) parental karyotypes are generally not analyzed, because the karyotypes are normal in virtually all cases. After having one child with Down syndrome, a mother's recurrence risk for another affected child is approximately 1% higher than her age-specific risk.[28,29] This fact is especially significant in younger mothers, whose age-specific risks are low. If a *de novo* translocation resulting in Down syndrome is found, the recurrence risk is less than 1%. If the mother is found to carry a constitutional balanced Robertsonian translocation, the risk of another translocation Down syndrome fetus is approximately 15% at the gestational age when amniocentesis is offered and 10% at birth. However, if the father is the translocation carrier, the recurrence risk is significantly smaller, approximately 1% to 2%.[30] While array-based diagnostic techniques will identify the copy number change associated with the trisomy, structural rearrangements such as Robertsonian translocations are not detected. In this situation, a karyotype will provide information regarding the mechanism of the trisomy, which is needed for accurate recurrence risk counseling.

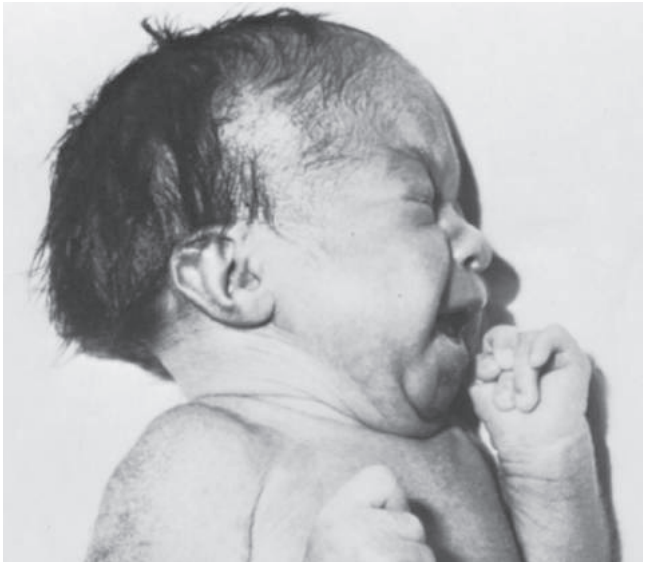
Trisomy 18 (Edwards Syndrome)

Trisomy 18 is encountered in 1.07 cases per 10,000 live births and 4.08 cases per 10,000 births overall, and it is associated with a high rate of intrauterine demise.[31] It is estimated that only 5% of conceptuses with trisomy 18 survive to birth. The rates of pregnancy loss have been estimated between 70% and 72% in cases with a viable fetus at 12 weeks, and 59% between 24 weeks and term.[32,33] Findings on prenatal ultrasonography can raise suspicion for the disorder—growth retardation, oligohydramnios or polyhydramnios, heart defects, myelomeningocele, clenched fists, and limb anomalies. Diagnostic testing is recommended when prenatal ultrasonography findings are suggestive of this condition. Maternal serum screening can show low values for alpha fetoprotein, unconjugated estradiol, and total human chorionic gonadotropin. The PPV of NIPS for trisomy 18 is currently 45% for a 36 year-old woman at 16 weeks' gestation (https://www.perinatalquality.org/Vendors/NSGC/NIPT/), thus high resolution anatomy ultrasounds and diagnostic testing by amniocentesis are currently recommended for follow up.[7]

Clinical Features

The classical phenotypic features reported at birth include intrauterine growth restriction (1500 to 2500 g at term), small narrow cranium with prominent occiput, open metopic suture, low-set posteriorly rotated ears, and micrognathia with small mouth. Characteristic clenched hands with overlapping digits, an excess of arches on dermatoglyphic examination, hypoplastic nails, and "rocker-bottom" feet or prominent heels with convex soles (Fig. 28.5) are described. Additional malformations encountered in this syndrome include congenital heart disease (ASD, VSD, PDA, pulmonic stenosis, aortic coarctation) in over 70% of patients,[34] cleft palate, clubfoot deformity, renal malformations, brain anomalies, choanal atresia, eye malformations, vertebral anomalies, hypospadias, cryptorchidism, and limb defects, especially radial ray defects.

Historically, the prognosis in this disorder is extremely poor with survival estimates of 37.2% at 28 days and 13.4% at one year.[35] Death is related to central apnea, infection, and congestive heart failure. The newborn period is characterized by poor feeding and growth, typically requiring tube feedings. Universal poor growth and profound intellectual disability have been documented, with developmental progress typically leveling at that of a 6 to 8-month-old infant.[34] Malignant tumors such as



• **Fig. 28.5** Newborn with trisomy 18, showing prominent occiput, characteristic facial appearance, and clenched hands.

hepatoblastoma and Wilms tumor have been described in some survivors.[36] There is emerging evidence that various interventions can improve the survival and quality of life for the child and the family, and this should be discussed with the parents during a prenatal or postnatal visit.

Families have indicated a desire to be able to take the infant home and spend time as a family, understanding the dismal prognosis. Many institutions have determined specific protocols typically based on each infant's clinical findings such as their ability to breathe without mechanical ventilation, for example, when considering surgical interventions such as cardiac repair. In general, those who do not require mechanical ventilation have less complex cardiac defects and do not show other major malformations, (e.g. omphalocele) benefit the most from intervention.[37] However, it is important to evaluate each infant on an individual basis during such considerations and have a team-based plan involving neonatology, cardiology, cardiac surgery, genetics, and other appropriate specialists when considering intervention.

Genetic Counseling

The estimate of the recurrence risk for trisomy 18 in a future pregnancy is a 1% risk over the maternal age-specific risk for any viable autosomal trisomy. Trisomy occurring from a structural rearrangement, such as a translocation, warrants parental karyotype analysis before the recurrence risk can be assessed.

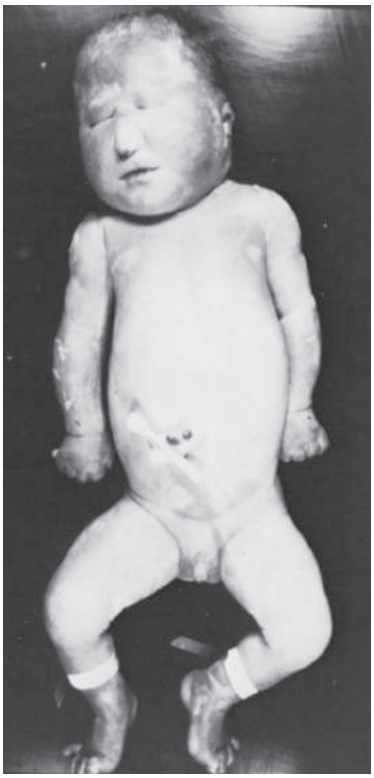
Trisomy 13 (Patau Syndrome)

The frequency of trisomy 13 is estimated to be 0.55 per 10,000 live births and 1.68 per 10,000 births overall.[31] It has been estimated that approximately 2% to 3% of conceptions with trisomy 13 survive to birth, with rates of pregnancy loss estimated at 49% to 50% for cases with a viable fetus at 12 weeks and 35% between 24 weeks and term.[32,33] As with other trisomies, abnormal NIPS findings or the presence of fetal ultrasonographic findings should prompt diagnostic testing by CVS or amniocentesis that can result in a prenatal diagnosis of trisomy 13. Currently, the calculated PPV of NIPS for trisomy 13 is 26% for a 36-year-old woman at 16 weeks' gestation, underscoring the need for appropriate genetic counseling and formal diagnostic testing.

Clinical Features

Trisomy 13 is associated with midline anatomic malformations including congenital heart disease, cleft palate, holoprosencephaly, renal anomalies, and postaxial polydactyly (Fig. 28.6). In addition, microcephaly, eye anomalies, and scalp defects can suggest the diagnosis. Brain malformations including holoprosencephaly are found in more than half of patients and can feature concomitant seizure disorders. Microcephaly, split sutures, and splayed fontanelles are encountered. A scalp defect (cutis aplasia) is relatively specific to the disorder, found in 50% of cases. Eye malformations, including iris colobomas and hamartomatous cartilage "islands," can be seen on fundoscopic examination.

Congenital heart disease is present in approximately 80% of patients, commonly VSD, ASD, PDA, or dextrocardia are seen. Minor anomalies of the extremities, such as postaxial polydactyly, single palmar creases, and hyperconvex narrow fingernails, are also seen. The fingers can be flexed or overlapped with camptodactyly noted. An increased frequency of nuclear projections in neutrophils, giving a drumstick appearance similar to that of Barr bodies, has been described. This finding would be especially striking in males, in whom Barr bodies would not be expected.



• **Fig. 28.6** Stillborn with trisomy 13. The facial appearance is that of cebocephaly, which is associated with holoprosencephaly. There is an extra digit on the ulnar border of the right hand.

As with trisomy 18, prognosis for the fetus with trisomy 13 is extremely poor, with survival estimates of 25.5% at 28 days and 11.5% at one year.[35] Intellectual disability is profound, and many patients have blindness and deafness as well. Feeding difficulties are typical. Similar to trisomy 18, specific interventions can increase the survival of some trisomy 13 infants and may improve their overall quality of life. A discussion with the parents should be considered regarding these possibilities but tailored to the specific defects seen in each individual infant. An institutional assessment protocol can be a helpful approach to determining when intervention may be offered.[37–39] For cases of trisomy 13 and trisomy 18 that are recognized for their exceptionally poor outcomes, early involvement of providers with experience in palliative care approaches can be beneficial for both the infant's family as well as the primary neonatal care team.

Genetic Counseling

Recurrence risk data suggest that, as with trisomy 18, the chances of having a child with *any* trisomy after a pregnancy affected by trisomy 13 is rare. The estimated risk is 1% higher than the maternal age–related risk for the recurrence of any viable autosomal trisomy in a subsequent pregnancy. Parental karyotypes are recommended in cases where a translocation has been identified in the affected infant in order to provide accurate recurrence risk counseling.

45,X (Turner Syndrome)

In early female embryogenesis, two X chromosomes are required for normal development. Turner syndrome, a phenotype associated with loss of all or part of one copy of the X chromosome in

a female conceptus, occurs in approximately 1 in 2500 female newborns. The 45,X karyotype or loss of one entire X chromosome accounts for approximately half of the cases. A variety of X chromosome anomalies—including deletions, isochromosomes, ring chromosomes, and translocations—account for the remainder. It is important to note that approximately 0.1% of fetuses with a 45,X complement survive to term; the vast majority (>99%) are spontaneously aborted underscoring the requirement for two normal X chromosomes during early embryonic development. Additional studies have shown that in approximately 80% of cases, it is the paternally derived X chromosome that is lost.[40]

Clinical Features

There is wide phenotypic variability in patients with Turner syndrome. Features present at birth include short stature, webbed neck, craniofacial differences (epicanthal folds and high arched palate), hearing loss, shield chest, renal anomalies, lymphedema of the hands and feet with nail hypoplasia, and congenital heart disease. Typical cardiac defects include bicuspid aortic valve, coarctation of the aorta, valvular aortic stenosis, and mitral valve prolapse.

Growth issues, especially short stature, are the predominant concern in childhood and adolescence; the mean adult height of patients with Turner syndrome is 135 to 150 cm without treatment. In cases with evidence of growth failure, growth hormone therapy is recommended with initiation of treatment around 4 to 6 years of age and preferably before 12 to 13 years.[41] Primary ovarian failure caused by gonadal dysplasia can result in delay of secondary sexual characteristics and primary amenorrhea. Cyclic hormonal therapy can be used to reflect the process of normal puberty[42] to aid the development of secondary sex characteristics and menses as well as to support bone mass. Infertility, related to gonadal dysplasia, is well described and has been successfully treated with assisted reproduction techniques and donor oocytes. It is important to evaluate the patient for structural cardiovascular defects before pregnancy.

In terms of intellectual development, specific difficulties with spatial and perceptual thinking can lead to a lower performance scores on neuropsychological testing; however, this syndrome is not characterized by intellectual disability.

Triploidy (69,XXX or 69,XXY)

As its name implies, triploidy is a karyotype containing three copies of *each* chromosome. The mechanisms that lead to this state include fertilization of the egg by two different sperm (dispermy) and complete failure of normal chromosome separation in maternal meiosis. The vast majority of triploid fetuses are spontaneously aborted, accounting for up to 15% of chromosomally abnormal pregnancy losses. Live births of affected fetuses are rare, and reports of survival beyond infancy are rarer still. Mosaicism with combinations of diploid and triploid cells (mixoploid) has also been documented. Malformations, including hydrocephalus, neural tube defects, ocular and auricular malformations, cardiac defects, and 3 to 4 syndactyly of the fingers are known findings. In cases where the extra set of chromosomes is paternal in origin (diandry), the placenta is often abnormal, typically large and cystic (partial hydatidiform moles). In cases where the extra set of chromosomes is maternal in origin (digyny), the placenta is small and non-cystic, with a severely growth-restricted fetus and relative macrocephaly.

Deletion Syndromes

In addition to the aneuploid conditions described above, partial monosomy of a chromosome can lead to recurrent recognizable patterns of malformation. Three well-described syndromes that are associated with the deletion or loss of genetic material from the short, or *p* arms of chromosomes 1, 4, and 5 are discussed below. All of these syndromes are associated with heterozygous deletions that involve the loss of many genes located in a specific region, resulting in their haploinsufficiency. While the three disorders below are recurrent, the deletion sizes vary, contributing to clinical variability since the gene content of the deleted region can be quite different from patient to patient. In general, larger deletions with more genes showing copy number loss tend to have more severe clinical consequences.

Chromosome 1p Deletion Syndrome (1p–)

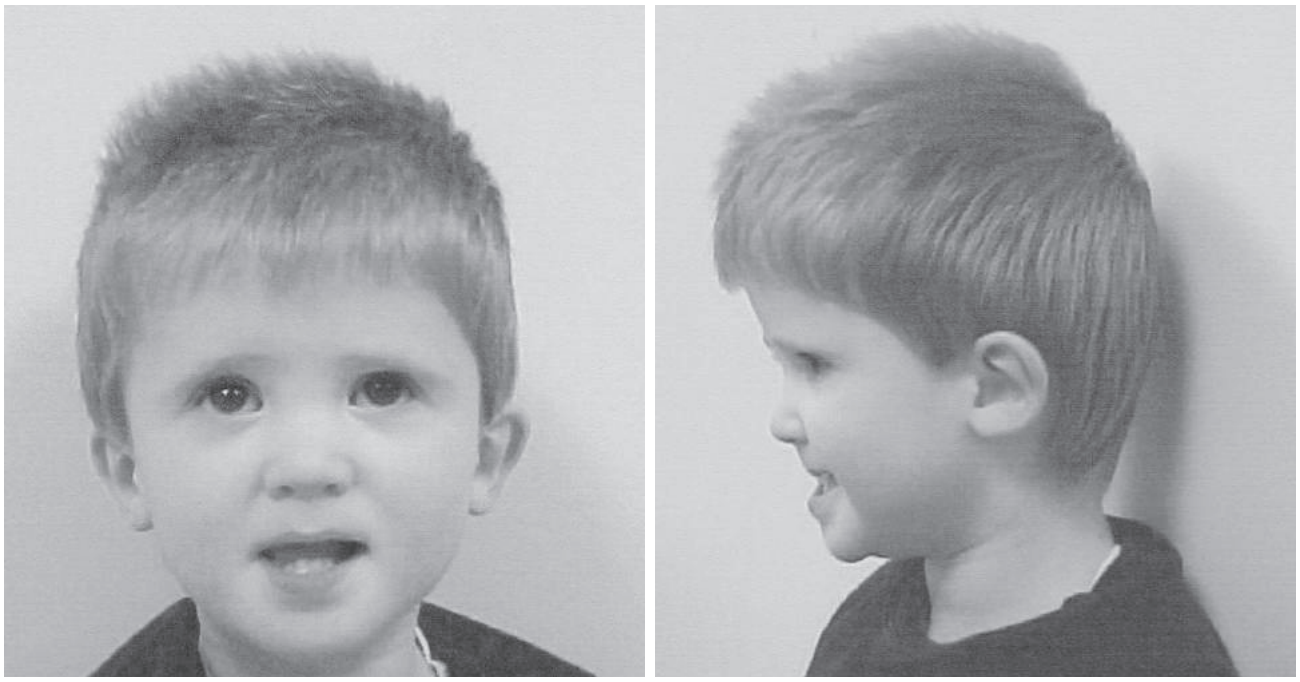
Monosomy for the distal short arm of chromosome 1, or deletion 1p36, has been associated with a constellation of clinical findings. A characteristic facies consisting of frontal bossing, large anterior fontanel, flattened midface with deep-set eyes, and developmental delay has been described (Fig. 28.7). Orofacial clefting, hypotonia, seizures, deafness, and cardiomyopathy are also noted.

This deletion syndrome is estimated to occur in approximately 1 in 5000 live births and is the most frequently occurring subtelomeric deletion.[43] Greater recognition of the phenotype and widespread use of chromosomal microarrays has led to improved diagnosis of this condition. Most deletions arise *de novo* in the patient with approximately 3% attributable to malsegregation of a balanced parental translocation. The size of the deletion differs amongst patients, from submicroscopic (<5 Mb) to large, cytogenetically visible deletions larger than 30 Mb. A genotype-phenotype correlation has been described with certain genes within the deleted region being associated with different aspects of the phenotype.[44]

Wolf–Hirschhorn Syndrome (4p–)

Distal deletions in the short arm of chromosome 4 are associated with a recognizable pattern of malformation. This syndrome is estimated to occur in 1 in 50,000 to 1 in 20,000 births[45] and has features including intrauterine growth restriction, microcephaly, midline structural defects such as cleft lip and cleft palate, cardiac septal defects, and hypospadias. The characteristic facial features are described as resembling a *Greek helmet*, as evidenced by hypertelorism with epicanthi, a high forehead with a prominent glabella, and a beaked nose. Prominent, low-set ears are also seen. Hypotonia, failure to thrive, and developmental delay are common, with approximately 30% mortality in the first year of life. Many patients have lived well into childhood and even into adulthood, although profound growth impairment and intellectual disability are usually described and are often accompanied by seizures.

The 4p deletions can be cytogenetically visible on karyotype analysis, although small submicroscopic deletions have also been described and range from 2 Mb to 30 Mb.[46] In cases where the clinical features are suggestive of the syndrome but the karyotype is not revealing, further cytogenetic analysis has been performed using specific 4p FISH probes or more commonly, chromosomal microarray. Microarray analysis also provides information about the relative size and gene content of the deleted region.



• **Fig. 28.7** Child with deletion in chromosome 1p36.

Genotype-phenotype correlations and critical regions for different aspects of the phenotype have been described.[47] Approximately 55% of patients have a 4p deletion as the only cytogenetic abnormality, while others have more complex abnormalities including ring chromosome 4, mosaicism for 4p loss, or an unbalanced translocation involving chromosome 4.[45] More than 80% of 4p deletions arise *de novo* in the patient with minimal risk of recurrence. In the 10% to 15% of cases resulting from a translocation, analysis of parental samples is indicated for appropriate recurrence risk counseling.

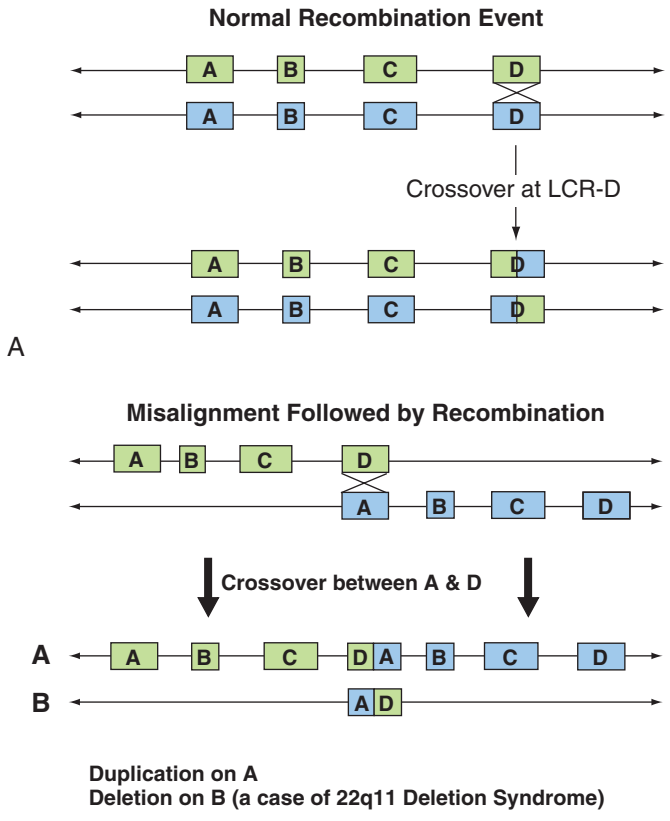
Cri du Chat Syndrome (5p–)

Partial monosomy of chromosome 5p is seen in approximately 1 in 15,000 to 1 in 50,000 live births[48] and is associated with a multiple congenital anomaly syndrome named for the unusual cry of the affected babies, described as similar to that of a cat or *cri du chat*. The constellation of features associated with this disorder includes low birth weight, microcephaly, round face, hypertelorism or telecanthus, downward-slanting palpebral fissures, epicanthi, and broad nasal bridge. Hypotonia, laryngomalacia, and cardiac defects are also seen, including ASD, VSD, and tetralogy of Fallot. Early issues include failure to thrive and pronounced developmental delay. The unusual cry resolves during infancy, and survival into adulthood is possible but is often marked by severe intellectual disability. Intensive therapy appears to provide benefit, and more sensitive measures of cognition demonstrate clearly better receptive language skills than expressive language ability. Therefore, affected children may understand more complex verbal language than their expressive skills can demonstrate.[49]

It is estimated that almost 100 genes are lost when the putative critical region from 5p15.2 to p15.33 is deleted,[50,51] and different regions associated with different aspects of the phenotype have been described.[48] Close to 90% of 5p deletions arise *de novo* in the affected child, incurring a minimal risk of recurrence (<1%). The remainder arise from malsegregation of a balanced rearrangement in a carrier parent, which would be associated with a risk of recurrence of an unbalanced karyotype in a future liveborn infant (risk varies depending on the specific translocation). Parental chromosome analysis is indicated for proper recurrence risk counseling.

Segmental Duplications and Microdeletion/Microduplication Syndromes

A greater appreciation for the complexity of the human genome and its structure was afforded with the completion of the human genome sequence. This work focused attention on regions of the genome susceptible to rearrangement. The presence of such regions appears to have a significant role in the origin of several genetic disorders.[11,12] These disorders result from inappropriate dosage of crucial genes in a given genomic segment via structural mechanisms (deletion or duplication) and in some cases, functional mechanisms (e.g. imprinting). Many of the regions prone to recurrent rearrangements have a common element: the presence of large, chromosome-specific segmental duplications. These repeat structures appear to mediate misalignment and aberrant crossover during recombination, resulting in inversions, deletions, and duplications within chromosomes (Fig. 28.8). Segmental duplications are large, low copy repeat structures mostly unique to humans and are localized to single chromosomes or within a single chromosomal band. Examples of the genomic disorders they cause include hemophilia A (inversion of Xq28), Sotos syndrome (where a number of patients have a deletion in 5q35), Smith–Magenis syndrome (deletion of 17p11.2), Charcot–Marie–Tooth disease (interstitial duplication in 17p12), and the reciprocal deletion of this same region in 17p12, leading to hereditary neuropathy with liability to pressure palsies, and a small percentage of patients with neurofibromatosis type I (deletion involving 17q11.2).



• **Fig. 28.8** (A) Alignment of low copy repeats before exchange. (B) Misalignment of low copy repeats before exchange can result in rearrangement. *LCR*, Low copy repeats.

In this section, we highlight several deletion syndromes that occur on chromosomes whose underlying genomic structure contains segmental duplications. Since the deletion endpoints coincide with the locations of the segmental duplications, these deletions tend to have recurrent deletion sizes and gene content. These include Williams–Beuren syndrome (involving 7q11.2), Prader–Willi syndrome (PWS) or Angelman syndrome (involving an imprinted region of 15q11 through 15q13), and 22q11.2 microdeletion syndrome (also known as DiGeorge or velocardiofacial syndrome). The latter is the most commonly occurring microdeletion syndrome in humans. It is important to note that in several of these microdeletion syndromes, reciprocal duplications of the exact same region may also occur (see Fig. 28.8) and can show a different phenotype than the deletion of the same region. In general, duplication syndromes cause fewer anatomic defects and show a wider phenotypic variability, but they are often characterized by developmental delays with or without behavioral abnormalities.

More recently recognized recurrent microdeletion syndromes (e.g., 1q21.1, 3q29, 15q13.3, 16p11.2, and 17q21) were identified because of the increased use of chromosomal microarrays in the clinical and research settings. These regions are similarly flanked by segmental duplications, likely predisposing to their rearrangements. Many of the patients with these particular rearrangements do not receive a clinical diagnosis in the newborn period, since they may not present with significant anatomic malformations or dysmorphic features that prompt a genetic evaluation in the NICU; however, they can present in early childhood with variable clinical findings. Typically there is developmental delay, behavioral abnormalities (such as autism spectrum disorder), or intellectual disability. Many of these syndromes are complicated by incomplete penetrance, meaning that unaffected family members may also carry the genomic imbalance, which can be challenging for genetic counseling.

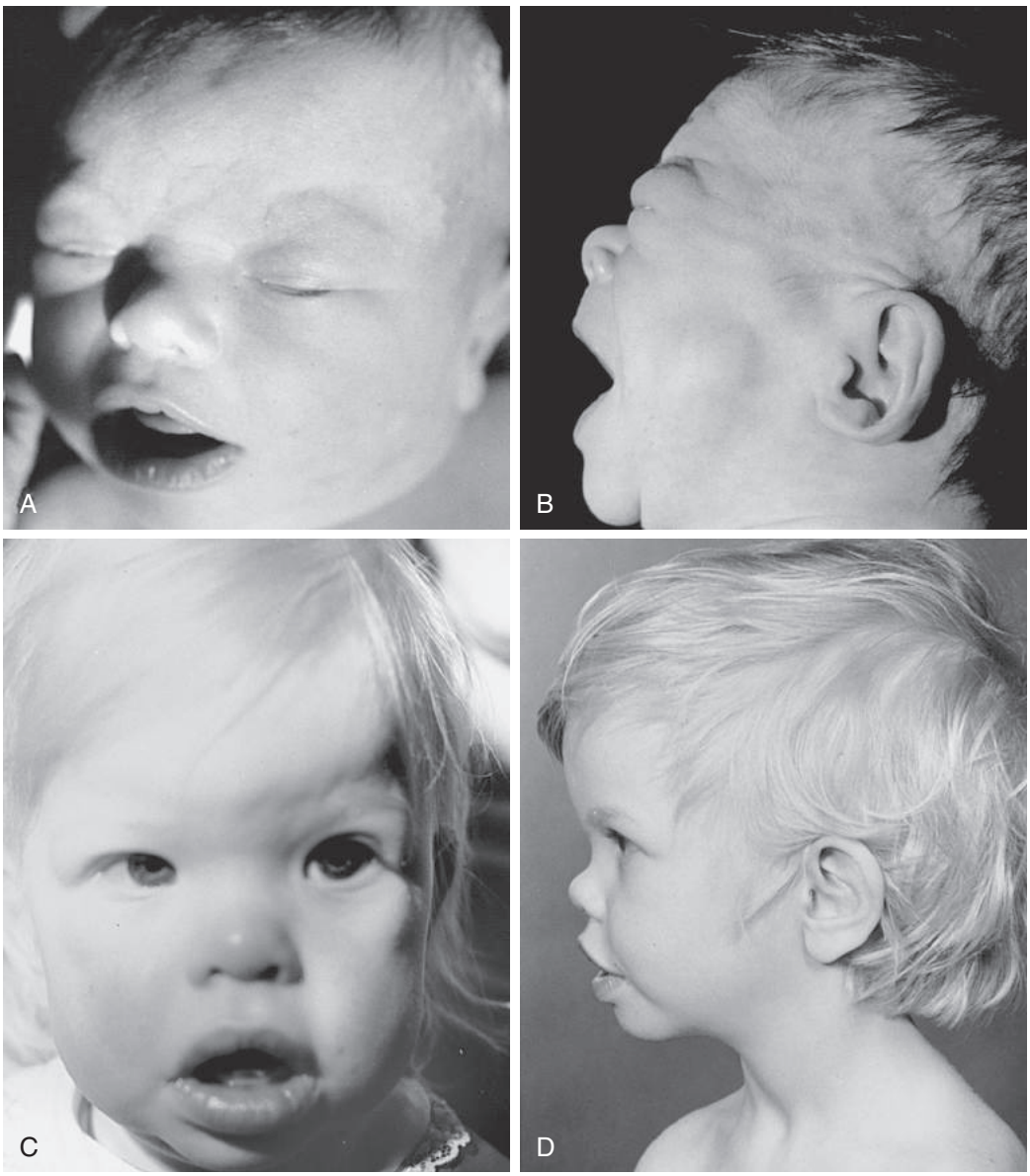
When an abnormality is identified by microarray analysis, the genes located in the affected region can be identified. It is then possible to decide what role, if any, the affected genes have in the observed phenotype. For example, deleted genes could be identified that may predispose a patient to cancer because of a germline loss of one copy of a tumor suppressor gene,[52] prompting careful surveillance for tumor formation. This underscores the utility of early identification of underlying chromosomal disorders for anticipatory guidance in clinical care.

Williams–Beuren Syndrome (7q11.2 Deletion)

The estimated incidence of Williams–Beuren syndrome (also known as Williams syndrome) is 1 in 10,000 live births. The phenotype has a variable spectrum but usually consists of distinctive facies, growth and developmental delay, cardiovascular anomalies, and occasionally infantile hypercalcemia (Fig. 28.9). Babies with Williams–Beuren syndrome usually show some degree of intrauterine growth restriction with mild microcephaly. Facial features include epicanthal folds with periorbital fullness of subcutaneous tissues, flat midface, anteverted nostrils, long philtrum, thick lips, large open mouth, and stellate irises that may not be discernible at birth. Many infants have cardiovascular anomalies; supravalvular aortic stenosis (SVAS) is the most commonly described defect, seen in more than 50% of cases. Pulmonary artery stenosis is also often encountered. It is interesting to note that isolated SVAS can also exist as a separate autosomal dominant trait and has been shown to occur from mutations within the elastin gene (*ELN*), which is located in the Williams syndrome region in chromosome 7q11.2. Patients with Williams–Beuren syndrome, have a deletion of 7q11.2 and are typically missing one entire copy of the elastin gene as well as other genes.

Hypercalcemia, which is manifested in approximately 10% of patients with this disorder is severe and persists through infancy. Umbilical and inguinal hernias are also associated features. Issues in infancy include feeding and growth problems, with pronounced irritability and colicky behavior. Hoarse voice, strabismus, hypertension, and joint mobility restrictions may develop later in childhood. In terms of development, the typical mild to moderate intellectual disability can be masked by relatively advanced language skills, although gross motor and visual–motor integration skills are typically affected. Attention-deficit disorders are common, and a characteristic outgoing personality is often described in affected children.

Many of the classic features of Williams–Beuren syndrome are not clearly discernible in the newborn period, but the diagnosis should be suggested in any child with SVAS, hypercalcemia, and facial features consistent with the disorder. The diagnosis can be confirmed quickly by chromosomal microarray analysis or FISH using probes specific for the deleted region of 7q11.2. Because the condition is typically sporadic and most deletions arise *de novo*, the risk of recurrence in subsequent pregnancies is minimal. An affected adult, however, would pass on the condition in an autosomal dominant manner, with a 50% risk of the disorder in his or her child.

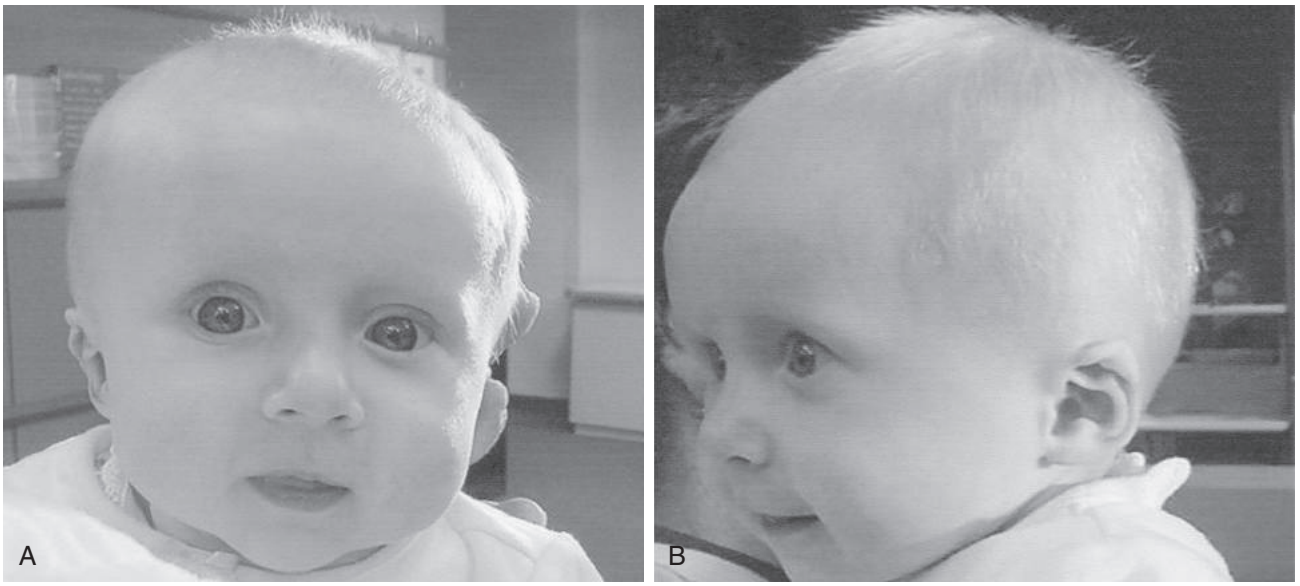


• **Fig. 28.9** Williams–Beuren syndrome. (A) Neonate with a coarse face, periorbital fullness, wide mouth, and thick lips with decreased Cupid's bow. (B) Neonate profile showing periorbital fullness, flat nasal bridge with full tip, and prominent cheeks. (C) Infant with periorbital fullness, flat nasal bridge, thick lips with decreased Cupid's bow, pouty lower lip, and low-set, full cheeks. (D) Infant profile showing dolichocephaly (increased anteroposterior diameter of head), a higher nasal bridge than in the neonate, full nasal tip, pouty lower lip, long neck, sloping shoulders, and part of pectus excavatum.

22q11.2 Deletion Syndrome

A deletion of 22q11.2 has been identified in most patients with the classically termed conditions DiGeorge, velocardiofacial, and conotruncal anomaly face syndromes, leading to the realization that these clinical entities all reflect features of the same genomic disorder.[53] The list of findings associated with 22q11.2 deletion syndrome is extensive and differs among patients even when the deletion sizes appear identical. Estimates indicate that 22q11.2 microdeletion syndrome occurs in approximately 1 in 1000 fetuses.[54] This disorder is the most common microdeletion syndrome occurring in humans, seen in 1:3000 live births and is a significant health concern in the general population.

The phenotype is characterized by a conotruncal cardiac anomaly and often aplasia or hypoplasia of the thymus and parathyroid glands. Most patients with a deletion can receive a diagnosis as newborns or infants with significant cardiovascular malformations, including interrupted aortic arch type B, truncus arteriosus, or tetralogy of Fallot, along with functional T-cell abnormalities, and hypocalcemia. In addition, facial dysmorphia may be present (Fig. 28.10), including hooded eyelids, hypertelorism, overfolded ears, bulbous nasal tip, a small mouth, and micrognathia. Since the initial report by DiGeorge in 1968,[55] the spectrum of associated clinical features has been expanded to include anomalies such as palate defects (overt or submucous clefts, velopharyngeal incompetence), vascular rings, feeding and



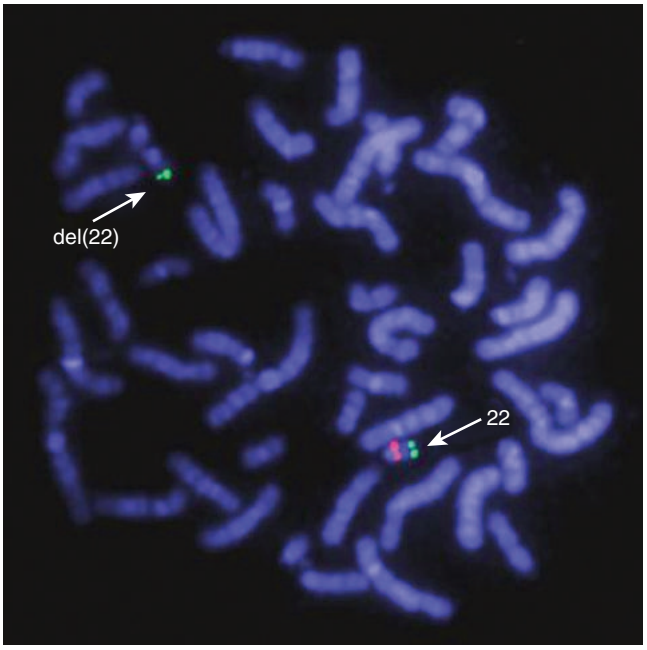
• **Fig. 28.10** Facial differences associated with 22q deletion syndrome. (A) Frontal view showing upslanting palpebral fissures, bulbous nose, and small chin. (B) Profile view showing deep set eyes and overfolded superior helices.

swallowing problems, gastroesophageal reflux, renal agenesis, and hypospadias.

Developmental delays or learning disabilities have been reported in most patients with 22q11.2 deletion syndrome, and a wide range of developmental and behavioral findings have been observed in young children.[56] In the preschool years, affected children were most commonly found to be hypotonic and developmentally delayed with language and speech difficulties. Severe or profound retardation was not seen, and one-third of patients functioned within the low average range.[56]

The majority of patients (80% to 90%) have the same large deletion, approximately 2.4 to 3 Mb that was historically detected by FISH (Fig. 28.11), but more commonly now by chromosomal microarray. This "typical" deletion includes approximately 50 genes and 7 micro ribonucleic acids.[53] The size of the deletion remains unchanged when it is inherited from an affected parent. However, the phenotype can be widely variable, even among affected members of the same family. Although smaller recurrent deletions that are half the size of the common deletion occur (1.5 Mb), a smaller size does not necessarily correspond to milder symptoms, making genotype–phenotype correlations difficult.

Most 22q11 deletions occur as *de novo* events, with less than 10% of them inherited from an affected parent. The prevalence of these de novo 22q11.2 deletions indicates a high rearrangement rate within this genomic region that is related to the presence of recombination-permissive segmental duplications in 22q11.[11,12,57]



• **Fig. 28.11** Fluorescence in situ hybridization study of a 22q deletion. The arrows point to both copies of the 22nd chromosome. One chromosome 22 shows a hybridization pattern involving a control probe *(green)* and the 22q11.2 region probe *(red)*. The other chromosome 22 shows a hybridization pattern of a control probe *(green)* and absent 22q11.2 region probe (*red*), suggesting this region is deleted. (Courtesy of Beverly S. Emanuel.)

Additional Microdeletion and Microduplication Syndromes

As mentioned previously, many genetic syndromes have been described with the increased use of chromosomal microarrays.[58,59] Several of the regions are also flanked by segmental duplications, which is the likely reason for their prevalence in diverse patient populations, and the occurrence of reciprocal rearrangements (deletion or duplication) of the same chromosomal region. Almost all of the patients with these more recently recognized deletions or duplications were not initially identified on the basis of their clinical features but were instead ascertained by microarray analysis.

Deletions of chromosome 1q21.1 with a size of approximately 1.35 Mb have been seen in patients with variable presentations, including developmental and behavioral abnormalities, mild facial dysmorphia, and microcephaly.[60,61] In the 3q29 microdeletion

syndrome, an approximately 1.6 Mb deletion was initially discovered[62] in patients with mild to moderate intellectual disability, microcephaly, and nonspecific facial dysmorphia. Duplications of this region have also been described in patients with developmental delays, intellectual disability, and microcephaly.[63] Reciprocal *duplications* of the region deleted in Williams–Beuren syndrome (7q11.23) are characterized by distinctive facial features, congenital anomalies, and intellectual and developmental disabilities including *poor* expressive speech in contrast to the deletion of the same region.[64]

The chromosome 15q13.3 region is distal to the more commonly known 15q11-q13 locus associated with Prader–Willi and Angelman syndromes and is prone to several recurrent deletions/duplications, with different breakpoints corresponding to the locations of segmental duplications in the region.[65] The most extensively documented is a 1.5 to 2 Mb deletion of 15q13.3, which is associated with variable phenotypes but typically includes seizures or abnormal electroencephalograms.[66] Interestingly, this deletion is often maternally inherited, suggesting that there might be an imprinting mechanism involved. A smaller 680-kilobase (kb) deletion within this region has also been described in patients with a range of neurobehavioral phenotypes.[67] Deletions of 16p12.2 can manifest as developmental and intellectual disability, cardiac malformations, epilepsy, hearing loss, renal and genital anomalies (the latter in males), and cleft lip/palate.[68] A recurrent 550-kb deletion of 16p11.2 has been described in approximately 1% of patients with autism[69] and its reciprocal duplication is associated with attention deficit–hyperactivity disorder and schizophrenia.[70,71] Chromosome deletions of 17q21.31 of approximately 500 to 650 kb were also found in patients with developmental delays, learning disabilities, and variable facial dysmorphia. Mutations of *KANSL1*, located within the 17q21.31 interval, can also be associated with the same phenotype.[72]

Disorders of Imprinted Chromosomes

A growing recognition of mechanisms regulating gene expression has emerged in the last few decades. Two of the most exciting concepts with important clinical correlates are imprinting and *UPD or uniparental disomy*. The term *genomic imprinting* implies that a whole region of a chromosome or a group of genes in a given region is subject to a difference in their expression that depends on whether they reside on the maternally inherited or the paternally inherited chromosome. In these cases, a genetic disorder might manifest itself on the basis of whether the genomic region was inherited maternally or paternally. The genes in an imprinted region are not necessarily mutated, but they are epigenetically marked such that the cell can distinguish between the maternal and paternal copies and coordinate expression on the basis of that distinction. At the molecular level, it appears that differences in the methylation of the DNA, and its replication and regulation at the transcriptional level, appear to be involved in this mechanism. The process of imprinting has become an area of important research initiatives, and there are now more than 270 known or predicted genes and chromosomal regions thought to be important in human disease (http://www.geneimprint.com/site/genes-by-species).

Prader–Willi Syndrome

It has been demonstrated that occasionally, instead of one copy of each chromosome being inherited from each parent, both copies of a given chromosome or chromosomal region can come from the same parent. This phenomenon, known as *uniparental disomy* or *UPD*, is associated with advanced maternal age. It becomes a significant issue when the chromosome involved is imprinted or has regions in it that are imprinted. UPD can occur as isodisomy, when two identical copies of a chromosome are inherited from the same parent (meiosis II error) or heterodisomy, when two different copies of a chromosome are inherited from the same parent (meiosis I error).

PWS involves the loss of activity from the paternally derived proximal long arm of chromosome 15 (15q11 to 15q13). This loss can occur through deletion or disruption of this region or through maternal UPD such that no paternal chromosome 15 is present.[73] Newborns with PWS have pronounced central hypotonia, hyporeflexia, and a weak cry. The poor tone manifests itself as sucking and swallowing difficulties that can lead to failure to thrive and the need for feeding tubes in infancy. Facial differences that have been described include bifrontal narrowing, almond-shaped eyes, and a small, downturned mouth. Genitalia are often hypoplastic, with cryptorchidism common in boys with this syndrome. The commonly reported small hands and feet are not always demonstrated in the newborn. Strabismus and hypopigmentation relative to the family are also common.

For infants with PWS, a history of poor fetal activity during the pregnancy can often be elicited, especially if the mother has had prior pregnancies as a comparison. Consistent with the hypotonia, breech presentation and perinatal insults are found more frequently than usual. The extreme hypotonia begins to abate in the first year of life and motor development improves, although developmental delay is the rule, especially for gross motor skills and speech. The feeding improves in the first few years of life and gives way to often uncontrollable hyperphagia and obesity. This issue and other behavioral problems, including severe temper tantrums, obsessive–compulsive disorder, and autism spectrum disorders, are encountered throughout life. Most patients manifest mild to moderate intellectual disability. Early diagnosis and recognition of the various medical issues along with preemptive implementation of behavioral therapy are essential components of the optimal management of these issues.

Deletions of the region critical in PWS have been demonstrated in up to 70% of patients. The deletion can be detected by a chromosomal microarray which has largely replaced FISH analysis using a probe specific for this region of chromosome 15. As these tests cannot determine whether the deletion involves the paternal or the maternal allele, further testing such as methylation analysis is required to distinguish it from Angelman syndrome (see below). A small number of patients have a disruption of this area as the result of a chromosomal translocation. To date, no single gene in this region has been implicated as the cause, but five genes are known to have paternal-only expression of protein-encoding genes (*MKRN3, MAGEL2, NECDIN, SNURF*, and *SNRPN*), as well as several paternally expressed noncoding RNA genes. It has been noted, however, that patients who have PWS as a result of a deletion of the region are more likely to be hypopigmented relative to their family. This feature has been attributed to deletion of a gene involved in pigmentation, *OCA2*.[74] Recurrence risks are negligible in cases in which de novo deletions are found and sporadic occurrence is usually encountered. In addition to hypopigmentation, patients with a deletion have a higher risk of sleep disturbance, speech defects, and need for feeding support.[75]

While large chromosome-specific segmental duplications are found in 15q11 and have been implicated in mediating the recurrent deletion of this genomic region (reviewed in[11,12]), approximately 20% to 25% of patients with PWS show maternal UPD that can be detected by means of a molecular assay designed to

assess specific methylation differences between maternal and paternal alleles. SNP microarray may detect UPD in cases of isodisomy, but not heterodisomy (which is relatively common in PWS), and microarray cannot distinguish paternal from maternal UPD without further testing including parental samples. Methylation analysis findings are abnormal in more than 99% of affected individuals but will not determine whether the cause is a deletion or maternal UPD. Further study is required if an abnormal methylation result is obtained. A maternal age effect has been demonstrated in UPD cases, and recurrence risks in families without deletions are estimated at 1 in 1000.

Angelman Syndrome

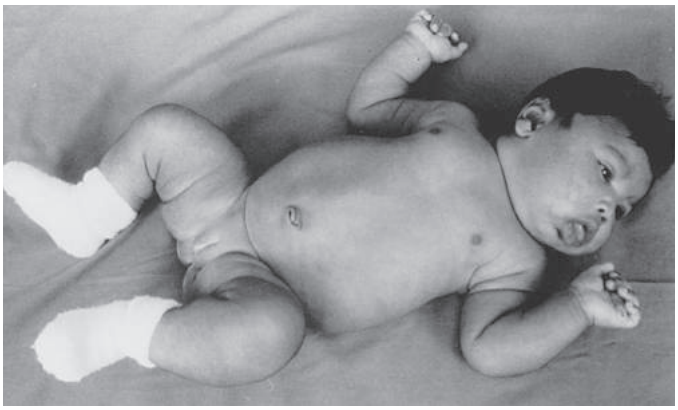
Loss of genetic material from the 15q11 to 15q13 region from the maternal copy of chromosome 15 is associated with Angelman syndrome. Clinical features are not evident in the newborn period and infancy but include significant intellectual disability, seizures, ataxic gait, tongue thrusting, inappropriate bursts of laughter, and facial differences, including protruding jaw, wide mouth, thin upper lip, and widely spaced teeth. The intellectual disability and hypopigmentation overlap with the features of PWS, but Angelman syndrome is a distinct entity.

Seventy percent to 75% of patients have a deletion of 15q that is detectable by chromosomal microarray (or specific FISH). A small percentage (3% to 5%) have evidence of paternal isodisomy (two paternal copies) of this region of chromosome 15, with no apparent maternal chromosome contribution. Unlike PWS, Angelman syndrome has been associated with mutations in a single gene, *UBE3A* (which encodes an enzyme involved in the ubiquitin pathway of protein degradation) that has been detected in up to 10% of patients. In addition, mutations of an imprinting center locus on chromosome 15 are associated with 1% to 2% of Angelman phenotypes.[76] Methylation analysis can be performed and will detect abnormalities in approximately 75% to 80% of patients because of a deletion or UPD. If methylation analysis findings are normal but Angelman syndrome is still suspected, *UBE3A* sequence analysis should be considered. The vast majority of cases result from a sporadic event, and the risk of recurrence can be best evaluated once the genetic mechanism has been determined for a given patient.

Beckwith–Wiedemann Syndrome

Beckwith–Wiedemann syndrome affects approximately 1 in 14,000 newborns and manifests itself as an overgrowth syndrome in the neonatal period. The characteristic findings are macrosomia, abdominal wall defect, and macroglossia (Fig. 28.12). Affected babies are large for their gestational age with proportionate length and weight. Infants of mothers with diabetes also manifest macrosomia but are more likely to have a weight disproportionately greater than length. Advanced bone age is also noted in Beckwith–Wiedemann syndrome. Hemihypertrophy caused by asymmetric growth is common, as is visceromegaly of various organs, including the spleen, kidneys, liver, pancreas, and adrenal glands.

Other characteristic features of the syndrome are macroglossia, linear creases of the earlobe with indentations on the posterior helix, and severe hypoglycemia. Although the hypoglycemia responds quickly to therapy, it can be present for several months; therefore recognition of the condition and immediate therapeutic intervention are critical in these cases. The hypoglycemia resolves spontaneously with age, and the physical diagnostic features also become less prominent with age, making the diagnosis more difficult to ascertain.



• **Fig. 28.12** Macrosomic infant with macroglossia and lax abdominal musculature. These findings are typical of Beckwith–Wiedemann syndrome. (From Viljoen DL, Jaquire Z, Woods DL. Prenatal diagnosis in autosomal dominant Beckwith-Wiedemann syndrome. Prenat Diagn. 1991;11:167–175.)

Equally important is the establishment of routine ultrasonographic surveillance at regular intervals, because children with Beckwith–Wiedemann syndrome are at increased risk of malignant tumors, especially Wilms tumor. The estimated risk is as high as 8% for patients with hemihypertrophy. Recommended screening for children with BWS includes full abdominal ultrasound and monitoring of serum alpha fetoprotein every three months until age 4 (screening for both Wilms tumor and hepatoblastoma) followed by renal ultrasound including the adrenal glands every three months until age 7 years.[77]

Although most cases of Beckwith–Wiedemann syndrome appear to arise de novo, up to 15% may be familial. In familial cases, the transmission is autosomal dominant, because of mutations in the maternally inherited *CDKN1C* gene in 40% of familial cases but only in 5%–10% of *de novo* cases. In addition, this region of the genome (11p15.5) is differentially imprinted such that certain genes are expressed only from the maternal allele, and others only from the paternal allele. The insulin-like growth factor type 2 gene (*IGF2*) is located in this region and encodes an important factor involved in fetal growth which is paternally expressed. Overexpression of the paternal allele results in an imbalance of expression leading to the overgrowth and tumor formation encountered in these patients. Paternal UPD has proved to be a mechanism involved in 10% to 20% of sporadic cases of Beckwith–Wiedemann syndrome. Methylation abnormalities at two distinct genetic loci within 11p15 account for approximately 60% of patients and is related to the overexpression of the *IGF2* gene. Therefore, all the available testing methods combined can detect the cause in approximately 85% of patients with Beckwith–Weidemann syndrome. The recurrence risk for future affected siblings or offspring of the proband depends on the specific genetic abnormality causing the disorder and can range from low (UPD, methylation abnormality) to as high as 50% (*CDKN1C* mutation).

Russell–Silver Syndrome

Russell–Silver syndrome presents in neonates with intrauterine growth retardation followed by postnatal growth deficiency. The head size is usually normal, causing a relative macrocephaly that

may have the appearance of hydrocephalus. Facial features can include a broad and prominent forehead, triangular-shaped face with a small chin, and downturned corners of the mouth. The fingers can show brachydactyly, camptodactyly, or more commonly fifth-finger clinodactyly. Other concerns involve limb-length discrepancy, delayed bone age, café au lait macules, hypospadias in males, developmental delays, diaphoresis, and hypoglycemia during the first 3 years of life. These patients are often examined by a geneticist as a toddler with growth retardation, proportionate short stature, and normal head circumference. When one is evaluating patients with growth retardation, it becomes important to know the prenatal and postnatal growth parameters, because they might provide a clue to the diagnosis of Russell–Silver syndrome.

The molecular mechanisms underlying the pathogenesis show that Russell–Silver syndrome is likely caused by abnormalities of imprinted genes. Maternal UPD of chromosome 7 is present in 7% to 10% of patients, and the symptoms are likely caused by overexpression of the maternal *GRB10* gene, which suppresses the activity of various growth factor receptors. In approximately 35% of patients, imprinting abnormalities of 11p15.5 occur because of a loss of the paternally expressed *IGF2* gene, leading to decreased prenatal and postnatal growth. This finding contrasts with that for some patients with Beckwith–Wiedemann syndrome, in whom *IGF2* is overexpressed, causing increased growth. For that reason, patients with Russell–Silver syndrome do not have a significantly increased risk of neoplasia compared with patients with Beckwith–Wiedemann syndrome, and routine cancer surveillance protocols are typically not recommended.

Future Directions: Characterization of Structural Variation by Genome Sequencing

Microarray-based testing has played a significant role in closing the gap between what is visible by karyotype/FISH and changes occurring at the single-nucleotide level that are routinely assayed by sequencing. However, there are still limitations to array-based testing in terms of the size of the events that can be detected and the precision of the breakpoints that can be identified. Currently, a growing number of technological developments is allowing for the detection of copy number variants using sequencing, which offers higher resolution and precision than arrays and provides the added benefit of being able to detect balanced chromosome rearrangements.[78] Exome sequencing, which is a widely-used technology for detection of single nucleotide variants and small insertions/deletions, is limited to the interrogation of the coding regions of the genome ("exons"). In contrast, whole genome sequencing provides more uniform coverage that includes inter- and intra-genic regions. Genome sequencing has shown the ability to accurately detect CNVs for clinical diagnosis and may ultimately replace array-based testing.[79,80] Other developments facilitating the detection of structural variation include long-read sequencing, which allows for sequencing of longer DNA molecules[81] and alternatives to sequencing such as optical genome mapping, in which DNA is labeled at specific sequence motifs and then linearized and passed through nanochannels, which allows large molecules of DNA to be imaged.[82–84] Whatever the method by which CNVs are detected, similar challenges will be faced in interpreting and assigning clinical significance to these variants, in particular CNVs involving intronic or non-coding regions of the genome.

Summary

This chapter has summarized the rapidly expanding field of chromosomal and genomic disorders, concentrating on those that commonly manifest themselves in the newborn period. The widespread development and clinical implementation of molecular cytogenetic techniques have allowed the identification of subtle rearrangements that were previously undetectable. These advances now enable the discernment of new syndromes in which the chromosomal anomaly may be defined before a characteristic phenotype is recognized. In addition, a greater understanding of the role of segmental duplications and their effects on human genetic disorders as well as of the influence of mechanisms that regulate gene expression, such as imprinting, is emerging. The tremendous advances in genomics led by the completion of the Human Genome Project and the development of new molecular diagnostic tools present new challenges for clinicians to better diagnose, understand, and care for patients with genetic disorders and their families.

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29 Inborn Errors of Carbohydrate, Ammonia, Amino Acid, and Organic Acid Metabolism

J. LAWRENCE MERRITT II AND RENATA C. GALLAGHER

Acute, life-threatening disease during the newborn period is a feature of many inborn errors of metabolism, including those of ammonia, carbohydrate, amino acid, fatty acid, ketone, and mitochondrial energy metabolism. Therefore, it is critical that neonatologists are familiar with the clinical symptoms, laboratory findings, methods of diagnosis, and empiric—as well as specific—management of each of these classes of disease (Table 29.1). Importantly, newborn screening (NBS) is only available for some disorders within each class. The use of NBS has resulted in presymptomatic identification, allowing early institution of therapy and improved outcome for many affected individuals. However, clinical presentation before the development of symptoms may still occur because of environmental and biologic factors, as well as local NBS and follow-up protocols. Factors that prevent or delay identification by NBS include when the disease is not detected by NBS or is incompletely ascertained, when the newborn develops symptoms before the NBS result being reported, and when follow-up testing has not been completed. Familiarity with these diseases and their characteristic signs and symptoms is critical for early clinical recognition and the initiation of potentially life-saving empiric management (Table 29.2). First-tier testing, including biochemical and analyte testing, is indicated although broad DNA sequencing, either exome or genome, has demonstrated utility in diagnosis of genetic and metabolic disease in critically ill children in the neonatal intensive care unit, and is increasingly being adopted as first- or second-tier testing.[1] Inborn errors of metabolism should also be considered in infants who develop symptoms outside of the immediate newborn period, as each of these disorders can have a later-onset presentation.

Carbohydrate Metabolism Disorders

Galactosemia

Elevated blood galactose, or *galactosemia*, is a result of a defect in one of three enzymes of the galactose metabolic pathway that converts galactose to glucose (Fig. 29.1). The disorder most clinically relevant in the newborn period is severe *galactose-1-phosphate uridyltransferase* (GALT) *deficiency*. It is a cause of neonatal jaundice and coagulopathy and is life threatening. It is commonly referred to as "classic galactosemia." This condition is the primary target of NBS for galactosemia. The other two enzyme defects that cause elevated blood galactose are *galactokinase* and *uridine diphosphate galactose-4-epimerase* deficiencies. Clinical features associated with each disorder are described. In classic galactosemia, with the ingestion of lactose, a disaccharide of glucose and galactose, the substrate of the enzyme galactose-1-phosphate accumulates, as does galactose and the secondary metabolites galactitol and/or galactonate. Elevations of galactitol may cause characteristic "oil drop" cataracts that may be present at birth. The roles of the other metabolites in pathogenesis of the liver, kidney, brain, and ovarian dysfunction of severe GALT deficiency are not understood. They likely include the deficiency of galactose-1-phosphate conjugated to uridine, as well as toxicity of accumulating metabolites.

The frequency of classic galactosemia is estimated to be 1 in 60,000 to 1 in 75,000 births in the United States and Europe.[2] There are also milder forms of unclear clinical significance, such as Duarte variant galactosemia, with enzyme activity of roughly 25% of wild type, which are frequently identified by abnormal NBS but are currently believed not to require treatment.[3]

NBS methods for identification of classic galactosemia vary by state and include measurement of "total galactose" (galactose plus galactose-1-phosphate) and GALT enzyme activity. Testing after an abnormal NBS may include DNA analysis to identify common mutations in the *GALT* gene. In states that measure analytes, children with galactokinase or epimerase deficiencies may be identified. These disorders will not be detected through screening for classic galactosemia through deficient GALT enzyme activity alone. The accuracy of NBS is dependent on information provided by the ordering nursery, as transfused red blood cells may cause a false-negative NBS for galactosemia. Transfusion of red blood cells will also impact detection of hemoglobinopathies. Thus, infants transfused before NBS require follow-up testing for galactosemia and hemoglobinopathies at least 4 weeks after transfusion. Also, infants who have not received a lactose-containing feeding (i.e., breast milk or non-soy-based formulas) before screening may not have elevated galactose levels and may have false-negative NBS if

TABLE 29.1 Presentations of Inborn Errors of Metabolism

	Newborn/Early Onset	Acute Presentation	Chronic Presentation	Currently on Newborn Screening Panels
Carbohydrates				
Galactosemia	Yes	Yes	Yes	Yes
Epimerase deficiency	Rare	Yes	Yes	Possibly
Galactokinase deficiency	No	No	Yes	Possibly
GSD Ia and Ib	Rare	Yes	Yes	No
GSD II	Yes	Yes	Yes	Possibly
GSD IV	Yes	Yes	Yes	No
Hereditary fructose intolerance	No	Yes	Yes	No
Fructose-1,6-bisphosphatase deficiency	No	Yes	No	No
Urea Cycle Disorders				
All types	Yes	Yes	Yes	Not all
Transient hyperammonemia of the newborn	Yes	Yes	No	No
Aminoacidemias				
MSUD	Yes	Yes	Yes	Yes
Tyrosinemia type 1	No	Rare	Yes	Yes
Nonketotic hyperglycinemia	Yes	Yes	Yes	No
Cystathionine β-synthase deficiency	Rare	Yes	Yes	Yes
Remethylation disorders	Yes	Yes	Yes	Possibly
Phenylketonuria	No	No	Yes	Yes
Organic Acidemias				
Methylmalonic acidemia(s)	Yes	Yes	Yes	Yes
Propionic acidemia	Yes	Yes	Yes	Yes
Isovaleric acidemia	Yes	Yes	Yes	Yes
Holocarboxylase synthase deficiency	Yes	Yes	Yes	Yes
Biotinidase deficiency	No	Possibly	Yes	Yes
Glutaric acidemia type 1	Rare	Yes	Yes	Yes
Fatty Acid Oxidations Disorders				
MCADD	Yes	Yes	Yes	Yes
VLCADD	Yes	Yes	Yes	Yes
SCADD	No	Rare	Rare	Yes
LCHADD and TFP	Yes	Yes	Yes	Yes
CTD	Yes	Yes	Yes	Yes
CPTI	Yes	Yes	Yes	Yes
CACT	Yes	Yes	Yes	Yes
CPTII	Yes	Yes	Yes	Yes
MADD	Yes	Yes	Yes	Yes

Continued

TABLE 29.1 Presentations of Inborn Errors of Metabolism—cont'd

	Newborn/Early Onset	Acute Presentation	Chronic Presentation	Currently on Newborn Screening Panels
Ketone Metabolism Disorders				
Mitochondrial acetoacetyl-CoA thiolase deficiency	Yes	Yes	Rare	Yes
HMG-CoA lyase deficiency	Yes	Yes	Yes	Yes
Succinyl-CoA 3-ketoacid-CoA transferase deficiency	Yes	Yes	Rare	No
Mitochondrial Disorders				
Primary lactic acidosis	Yes	Yes	Yes	No
Pyruvate dehydrogenase complex deficiency	Yes	Yes	Yes	No
Pyruvate carboxylase deficiency	Yes	Yes	Yes	No
Electron chain deficiencies	Yes	Yes	Yes	No
Leigh disease	Rare	Yes	Yes	No
Pearson syndrome	Yes	Yes	Yes	No
Barth syndrome	Yes	Yes	Yes	No

CACT, Carnitine acylcarnitine translocase deficiency; *CoA*, coenzyme A; *CPTI*, carnitine palmitoyltransferase type I deficiency; *CPTII*, carnitine palmitoyltransferase type II deficiency; *CTD*, carnitine transporter deficiency; *GSD*, glycogen storage disorder; *HMG*, 3-hydroxy-3-methylglutaryl; *LCHADD*, long-chain acyl-CoA dehydrogenase deficiency; *MADD*, multiple acyl-CoA dehydrogenase deficiency; *MCADD*, medium-chain acyl-CoA dehydrogenase; *MSUD*, maple syrup urine disease; *SCADD*, short-chain acyl-CoA dehydrogenase deficiency; *TFP*, trifunctional protein deficiency; *VLCADD*, very long chain acyl-CoA dehydrogenase deficiency.

TABLE 29.2 Treatments for Inborn Errors of Metabolism

	Dietary	Medications	Vitamin Supplementation	Other(Dialysis, Transplantation)
Carbohydrates				
Galactosemia	Yes	No	Yes	–
Epimerase deficiency	Yes	No	Yes	–
Galactokinase deficiency	Yes	No	Yes	–
GSD Ia and Ib	Yes	Yes	Yes	Liver, kidney Txp
GSD II	Yes	Yes	No	–
GSD IV	Yes	No	No	Liver, cardiac Txp
Hereditary fructose intolerance	Yes	No	No	–
Fructose-1,6-bisphosphatase deficiency	Yes	No	No	–
Urea Cycle Disorders				
All types	Yes	Yes	Yes	Liver Txp, HD
Transient hyperammonemia of the newborn	No	Yes	No	HD
Aminoacidemias				
MSUD	Yes	No	Yes	Liver Txp, HD
Tyrosinemia type 1	Yes	Yes	No	Liver Txp

Continued

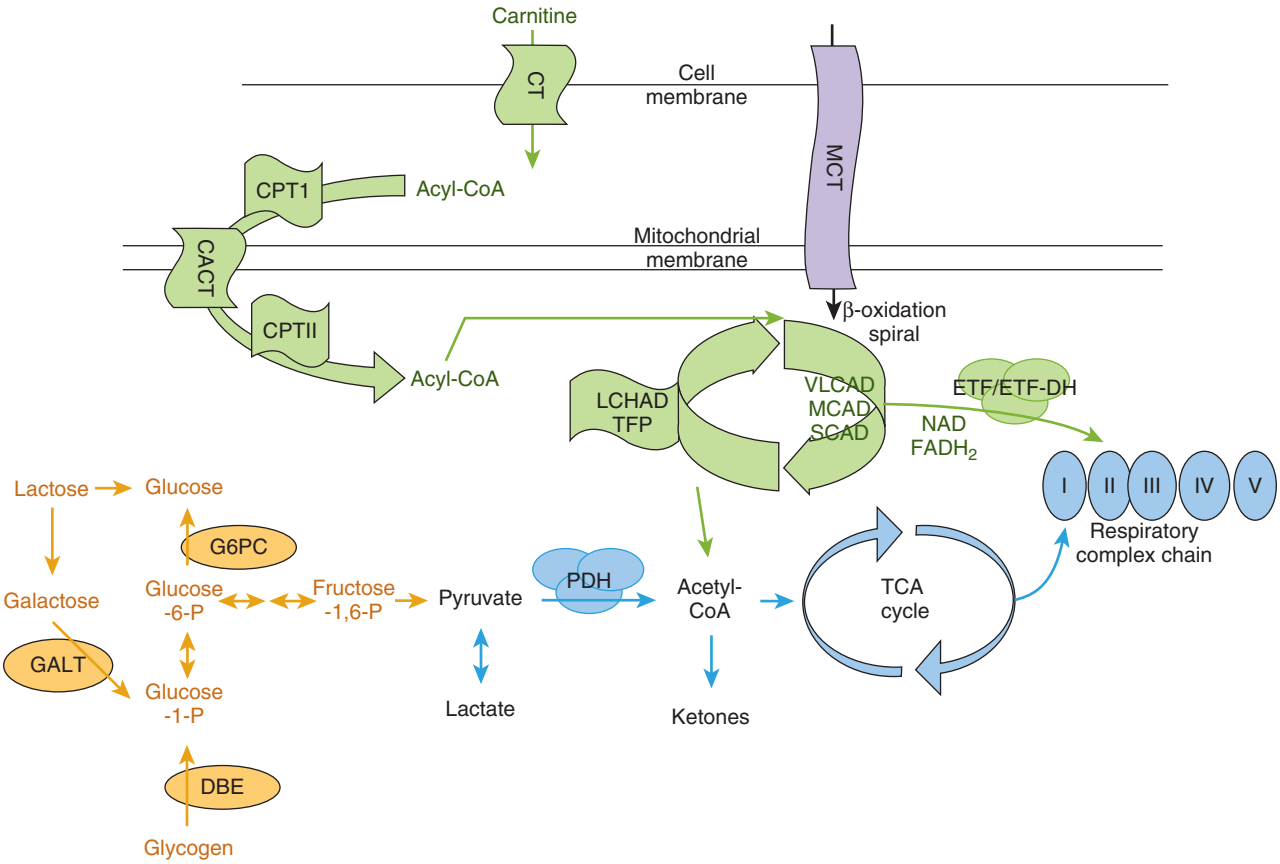
TABLE 29.2 Treatments for Inborn Errors of Metabolism—cont'd

	Dietary	Medications	Vitamin Supplementation	Other(Dialysis, Transplantation)
Nonketotic hyperglycinemia	No	Yes	No	–
Cystathionine synthase deficiency	Yes	Yes	Yes	–
Remethylation disorders	Yes	Yes	Yes	HD
Phenylketonuria	Yes	Yes	No	–
Organic Acidemias				
Methylmalonic acidemia	Yes	Yes	Some forms	Liver Txp, HD
Propionic acidemia	Yes	Yes	No	Liver Txp, HD
Isovaleric acidemia	Yes	Yes	Yes	HD
Holocarboxylase synthase deficiency	Variable	No	Yes	–
Biotinidase deficiency	No	No	Yes	–
Glutaric acidemia type 1	Yes	Yes	Yes	–
Fatty Acid Oxidations Disorders				
MCADD	Variable	Yes	No	–
VLCADD	Yes	Yes	No	Cardiac Txp
SCADD	No	Variable	No	–
LCHADD and TFP	Yes	Yes	No	Cardiac Txp
CTD	No	Yes	No	–
CPTI	Yes	Yes	No	–
CACT	Yes	Yes	No	–
CPTII	Yes	Yes	No	–
MADD	Yes	Yes	Yes	–
Ketone Metabolism Disorders				
BK thiolase deficiency	Yes	Yes	No	–
HMG-CoA lyase deficiency	Yes	Yes	No	–
SCOT deficiency	Yes	Yes	No	–
Mitochondrial Disorders				
Primary lactic acidosis	No	No	Some forms	–
Pyruvate dehydrogenase complex deficiency	Yes	Yes	Yes	–
Pyruvate carboxylase deficiency	No	Yes	No	–
ETC defects	No	Variable	Some forms	–
Leigh disease	No	Variable	Some forms	–
Pearson syndrome	No	Yes	Yes	–
Barth syndrome	Yes	Yes	Variable	Cardiac Txp

BK, Beta-keto or mitochondrial acetoacetyl-CoA; *CACT*, carnitine acylcarnitine translocase deficiency; *CoA*, coenzyme A; *CPTI*, carnitine palmitoyltransferase I; *CPTII*, carnitine palmitoyltransferase II; *CTD*, carnitine transporter deficiency; *ETC*, electron transport chain; *HD*, hemodialysis; *HMG*, 3-hydroxy-3-methylglutaryl; *LCHADD*, long-chain acyl-CoA dehydrogenase deficiency; *MADD*, multiple acyl-CoA dehydrogenase deficiency; *MCADD*, medium-chain acyl-CoA dehydrogenase deficiency; *SCADD*, short-chain acyl-CoA dehydrogenase deficiency; *SCOT*, succinyl-CoA 3-ketoacid-CoA transferase; *TFP*, trifunctional protein deficiency; *Txp*, transplantation; *VLCADD*, very long chain acyl-CoA dehydrogenase deficiency.

the screening method is analyte rather than enzyme-based. False-positive NBS results can occur in hot weather if the screening method is enzyme activity, as the enzyme is denatured in heat. Mutation analysis and enzyme activity help identify neonates with classic galactosemia (<1% control GALT activity), who have a poorer prognosis than those with variant (nonclassic) galactosemia (1% to 10% control GALT activity).[4]

At presentation, total blood galactose levels may be elevated with elevated red blood cell galactose-1-phosphate (Gal-1-P) and urine galactitol levels. During this phase of severe hypergalactosemia, positive reducing substances will be present in urine, but these resolve within hours with dietary restriction of galactose. There may be factitious elevation of glucose in affected individuals, as measured by bedside glucometers.[5] Following the initiation



• **Fig. 29.1** Overview of metabolism of carnitine transport, fatty acid oxidation, glucose, TCA cycle, and mitochondrial respiratory chain. *CACT*, Carnitine acylcarnitine translocase; *CT*, carnitine transporter; *CPTI*, carnitine palmitoyltransferase I; *CPTII*, carnitine palmitoyltransferase II; *$FADH_2$*, flavin adenine dinucleotide; *DBE*, debranching enzyme; *GALT*, galactose-1-phosphate uridyltransferase; *Acyl-CoA*, long-chain fatty acid attached to Coenzyme A; *LCHAD*, long-chain 3-hydroxy acyl-CoA dehydrogenase; *ETF/ETF-DH*, electron transfer flavoprotein/electron transfer flavoprotein dehydrogenase; these are deficient in MADD; *MADD*, multiple acyl-CoA dehydrogenase deficiency; *MCAD*, medium-chain acyl-CoA dehydrogenase; *MCT*, medium-chain triglyceride; *NAD*, nicotinamide adenine dinucleotide; *PDH*, pyruvate dehydrogenase; *SCAD*, short-chain acyl-CoA dehydrogenase; *TCA*, tricarboxylic acid; *TFP*, trifunctional protein; *VLCAD*, very long chain acyl-CoA dehydrogenase.

of treatment with a lactose-free diet, Gal-1-P levels decrease but rarely normalize, remaining elevated for the lifetime of the patient. There is a target range for treatment, and maintenance of Gal-1-P levels in treatment range through dietary restriction of galactose is the current goal of short- and long-term dietary therapy.[6]

Untreated classic galactosemia may present with severe multiorgan (liver and kidney) disease in the first days to weeks of life. The most common clinical symptoms are vomiting, poor feeding, failure to thrive, and lethargy, with jaundice and hepatomegaly on physical examination. About 10% of affected neonates develop sepsis, often due to *Escherichia coli*; other causative organisms include *Klebsiella* and *Enterobacter*.[4] Characteristic laboratory findings are indirect hyperbilirubinemia and apparent liver failure with markedly elevated coagulation parameters out of proportion to the transaminase elevations. With continued lactose/galactose ingestion, liver disease can progress to cirrhosis with portal hypertension and splenomegaly. Renal Fanconi syndrome may also develop. Cataracts may develop in the first few weeks of life in untreated individuals, while some neonates are born with cataracts.

Management of the liver disease entails dietary restriction of galactose and supportive care. The coagulopathy resolves over several days. A lactose-free formula should be initiated as soon as classic galactosemia is suspected as this can be life-saving. An early change to soy formula may mask the symptoms of the disease. With early dietary restriction, improvement has been seen in severe clinical presentations and in neonatal mortality.[2] While growth and feeding return to normal, treated individuals may develop long-term complications including speech and language disorders, below average intelligence and cognitive functioning, cataracts, primary ovarian insufficiency (in most females), decreased bone mineral density, tremor, dystonia, coordination problems, or severe ataxia.[2,7,8] The cause of these complications is unknown, and they may not be improved by dietary restriction.[9,10]

Patients with classic galactosemia are advised to continue a lactose-restricted diet for their entire lives, but this diet does have significant risk of calcium and vitamin D deficiencies, and these must be supplemented. Dairy and high galactose nondairy foods including fruits, legumes, and vegetables are restricted. Not all

galactose is exogenous; there is some endogenous galactose production. Treatment compliance is monitored through frequent assessment of Gal-1-P levels. The restriction of galactose-containing fruits and vegetables may not be necessary lifelong.[11]

NBS has changed the clinical outcome of classic galactosemia and when results are provided as early as 3 to 4 days of life—before significant clinical symptoms—hospitalization is often avoided. A systematic review of NBS for galactosemia in Europe identified significant variability in galactosemia screening methods, cutoff values, and screening ages. Mortality ranged from 0% to 100% with no agreement regarding treatment of variant forms, or timing of clinical follow-up, and evidence in favor of NBS was considered insufficient. The greatest confounder of the cost-benefit assessment of NBS was the false-positive testing rate and the effects of these test results on families.[2]

Epimerase Deficiency Galactosemia

Uridine diphosphate-galactose 4-epimerase (GALE) *deficiency* has several forms. Generalized GALE deficiency may present similarly to GALT deficiency when the enzyme is deficient in all tissues. Peripheral or intermediate forms of GALE deficiency are associated with deficient enzyme activity in red blood cells, with normal or only partially decreased enzyme activity in other tissues (i.e., white blood cells or fibroblasts). For the peripheral or intermediate forms, children receiving a normal, lactose-containing diet will not become symptomatic. GALE deficiency may be detected on newborn screen in states that test for classic galactosemia by analyte. These children have elevated Gal-1-P levels with normal GALT enzyme testing. Individuals with generalized disease can develop severe liver and renal disease if untreated, and they can be diagnosed with GALE deficiency through assessment of activity in red blood cells. GALE deficiency is treated identically to GALT deficiency, when symptomatic.[12]

Galactokinase Deficiency

Galactokinase (GALK) *deficiency* causes elevated galactose with a normal Gal-1-P level. Affected individuals also have elevated galactitol and may develop dense cataracts if untreated. GALK deficiency is also associated with pseudotumor cerebri, but the disease does not cause systemic effects.[13] As for GALE deficiency, GALK deficiency may be detected on newborn screen in states that test for classic galactosemia by analyte. GALT enzyme testing is normal. Cataracts may develop in the neonatal period, and early treatment may improve or resolve cataracts if infants are treated with galactose restriction before 4 to 8 weeks of life.[14] A recent evaluation of the GalNet registry has identified additional complications in neonates of elevated transaminases, bleeding diathesis, and encephalopathy.[10] Dietary galactose restriction may be necessary but is milder than that required for GALT deficiency.

Glycogen Storage Diseases

Glycogen storage diseases (GSDs) are due to abnormalities in glycogen synthesis or utilization for energy production. They are divided into types primarily affecting the liver (types I, IIIb, IV, VI, and IX), the muscles (types II, V, and VII), or mixed (type IIIa and forms of IX). Hypoglycemia is often a presenting symptom of the liver-based GSDs. Most GSDs are inherited in an autosomal recessive manner, except GSD IXa and IXd (hepatic and muscle phosphorylase kinase deficiencies, respectively), which are X-linked. Severe GSD II is associated with an infantile cardiomyopathy and is the GSD that is most likely to be encountered in the neonatal intensive care unit. Treatments for GSDs are of variable efficacy given the spectrum of these diseases. Currently, there are multiple clinical trials underway including gene therapy technologies for many types of GSDs, including GSD 1a and GSD II.

Hepatic Glycogen Storage Diseases

GSD I is due to glucose-6-phosphatase deficiency (von Gierke disease, Ia) or to deficient glucose-6-phosphate transport (Ib). Glucose-6-phosphatase plays a critical role in both glycogenolysis and gluconeogenesis (see Fig. 29.1). The frequency of GSD type I is estimated to be 1 in 100,000 births, with 80% of cases being type Ia. The frequency is 1 in 20,000 in Ashkenazi Jews due to a founder mutation.[15]

GSD I may not manifest in the neonatal period, as frequent newborn feeding may prevent symptomatic hypoglycemia and the development of hepatomegaly. Major clinical findings include failure to thrive, with an enlarged abdominal girth from hepatomegaly and hypoglycemia. Major laboratory findings are a rapid fasting hypoglycemia (typically within a few hours) with ketosis and lactic acidosis. Because of the high lactate level, individuals may appear relatively asymptomatic from the hypoglycemia. Hypercholesterolemia, hypertriglyceridemia, and hyperuricemia may be seen in older individuals. The disease also affects the kidneys and causes focal segmental glomerulosclerosis and progressive renal insufficiency. Patients with GSD Ib develop recurrent infections because of neutropenia and defective neutrophil function along with inflammatory bowel disease that may develop in the first year of life. Diagnosis is now often confirmed through DNA sequencing of the *G6PC* (GSD Ia) and *SLC37A4* (GSD Ib) genes, although liver biopsy for enzyme activity may also be performed.

Therapy focuses on the prevention of hypoglycemia and resultant brain damage and growth failure through frequent feedings and restriction of lactose and sucrose, as galactose and fructose derived from these feed into the blocked pathway.[15] Continuous nasogastric feedings or boluses of uncooked cornstarch are essential at night and often during the day and do improve glucose control and growth but do not completely correct other biochemical abnormalities. A comprehensive plan for treatment of intercurrent illnesses and emergencies is required. Neutropenia in type Ib may be treated with granulocyte colony-stimulating factor. Liver transplantation has been shown to improve metabolic control, fasting hypoglycemia, and growth.[16]

Muscular Glycogen Storage Diseases

The most common and significant form of muscular GSD is type II, commonly called *Pompe disease* (*acid alpha-glucosidase deficiency* [abbreviated as GAA], also known as *acid maltase deficiency* or *lysosomal α-1,4-glucosidase deficiency*). This GSD was the first identified lysosomal storage disease (LSD), as glycogen accumulates within the lysosome because of a defect in lysosomal-mediated glycogen degradation. Muscle pathology will demonstrate vacuolar myopathy with glycogen storage within lysosomes and free glycogen in the cytoplasm demonstrated by electron microscopy. The vacuoles are periodic acid-Schiff positive, digestible by diastase, and positive for acid phosphatase. As for some other LSDs, enzyme replacement therapy has been developed for Pompe disease and is the only currently effective therapy.

Pompe disease has an estimated incidence of 1 in 40,000 in the Netherlands, based on the country's screening for three common mutations in newborn blood spots. The incidence ranges from 1 in 57,000 for late-onset disease to 1 in 138,000 for classic infantile disease.[17,18] The classic infantile presentation of Pompe disease

is hypotonia and hypertrophic cardiomyopathy. Creatine kinase, lactate dehydrogenase, and aspartate aminotransferase are elevated. The electrocardiogram is abnormal with a short PR interval and giant QRS complex in all leads, suggesting biventricular hypertrophy (Fig. 29.2). Late-onset presentations are of myopathy and have been diagnosed as early as the second year of life. Diagnosis is made through the identification of decreased GAA activity in dried blood spots—from newborn screening or diagnostic testing—or in fibroblasts or muscle biopsy. Confirmation via sequencing of the *GAA* gene is recommended following any GAA enzyme testing due to the presence of pseudodeficiency alleles in which the in vitro enzyme activity is abnormal, but in vivo activity is normal, and to avoid muscle biopsy.[19]

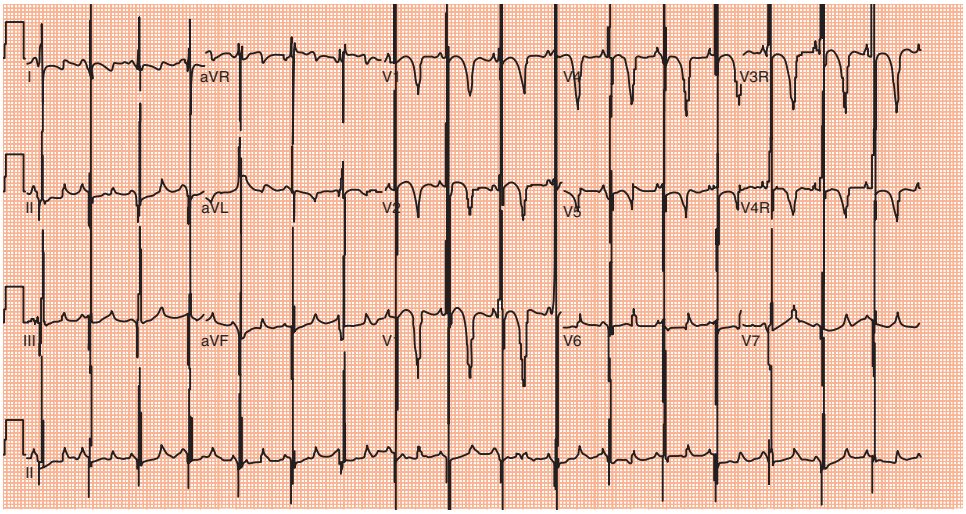
Decisions regarding which disorders are included on a state's NBS panel are made by each state, and many states now include Pompe disease due to the markedly improved outcome of infantile-onset Pompe disease (IOPD) with early treatment. NBS and early initiation of enzyme replacement therapy has demonstrated improvement in cardiac size, muscle pathology, growth, and gross motor function in affected individuals but not in arrhythmias such as Wolff-Parkinson-White or in dysphagia or osteopenia.[20–22] Long-term follow-up of early-treated individuals has demonstrated increased life span and increased ambulation with individuals not requiring mechanical ventilation and increased muscle strength and function.[23,24] Gene therapy is being investigated with promising results in a mouse model and with initial clinical trials being implemented.[25]

Andersen disease, or GSD IV, is due to deficiency of glycogen branching enzyme, expressed in multiple tissues, and may manifest primarily as hepatic or muscular disease, with involvement of the heart and/or the nervous system in up to five different clinical presentations. Two rare neuromuscular subtypes may present in the newborn period. The fatal perinatal neuromuscular subtype presents with fetal akinesia sequence with polyhydramnios, decreased fetal movement, fetal hydrops, and neonatal death or with hypotonia, muscular atrophy, arthrogryposis, and death in the neonatal period from cardiopulmonary failure.[26] The second congenital neuromuscular subtype presents with profound hypotonia, respiratory distress requiring mechanical ventilation, dilated cardiomyopathy, and death in early infancy. The classic GSD IV subtype is the progressive hepatic subtype. Children are often normal at birth but develop failure to thrive, hypotonia, and potentially progressive liver dysfunction leading to cirrhosis and cardiomyopathy requiring liver and heart transplantation, respectively.[27] Death may result from progressive cardiomyopathy despite liver transplantation. There is also a non-progressive hepatic subtype and a childhood neuromuscular subtype. GSD IV is a rare autosomal recessive disorder, and diagnosis is confirmed through DNA sequencing of the *GBE1* gene or by detection of abnormal enzyme activity in muscle, liver, or skin fibroblasts.

Fructose Metabolism

The primary disorder of fructose metabolism is *hereditary fructose intolerance* (HFI). This is a rare autosomal recessive disorder triggered by ingestion of fructose, sucrose, or sorbitol, which may present clinically when infants are weaned from breast milk or formula and juice or fruit are added to the diet or when they receive a formula that contains fructose.[28] Affected infants or neonates who are given sucrose solutions for pain relief during minor procedures may develop hypoglycemia, and a diagnosis of HFI should be considered in these cases. Clinical findings include pallor, lethargy, poor feeding, vomiting, loose stools, poor growth, hepatomegaly, and hypoglycemia, lactic acidemia, hyperuricemia, transaminase elevations, and positive urine reducing substances with ingestion of fructose. There are reports of neonates who developed life-threatening acute liver failure after receiving fructose-containing formulas.[29] Renal tubular dysfunction may be present. Diagnostic testing consists of measuring enzyme activity in liver tissue and/or sequencing of *ALDOB*. Treatment includes elimination of fructose, sucrose, and sorbitol from the diet and medications. In practice, complete elimination of these can be quite difficult but is necessary for optimal outcome. There is no current newborn screening for HFI.

Fructose-1,6-bisphosphatase deficiency is not a disorder of fructose metabolism. It is a disorder of gluconeogenesis, although as with other disorders of gluconeogenesis, therapy may include some limitation of dietary fructose. Patients may present in the newborn period with lactic acidosis and hypoglycemia when glycogen reserves are limited and then be clinically silent and present later (typically before 2 years of age) during times of fasting or following a fructose load. Acute crisis presents similarly to HFI and GSD Ia, and these are in the differential diagnosis for symptoms of acidosis and hypoglycemia. This is a very rare autosomal



• **Fig. 29.2** Pompe disease electrocardiogram.

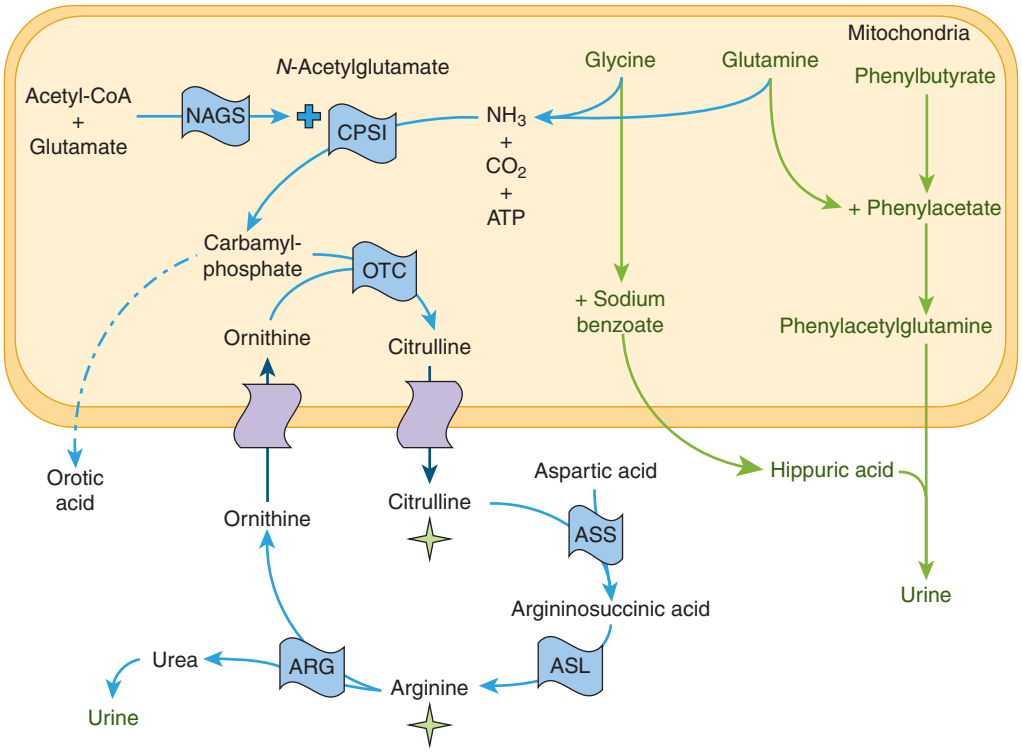
recessive disorder resulting from mutations in the *FBP1* gene, with an estimated incidence between 1 in 350,000 and 1 in 900,000.[30]

Urea Cycle Disorders

Removal of excess nitrogen is the function of the urea cycle. Urea cycle disorders (UCD) result from inhibition of the synthesis of urea from ammonia and classically manifest in the newborn period, although these may manifest at any age. Symptoms of hyperammonemia are provoked during episodes of protein catabolism (e.g., because of illness and poor oral intake) or dietary protein excess. Hyperammonemia is treated by controlling protein catabolism through dietary limitation of protein and removing offending toxic products (chiefly ammonia) with ammonia-scavenging medications or dialysis. UCDs are frequently classified as proximal (mitochondrial) and distal (cytoplasmic). The three proximal UCDs are N-*acetylglutamate synthase deficiency* (NAGS), *carbamyl phosphate synthetase I* (CPSI) *deficiency*, and *ornithine transcarbamylase deficiency* (OTCD). The distal UCDs are *argininosuccinate synthetase deficiency* (known as *ASS deficiency* or *citrullinemia type I*, *CIT1*), *argininosuccinate lyase (ASL) deficiency* (also known as *argininosuccinic aciduria*, ASA), and *arginase 1 deficiency*. Additionally, disorders of the urea cycle include two of mitochondrial membrane transport. Deficiency of the mitochondrial ornithine transporter 1 is a cause of hyperammonemia, hyperornithinemia, and homocitrullinuria syndrome, and deficiency of the mitochondrial aspartate–glutamate transporter is a cause of citrullinemia type II or citrin deficiency. A defect of a plasma membrane transporter affects the renal tubular transport of cationic amino acids, including lysine, arginine, and the amino acids required for urea cycle function, and results in *lysinuric protein intolerance*, a multisystem disorder that only rarely manifests with neonatal hyperammonemia. Incidence estimates of all UCDs are 1 in 35,000 births, with OTCD, the most common UCD, estimated at 1 in 56,500 births.[31] All UCDs are autosomal recessive except for OTCD, which is X-linked. Roughly 20% of heterozygote females manifest symptoms at some time in their life, and some of these present in the newborn period.

One complete turn of the urea cycle will produce a molecule of urea from two molecules of ammonia and one of bicarbonate. The nitrogen in ammonia is generated from the hepatic nitrogen pool of amino acids including glutamine, glutamate, and glycine. *N*-acetylglutamate is the product of the first enzyme in the cycle and is an essential activator of carbamyl phosphate synthetase I, which converts ammonia and bicarbonate into carbamyl phosphate. Ornithine and carbamyl phosphate are condensed by ornithine transcarbamylase to generate citrulline. Citrulline is combined with aspartate by argininosuccinate synthetase to create argininosuccinic acid. Fumarate is released from this by argininosuccinate lyase to create arginine. Urea is generated from arginine by arginase 1 and is excreted while ornithine reenters the urea cycle (Fig. 29.3).

Clinical symptoms in the newborn period are similar for all UCDs and are due to hyperammonemia. Severely affected



• **Fig. 29.3** Overview of urea cycle metabolism (*blue*) and nitrogen scavenger therapies (*green*). *Stars* indicate supplementation with citrulline or arginine. *Plus sign* indicates allosteric activator of carbamyl phosphate synthetase I. *Dashed line* indicates accumulation of orotic acid in ornithine transcarbamylase deficiency. *Purple* indicates transport between the mitochondrion and the cytosol. *ARG*, arginase; *ASL*, argininosuccinate lyase; *ASS*, argininosuccinate synthetase; *ATP* adenosine triphosphate; *CPSI*, carbamyl phosphate synthetase I; CO_2, carbon dioxide; *NAGS*; *N*-acetylglutamate synthase; NH_3, ammonia; *OTCD*, ornithine transcarbamylase.

newborns exhibit progressive alteration of level of consciousness with drowsiness and lethargy progressing to unresponsiveness, beginning after 24 hours of life. Typical symptoms include poor feeding, vomiting, hyperventilation (caused by ammonia elevation and resulting in a primary respiratory alkalosis), and temperature instability. There may be peripheral circulatory failure that progresses to multiorgan failure. Marked hyperammonemia causes acute encephalopathy, leading to seizures, coma, and death if untreated. Ammonia levels should be checked in any infant with these symptoms, which may mimic sepsis or intestinal obstruction, and, if elevated, should be treated rapidly. Later-onset presentations include recurrent emesis, ataxia, liver dysfunction or apparent failure with coagulopathy, postpartum psychosis, and other psychiatric symptoms such as aggression, agitation, mania, and personality changes.[32]

Without rapid treatment severe UCDs are almost always fatal or result in severe and irreversible brain damage. The primary goal of treatment is to remove excess ammonia, which is neurotoxic. The effects of ammonia include alterations in amino acid pathways, neurotransmitters, energy production, nitric oxide synthesis, axonal and dendritic growth, and signal transduction in the developing brain. Additionally, excess glutamine may cause cerebral swelling and edema in the mature brain.[33] Acute hyperammonemic episodes may be associated with transaminase elevation and synthetic liver dysfunction or apparent liver failure, and it is important to assess transaminases and coagulation parameters.

Individuals affected by proximal UCDs have been reported to present earlier in life, to have a higher peak ammonia level, and to have a longer average length of stay compared with patients with distal UCDs. Reports identify the age at first admission for hyperammonemia as less than or equal to 2 days in 55% of OTCD and CPSI patients and at less than 7 days in 84% of these patients.[34] For OTCD patients, nearly half of the males but only 4% of females will present between 0 and 30 days of age.[35]

The critical laboratory abnormality in a UCD is elevated plasma ammonia. While artifactual elevations may occur due to problems with sample collection and processing, hyperammonemia in a newborn is a medical emergency, and, if elevated, the test should be repeated, and additional evaluation and management initiated immediately. Normal plasma ammonia levels in newborns are as high as 110 µmol/L (although care should be taken to confirm units as some laboratories report mg/dL). A level of greater than 150 µmol/L (255 mg/dL) should prompt suspicion of, and evaluation for, an inborn error of metabolism in neonates. In older infants, children, and adults the reference range for ammonia is less than 35 µmol/L (60 mg/dL). In primary or secondary disorders of the urea cycle that present in the newborn period, ammonia levels may be in the thousands. The differential diagnosis of hyperammonemia in the newborn period includes urea cycle defects, organic acidemias, fatty acid oxidation disorders (FAODs), CA-VA (carbonic anhydrase VA) deficiency, and transient hyperammonemia of the newborn (THAN). In UCDs the hyperammonemia is often associated with a respiratory alkalosis caused by the effect of ammonia on the respiratory control centers in the brainstem. A primary respiratory alkalosis in a newborn should prompt a physician to order an ammonia level. Early involvement of a geneticist with experience in the evaluation and management of inborn errors of metabolism is critical. Specialized biochemical laboratory testing should include plasma amino acids, total and free plasma carnitine, plasma acylcarnitine profile, total plasma homocysteine, plasma B12 level, urine organic acids, urine amino acids, and a quantitative urine orotic acid to assess for UCDs, as well as fatty acid oxidation disorders and organic acidemias, which can also present with marked hyperammonemia in the newborn period.

In proximal UCDs, there is decreased citrulline on plasma amino acid analysis, while patients with distal UCDs have either elevated citrulline (in CIT1 and ASA deficiency), elevated argininosuccinic acid (in ASA deficiency), or elevated arginine (in arginase deficiency). In OTCD, increased urinary orotic acid is present and may be identified on urine organic acid analysis, but a quantitative value is recommended because of variability in detection. NBS for CIT1, ASA, and arginase deficiency is performed through measurements of citrulline (CIT1, ASA) and arginine (arginase deficiency) in blood spots as these are elevated in these conditions. Proximal UCDs are not well identified on NBS because of the poor sensitivity of low citrulline levels, and only a few states have started screening for proximal UCDs.[36] When a UCD is suspected, further evaluation through blood and urine metabolite testing and confirmation of a diagnosis is necessary through DNA testing. For OTCD this should include analysis for gene copy number and intronic variants through deep sequencing, but still may only detect mutations in about 90% of patients.[37] In some cases, liver biopsy and enzyme analysis of liver tissue are required.

Treatments with medications, dialysis, or liver transplantations will control ammonia levels, though neurologic outcomes are poorer when presenting blood ammonia levels are greater than 360 µmol/L.[38] Hemodialysis is the primary method for rapid removal of ammonia. Recent consensus guidelines have been published for hyperammonemia management through medical management and renal replacement therapy. Renal replacement therapy was recommended in cases of a rapidly increasing ammonia level greater than 150 µmol/L with a rapidly deteriorating neurological status, rapidly increasing levels greater than 300 µmol/L that are uncontrolled, or an ammonia level of greater than 400 µmol/L when medical measures are ineffective.[39] Continuous arteriovenous hemofiltration (CAVH) provides a lower clearance rate but has the added benefit of continuous use and a lesser likelihood of major swings in intravascular volume that can exacerbate an already fragile state and cerebral edema. Ammonia clearance with peritoneal dialysis is approximately one-tenth that of CAVH and is not recommended unless other methods of kidney replacement therapy are not available.[39,40] Ammonia is not cleared effectively by exchange transfusion.

Medical treatment of hyperammonemia through alternative pathway or nitrogen-scavenging therapies are effective and are critical in acute and chronic management (see Fig. 29.3). When hyperammonemia is recognized and a UCD suspected, alternative pathway therapy can be rapidly implemented before hemodialysis, continued throughout, and maintained afterward and transitioned from intravenous (IV) to oral therapy for chronic management. The only approved IV therapy for treatment of hyperammonemia is sodium benzoate plus sodium phenylacetate (Ammonul). Arginine becomes an essential amino acid in severe early urea cycle defects; it stimulates the CPSI enzyme and is required for urea cycle function as it is one of the amino acids that comprises the urea cycle. Thus, arginine should be provided IV for suspected neonatal OTCD or CPSI and is especially effective in patients with CIT1 and ASA. It should not be given in known or suspected arginase 1 deficiency. Ammonul is rarely stocked except in pharmacies of tertiary care metabolic centers, and 10% arginine HCl is commonly available.

Nitrogen-scavenging therapies can effectively help control hyperammonemia and should be considered when the ammonia level is greater than 150 µmol/L. When combined with other therapies, nitrogen-scavenging therapies are critical to the acute management of marked hyperammonemia. In acute management of hyperammonemia, Ammonul is given as a loading dose of 250 mg/kg (of sodium benzoate and of sodium phenylacetate) over 90 minutes followed by a 250 mg/kg dose over 24 hours by continuous IV infusion. Arginine hydrochloride should be provided in known or suspected cases of NAGS, CPSI, or OTCD and is given as a 250 mg/kg loading dose over 90 minutes followed by a 250 mg/kg 24-hour maintenance dose. The dose is 600 mg/kg for known or suspected CIT1 or ASA.[39,40] Also critical is reversal of catabolism, and at least age appropriate and even higher calories must be provided by IV glucose at high concentration and IV intralipid (once a disorder of fat metabolism has been excluded). Parenteral nutrition, including provision of catabolism-sparing essential amino acids, should be initiated when ammonia has been controlled, ideally within 24 to 36 hours of the initiation of treatment. This treatment should be performed in collaboration with a clinical biochemical geneticist with experience in the treatment of UCDs. An enzyme replacement therapy is now being investigated for arginase deficiency has shown improvements in baseline lower-limb spasticity, developmental delay, and previous hyperammonemic events.[41]

Chronic management of infants with UCDs consists of providing adequate dietary protein, which will require a combination of natural (whole) protein from a regular infant formula as well as a special metabolic formula consisting of only essential amino acids to decrease the nitrogen burden, oral/enteral ammonia-scavenging medications, and arginine or citrulline, depending on the defect and severity. Also critical is the prevention of protein catabolism during times of illness or other physiologic stress, and an emergency sick-day diet and emergency letter should be provided at hospital discharge. Children must be monitored frequently, and medications and diet adjusted to prevent hyperammonemia and to allow adequate growth without over-restriction of protein resulting in poor growth and provoking catabolism. Long-term management requires a multidisciplinary team of a clinical biochemical geneticist, biochemical nutritionist, and genetic counselors and, in some cases nurses, a neurodevelopmental pediatrician, a neurologist, and rehabilitative medicine specialist, depending on the presence and severity of early brain injury.

The height of the initial ammonia concentration is associated with cognitive impairment in the proximal UCDs, hyperornithinemia–hyperammonemia–homocitrullinuria syndrome, and citrin deficiency, as compared with the distal UCDs.[42] A 2005 review estimated a poor outcome with a mortality of 84% in neonatal-onset cases and 28% in late-onset cases, before the use of nitrogen-scavenging therapies in Europe.[43] When reviewing survival according to age, diagnosis, and first or recurrent episodes, the lowest survival is seen in male OTCD neonates at first episode. In an open label trial, newborns less than 30 days old had survival rates of 73% compared with 94% in infants greater than 30 days of age.[44] In a 2009 study, intellectual disability in neonatal-onset cases was reported at almost 50%, compared with historical estimates of 60% to 80%.[45] Currently, early liver transplantation in the first year of life, especially if initial rescue therapy has limited the peak and duration of hyperammonemia, is recommended.[46] Early recognition of hyperammonemia and rapid lowering of ammonia is critical to decreasing the extent and severity of early brain injury.[47]

Rare cases of carbonic anhydrase VA (CA-VA) deficiency are being more frequently confirmed through whole exome sequencing with mutations in the *CA5A* gene following neonatal presentations of hyperammonemia with elevated orotic acid and glutamine levels (similar on initial interpretation to a proximal UCD) with lactic acidosis and ketosis. Following treatment this disorder may have a good prognosis.[48]

Transient Hyperammonemia of the Newborn

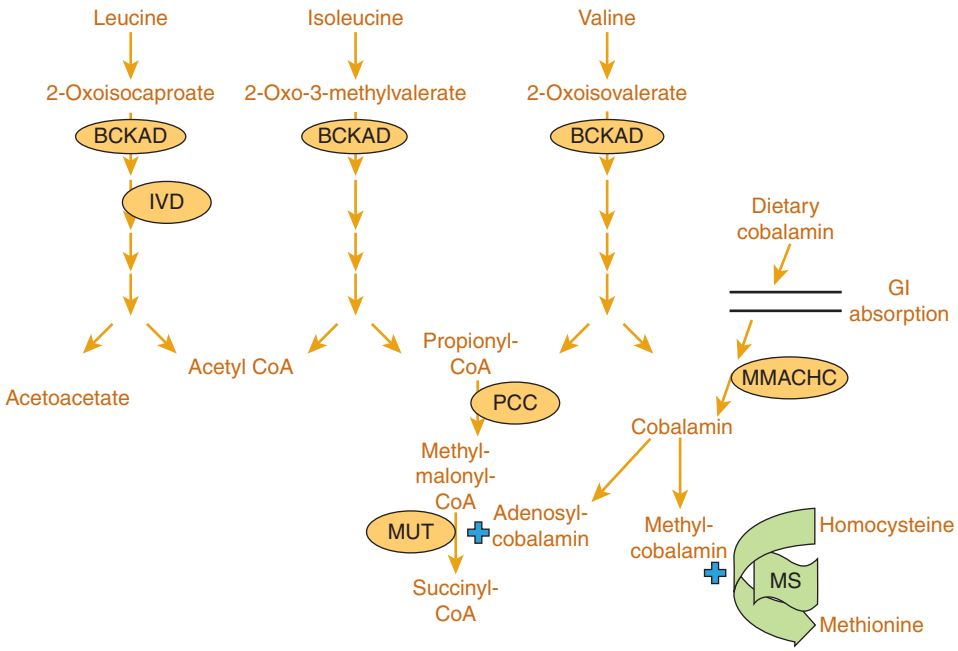
THAN is most common in preterm newborns less than 36 weeks gestational age that have a birth weight of less than 2.5 kg. Typically, it occurs following respiratory distress syndrome in the first 24 hours of life, and coma develops within the first 48 hours of life.[49] Serum ammonia levels can surpass 1500 µmol/L, and infants may require hemodialysis and protein restriction.[50]

The cause of this disease is unknown. When suspected, a Doppler ultrasound of the portal vein should be performed to evaluate for a clot in the portal vein. Plasma amino acid levels may have elevations of citrulline and arginine. The glutamine-to-ammonia ratio may distinguish this from a urea cycle defect (glutamine to ammonia ratio <1.6 in THAN, ensuring units are the same). There may be no respiratory alkalosis.[50] The mortality rate in THAN appears to be linked to the duration of coma; outcome can be good in surviving infants, and long-term treatment and protein restriction may not be necessary.

Amino Acid Metabolism Disorders

Maple Syrup Urine Disease

Maple syrup urine disease (MSUD) is a rare autosomal recessive inborn error of amino acid metabolism caused by branched-chain α-ketoacid dehydrogenase (BCKAD) complex deficiency. This enzyme is involved in the metabolism of the three branched-chain amino acids (BCAAs), leucine, isoleucine, and valine, at the step of conversion of each of their respective α-ketoacid derivatives into their decarboxylated coenzyme A (CoA) metabolites in the mitochondria (Fig. 29.4). The enzyme complex is composed of three components, E1, E2, and E3. E1 has two subunits, E1α and E1β, encoded by the *BCKDHA* and *BCKDHB* genes, respectively. E2 is dihydrolipoamide branched-chain transacylase (*DBT* gene). Mutations in the gene encoding the E3 subunit (*DLD*) cause a related but more severe disorder with lactic acidosis and elevations of pyruvate, alanine, and the α-ketoacids, as the E3 subunit is also a component of the pyruvate dehydrogenase (PDH) and α-ketoglutarate dehydrogenase complexes. MSUD occurs in 1 in 185,000 newborns, but in the old-order Mennonite communities of the United States in areas of eastern Pennsylvania, Kentucky, New York, Indiana, Wisconsin, Michigan, Iowa, and Missouri, the frequency is 1 in 358 due to a founder effect for a missense mutation (c.1312T>A in *BCKDA*, $E_1\alpha$).[51] The classic—and most frequent—presentation of MSUD is seen as early as 48 hours of life with poor feeding, irritability, lethargy, and a shrill and high-pitched cry. Symptoms rapidly progress to intermittent apnea, opisthotonus, and stereotyped movements described as "bicycling" or "fencing" alternating with hypotonia, as cerebral edema progresses. A bulging fontanelle may be present. As the illness progresses, coma, apnea, bradycardia, and respiratory failure will develop and usually result in death in the absence of specific



• **Fig. 29.4** Overview of branched-chain amino acid metabolism. *Plus sign* indicates allosteric cobalamin activator. *BCKAD*, Branched-chain α-ketoacid dehydrogenase; *MMACHC*, cobalamin C defect; *MS*, methionine synthase; *GI*; gastrointestinal; *IVD*, isovaleryl-CoA dehydrogenase; *MUT*; methylmalonyl-CoA mutase; *PCC*; propionyl-CoA carboxylase.

medical intervention. The odor of maple syrup in the cerumen may be detected after the first days of life and then in the saliva, breath, urine, and feces.

Neurologic progression is accompanied by, and due to, increasing elevations of the BCAA leucine, which is most readily assessed in plasma. Leucine and the related metabolite α-ketoisocaproic acid (α-KIC) may cause depletion of glutamine, glutamate, aspartate, and pyruvate. The mitochondrial respiratory chain may be inhibited by α-KIC and cause accumulation of lactic acid in the central nervous system (CNS).[52,53]

Neonates with MSUD present with lethargy and metabolic acidosis may develop or may be present initially. Untreated infants may develop ketonuria, which is separate from the ketoacidosis, and an elevated anion gap. Urine ketones may be negative in the presence of high levels of ketoacids. Quantitative plasma amino acid analysis and urine organic acid analysis should be diagnostic in the severe form of the disorder. The former demonstrates marked elevations of BCAAs and the presence of high levels of allo-isoleucine, a compound considered pathognomonic for MSUD. Analysis of urine organic acids will identify high levels of the relevant α-ketoacids in an ill child with the severe form of the disorder. Leucine may be in the thousands and elevated and rising levels require immediate treatment. This is a medical emergency due to the high risk of death and permanent neurologic damage. NBS results report "leucine," but this is the sum of leucine + isoleucine + hydroxyproline as these cannot be separated by tandem mass spectrometry without column chromatography. Ratios of leucine:alanine, leucine:phenylalanine, and valine:phenylalanine have improved the sensitivity and specificity of NBS as has implementation of a second-tier test of quantitation of allo-isoleucine after a high leucine value has been identified.[54]

An aggressive nutritional approach appears to work to lower leucine effectively in MSUD.[55] BCAA-free modified parenteral nutrition solution can be used in infants and older children with acute leucinosis but is rarely immediately available locally. This should be given in combination with IV glucose at high concentrations with IV intralipids. An insulin drip may also be necessary to curtail the effects of the catabolic stimulus and prevent hyperglycemia but must be carefully performed to avoid hypoglycemia. CAVH or hemodialysis may achieve more rapid normalization of the plasma BCAAs and their corresponding branched-chain ketoacids.

Neurologic outcomes in classic MSUD have improved with NBS, although the risk for subsequent brain injury or death remains, and long-term follow-up and continued vigilance are necessary to prevent injury.[52,56] Long-term neuropsychiatric assessments are showing that those who remain asymptomatic in the neonatal time period and in whom strict metabolic control is maintained can optimize their long-term mental health.[52] Liver transplantation appears to result in similar outcomes when compared with those who have not had transplantation, but liver transplant may prevent further neurocognitive impairment from prevention of injury during recurrent acute events.[57,58]

Long-term MSUD treatment focuses on a BCAA-free formula balanced with the provision of sufficient BCAAs to maintain normal growth and development. The goal is that plasma leucine, isoleucine, and valine levels are in the near-normal range, though this may be difficult to achieve outside of infancy. Affected individuals must be closely monitored, and careful management by a biochemical genetic nutritionist is critical. Care must be given to ensure adequate supplementation with isoleucine and valine as BCAA-free formulas may lead to over-restriction of these. Over-restriction of isoleucine can result in anemia and a severe exfoliative rash similar to acrodermatitis enteropathica. A rare thiamine-responsive variant of MSUD may show improved BCAA levels and a decreased need for protein restriction with thiamine supplementation.

Tyrosinemia Type 1

Tyrosinemia type 1 (TYR1), or *hepatorenal tyrosinemia*, is an autosomal recessive disorder caused by a deficiency of the enzyme fumarylacetoacetate hydrolase as a result of mutations in the *FAH* gene. This enzymatic reaction is the last in the catabolism of phenylalanine and tyrosine to fumaric acid and acetoacetate, and the accumulation of tyrosine is due to other accumulating metabolites. The primary metabolites that accumulate are maleylacetoacetic acid and fumarylacetoacetic acid, and these both result in the elevation of succinylacetone. This compound is pathognomonic for this disease and is the primary confirmatory metabolite identified on urine organic acid analysis. It is a more sensitive and specific marker than tyrosine in NBS but is not available in all NBS programs. The estimated incidence is 1 in 100,000 to 1 in 120,000 in the general population. The incidence is higher in specific populations with estimates of 1 in 60,000 to 1 in 74,000 in Norway and Finland, 1 in 16,000 in Quebec, and 1 in 1846 in the Saguenay-Lac Saint-Jean region of Quebec because of common founder mutations in these areas.[59]

The phenotype of TYR1 is variable. One presentation is of an acute, early-onset, severe liver disease at less than 2 months of age; there is also an infantile-onset presentation and a chronic presentation after 1 year of age. The acute, early-infantile presentation may be fatal with hepatomegaly, jaundice, elevated transaminases, and profound prolongations of prothrombin time and partial thromboplastin time. Affected individuals develop a renal Fanconi syndrome with generalized aminoaciduria, glycosuria, hypophosphatemia, hypouricemia, proteinuria, and an unusual urine odor of "boiled cabbage." Children with the chronic phenotype exhibit liver disease, hypophosphatemic rickets as a result of the renal Fanconi syndrome, cardiomyopathy (in 20% to 30%), and porphyria-like neurologic crises with abdominal pain, peripheral neuropathy, and respiratory failure.[59]

Plasma amino acid analysis will demonstrate elevated tyrosine levels, but this is not diagnostic as elevations of tyrosine and methionine are nonspecific and may be found in any disease causing liver dysfunction. Serum alpha fetoprotein levels are abnormally high. The identification of succinylacetone on urine organic acid analysis is diagnostic, and this may be detected within the first 12 hours of life.[60] As for most metabolic disorders, the characteristic metabolite abnormalities may not be present or detectable at all times.

NBS has allowed early treatment with 2-(2-nitro-4-trifluoromethylbenzoyl)-1,3-cyclohexanedione (NTBC), and this has improved clinical outcome.[61,62] Treatment for TYR1 includes NTBC, which inhibits *p*-hydroxyphenylpyruvate dioxygenase, a proximal enzyme in the pathway, reducing the accumulation of succinylacetone but resulting in increased tyrosine levels. NTBC improves liver and renal disease but requires the implementation of a low-tyrosine, low-phenylalanine diet for improved neurologic outcome.[63] Early treatment, ideally within the first month of life, results in a significant reduction in the development of acute liver disease, hepatomegaly, cirrhosis, hepatocellular carcinoma, renal dysfunction, rickets, and the need for liver transplantation.[61] Before the use of NTBC, most infants with the early-onset form of TYR1 died in early to late infancy. Unfortunately, patients treated with NTBC have shown impaired cognitive outcomes including a lower intelligence quotient, suboptimal executive functioning (working memory and cognitive flexibility), and social cognition (face recognition and the identification of facial emotion) when treated with a natural protein-restricted diet.[63,64] Dietary over-restriction leading to hypophenylalaninemia may be the cause of these neurocognitive deficits, poor growth, cortical myoclonus, and eczema, although these have been seen to improve or resolve following phenylalanine supplementation.[65]

One complication of TYR1 is the development of hepatocellular carcinoma, typically occurring in later presentations in older children. Monitoring through serial liver ultrasounds and alpha fetoprotein levels is necessary. Treatment with NTBC will improve the biochemical markers and liver dysfunction, but liver transplantation may still be necessary in those who are NTBC-resistant or who have chronic liver disease or poor quality of life.[61]

Other forms of tyrosinemia may be detected with elevated tyrosine levels on NBS or plasma amino acid analysis. Tyrosinemia type 2, or oculocutaneous tyrosinemia, presents with corneal tyrosine crystals, causing photophobia and hyperkeratotic plaques on the hands and soles of the feet. Tyrosinemia type 3 is extremely rare and has a variable phenotype including ataxia and mild mental retardation.[66] These are due to enzyme defects in tyrosine aminotransferase and *p*-hydroxyphenylpyruvate dioxygenase, respectively.

Transient tyrosinemia of the newborn is common in premature infants and is probably the most common disturbance of amino acid metabolism identified on NBS. It is due to delayed maturation of *p*-hydroxyphenylpyruvate dioxygenase or liver immaturity.

Nonketotic Hyperglycinemia

Glycine encephalopathy, also termed *nonketotic hyperglycinemia* (NKH), is an autosomal recessive disorder of the catabolism of glycine to carbon dioxide and ammonia.[67] The incidence is about 1 in 60,000. The glycine cleavage system is composed of four proteins, glycine decarboxylase (GLDC), amino-methyltransferase (AMT), the glycine cleavage H protein, and lipoamide dehydrogenase. These are also called the *P (pyridoxal-phosphate)*, *T (tetrahydrofolate)*, *H (hydrogen)*, and *L (lipoamide) proteins*, respectively, for the cofactor each utilizes. GLDC removes carbon dioxide, the AMT protein removes ammonia, the H protein removes the hydrogen, and the L protein regenerates the reduced form of the protein.[67] The majority of affected individuals have mutations in the *GLDC* or *AMT* genes.[68] The pathophysiology is likely related to glycine's role in the CNS as both an inhibitory and an excitatory neurotransmitter. The most common form of the disorder manifests in the first week of life as apnea and treatment refractory seizures associated with a burst-suppression pattern on electroencephalogram (EEG). This neonatal-onset form is associated with a very poor prognosis, even with early diagnosis and treatment.[68,69] There are milder forms of the disorder that manifest in the first months of life or later.

The diagnosis of NKH is based on both the absolute value of glycine in cerebrospinal fluid (CSF) and on the ratio of CSF glycine to plasma glycine. CSF and plasma amino acids must be obtained concurrently. However, the presence of blood in the CSF invalidates the results as CSF amino acid values will not be accurate.[70] CSF glycine is generally greater than 40 µmol/L in affected individuals, and a CSF-to-plasma glycine ratio of 0.08 or greater is considered diagnostic of NKH.[68] This disorder is not identified through NBS because of high false-positive rates of blood spot glycine levels.

One goal of treatment is to lower CSF glycine through high-dose sodium benzoate therapy. Benzoate is conjugated with glycine to form hippuric acid, which is excreted. In addition,

dextromethorphan, an *N*-methyl-D-aspartate (NMDA) receptor antagonist, is prescribed to counteract the activation of the NMDA receptor by glycine. Restriction of dietary protein to restrict the amino acid glycine is not an effective therapy. Other care is supportive as the majority of affected individuals have profound intellectual disability and develop spastic quadriplegic cerebral palsy. Mildly affected individuals with a later-onset presentation with autism have been described. Withdrawal of support in the newborn period has been performed because of the poor prognosis, but the existence of a "mimic" of the disorder associated with identical, but transient, biochemical and clinical features—and with a good outcome—may complicate this decision.[71]

Two classes of disorders that have a secondary effect on the glycine cleavage system have been described. One class was identified through candidate gene sequencing of individuals with defects in the glycine cleavage system that lacked mutations in the known causative genes.[72] The two classes are defects in lipoate synthesis, as lipoate is a cofactor of the L protein, and defects in iron–sulfur cluster biogenesis, as lipoate synthase is an iron–sulfur-containing protein. The disorders have unique clinical and biochemical features, and all have deficient glycine cleavage.[73] It is important to consider these variant disorders in a neonate with apparent NKH. DNA testing should be performed to confirm the correct diagnosis, as the results are necessary for accurate treatment, prognosis, and genetic counseling.

Hyperhomocysteinemias: Cystathionine β-Synthase Deficiency and Remethylation Disorders

In humans, through conversion of the essential amino acid methionine to homocysteine, a methyl donor is made available for multiple methylation reactions. Homocysteine may then either be metabolized to cysteine (the transsulfuration pathway) via cystathionine β-synthase or be remethylated back to methionine; defects in the relevant enzymes may lead to homocystinemia (or homocystinuria).

Classic homocystinuria is caused by an autosomal recessive deficiency of the cystathionine β-synthase enzyme as a result of mutations in the *CBS* gene and rarely presents in early infancy. The population frequency is estimated to be between 1 in 1800 and 1 in 900,000. NBS detects elevated methionine levels, but this will not detect all affected infants, particularly those with the pyridoxine-responsive form of the disease. Early treatment, which may include pyridoxine, dietary therapy and betaine, improves clinical outcomes, including ectopia lentis, myopia, mental impairment, a marfanoid body habitus, osteoporosis, thromboembolic events, and behavioral problems.[74] Infants may present with thrombosis, and homocysteine should be assayed for any thrombotic event, regardless of the NBS result.

Defective methionine remethylation may be due to methionine synthetase deficiency, methionine synthetase reductase deficiency, 5-methylenetetrahydrofolate reductase (MTHFR) deficiency, and other defects in cobalamin (a cofactor for methionine synthase) metabolism or transport. Newborns may come to clinical attention with encephalopathy with lethargy, feeding difficulties, hypotonia, seizures, pancytopenia with megaloblastic anemia, cardiomyopathy, and optic atrophy and retinopathy.[75,76] Previously thought to be rare, these disorders are now identified more frequently as a result of NBS, and false positives for these may be due to maternal nutritional cobalamin deficiency. The disorders of cobalamin intracellular processing or transport are identified by decreased methionine, elevated homocysteine, and/or elevated methylmalonic acid and C3-(propionyl)-acylcarnitine levels (see Methylmalonic Acidemia). Infants may quickly develop postnatal failure to thrive, and appropriate treatment, which may include hydroxocobalamin, betaine, folinic acid, carnitine, and methionine supplementation, is now resulting in improvements in clinical outcome.[75,76]

Patients with MTHFR deficiency may present as neonates with decreased consciousness, hydrocephalus, hypotonia, and feeding difficulties. There may be severe cortical atrophy and brain lesions caused by thromboses of the arteries or veins. This may present as an acute disorder of intoxication in the newborn period. Treatment with high-dose betaine, which enhances the remethylation of homocysteine to methionine through an alternate betaine–homocysteine methyltransferase pathway, is reported to improve or normalize development.[75,76]

Early detection of disorders of remethylation and of classic homocystinuria is possible through NBS, which utilizes a high methionine level to detect classic homocystinuria and a low methionine level to detect remethylation disorders. NBS is not fully sensitive or specific for these disorders. One cause of false positives is heterozygous mutations in the *MAT1A* gene causing hypermethioninemia, which may be benign. The sensitivity of NBS for cystathionine β-synthase deficiency is improved by using elevated methionine levels together with methionine:phenylalanine and methionine:total homocysteine ratios, although measurement of total homocysteine is not available universally. NBS for some remethylation disorders is possible by detection of low methionine levels with elevated C3-acylcarnitine levels, depending on the individual state programs.[76] Confirmatory DNA testing is available for all of the disorders.

Phenylketonuria

Phenylketonuria (PKU) is an autosomal recessive disorder resulting from deficiency of phenylalanine hydroxylase (PAH). The most severe form causes intellectual disability if untreated. It is the most common inborn error of amino acid metabolism, affecting about 1 in 10,000 northern European or east Asians, 1 in 2600 individuals in Turkey, and 1 in 4500 in Ireland, but only 1 in 143,000 in Japan and 1 in 200,000 both in Finland and among those of Ashkenazi Jewish ancestry.[77] The enzyme defect results in elevated levels of phenylalanine, which is the cause of the CNS injury, and decreased levels of tyrosine, which is of unclear clinical significance, but low levels may lead to supplementation. PAH requires the cofactor tetrahydrobiopterin (BH_4), and PAH enzyme deficiency may also result from a deficiency in the synthesis or recycling of BH_4. Disorders of BH_4 metabolism may result in elevated phenylalanine levels and should be ruled out when performing follow-up NBS testing, as treatment and outcomes differ.[78] BH_4 is also a cofactor for tyrosine hydroxylase, tryptophan hydroxylase, and nitric oxide synthase, and BH_4 deficiency also leads to impaired levels of L-dopa, dopamine, norepinephrine, melanin, serotonin, citrulline, and nitric oxide. PKU does not present with symptoms in the newborn period. It is a cause of intellectual disability, and there are no systemic manifestations. Treatment, however, should be instituted in the first several weeks of life for optimal outcome, and it is important that physicians caring for newborns are familiar with the evaluation and management of newborns with an abnormal NBS for PKU.

PKU was the first disorder for which NBS was implemented in the 1960s. NBS for PKU is a model for preventative and personalized medicine, as well as for public health screening and intervention. The blood spot phenylalanine level and the phenylalanine:tyrosine ratio will be elevated and detected by NBS. The blood spot should be collected after 24 hours of age following breastfeeding or formula feeding. Elevated urine phenyl ketones and phenylacetic acid result in a "mousey" urine odor. Diagnosis should be confirmed through plasma amino acid analysis and testing for BH_4 metabolism disorders in blood and urine. DNA sequencing of the *PAH* gene may provide helpful information for genetic counseling and treatment.

Untreated PKU patients will develop developmental and intellectual disabilities, eczema, hypopigmented skin and hair, and epilepsy in infancy and at older ages. Dietary phenylalanine restriction with strict compliance in order to maintain plasma phenylalanine levels in treatment range is necessary to prevent intellectual disability and other features. Dietary compliance may be difficult for teenagers and adults, but lifelong dietary treatment is necessary to avoid neuropsychiatric symptoms such as inattention, hyperactivity, depression, and anxiety.[79] Mothers who are poorly compliant during pregnancy and have phenylalanine levels above the recommended treatment range may have children with microcephaly, fetal growth restriction, congenital heart defects, and other malformations, known as *maternal PKU syndrome*. Attaining plasma phenylalanine levels within treatment range before the 8th week of pregnancy is essential and ideally should be achieved before pregnancy. Phenylalanine levels should be maintained between 120 and 360 µmol/L (or 2 to 6 mg/dL), the typical treatment range in the United States, throughout the pregnancy.[78] Plasma phenylalanine levels in untreated individuals with classic PKU are greater than 1200 µmol/L (20 mg/dL).

Dietary therapy for PKU is effective and is a low natural-protein diet supplemented with phenylalanine-free medical formula to ensure adequate total protein and micronutrients. An adequate amount of phenylalanine from whole protein in formulas and/or food is necessary for normal growth and protein synthesis, with a goal of maintaining plasma phenylalanine levels less than 360 µmol/L.[78] In patients with classic PKU, protein tolerance may be as low as 5 g of protein per day, requiring metabolic formula lacking phenylalanine to provide the majority of needed daily protein (0.9 to 1 g/kg/day for an adult, i.e., about 70 g for a 70 kg adult). This diet is difficult to maintain lifelong and other therapies have been developed. Sapropterin (a synthetic form of the BH_4 cofactor) may be beneficial in lowering phenylalanine levels in the 25% to 50% of patients who are sapropterin responsive. Other therapies include large neutral amino acids that compete for the same gut and brain transporter as phenylalanine in older children and adults, and lower brain phenylalanine levels, and use of an alternate enzyme, polyethylene glycol-conjugated phenylalanine ammonia lyase, that converts phenylalanine to transcinnamic acid, which is nontoxic and is excreted. The enzyme is given subcutaneously to reduce plasma phenylalanine levels and may result in patients being able to tolerate an unrestricted diet.[78]

Organic Acidemias

The classical organic acidemias (or acidurias, the terms are interchangeable) are characterized by systemic illness and presentation in the newborn period in the severe forms and include methylmalonic acidemia (MMA), propionic acidemia, and isovaleric acidemia. These are due to defects in pathways that affect the catabolism of one or more of the BCAAs (leucine, isoleucine, and valine) as well as other amino acids (see Fig. 29.4). An organic acid is any organic compound that contains a carboxy functional group but no α-amino group. They are intermediates in multiple pathways, including those of amino acid, fatty acid, cholesterol, and neurotransmitter metabolism. Disorders of fatty acid oxidation, ketone body metabolism, and lactic acid metabolism may also be detected through organic acid analysis.

Before NBS, these disorders were diagnosed only after patients became symptomatic, unless there was a known affected family member. While early presentation does occur before NBS results are available, with widespread NBS it is more common to be confronted with an asymptomatic or early symptomatic patient and a differential diagnosis that is based on the NBS results. Presenting symptoms of the classic organic acidemias in the neonate result from the progressive hyperammonemia, keto lactic acidosis, and hypoglycemia.

Methylmalonic Acidemia

Multiple genetic defects can lead to the elevation of methylmalonic acid. Isolated methylmalonic acidemia (MMA) is caused by methylmalonyl-CoA mutase enzyme deficiency because of mutations in the *MUT* gene. Adenosylcobalamin is a required cofactor for the mutase enzyme, and cobalamin (vitamin B_{12}) disorders of processing or transport may present with elevations of methylmalonic acid alone or in combination with elevated homocysteine, as homocysteine is also metabolized by a cobalamin-dependent enzyme, cystathionine β-synthase (see Hyperhomocysteinemias and Remethylation Disorders). Cobalamin is acquired through dietary sources and must be appropriately transported and then undergoes a series of intracellular modifications. Thus, impaired cellular cobalamin metabolism and other inherited defects in cobalamin transport or modification result in MMA. Inherited defects of cobalamin transport or modification may cause isolated MMA, or a combined defect with homocystinuria.[80,81] Methylmalonic acid is detected in the blood, CSF, and urine of affected individuals. The disorder is typically identified through urine organic acid analysis after an abnormal NBS or in a clinically presenting neonate. The precursors of methylmalonyl-CoA are primarily isoleucine and valine but additionally include methionine, threonine, thymine, and odd-chain fatty acids.[82]

There is a spectrum of severity of isolated MMA that ranges from severe, catastrophic newborn-onset disease to benign forms.[81] Patients presenting in the newborn period have a severe phenotype but may have a vitamin responsive form of the disorder (e.g., cobalamin A deficiency). Levels of homocysteine and vitamin B_{12} should always be assessed when elevated methylmalonic acid has been identified, and cobalamin should be provided empirically. The neonate appears well at birth but may rapidly progress as early as the second or third day of life to having problems with feeding and then develop vomiting, lethargy, and perhaps seizures. There may be tachypnea to compensate for metabolic acidosis. Crucial laboratory findings include metabolic acidosis with an increased anion gap, elevated lactate, elevated ketones, and elevated ammonia. The elevation of ammonia may be as high as that found in neonates presenting with early-onset UCDs, and specialized biochemical genetic laboratory testing is required for diagnosis and should include a urine organic acid analysis and a plasma amino acid analysis, a plasma acylcarnitine profile, and a quantitative orotic acid level. Ketonuria is uncommon in newborns, and the physician must always consider an organic acidemia in an acutely

ill newborn with ketosis. Other laboratory findings are thrombocytopenia, leukopenia, and anemia caused by effects of the metabolite on hematopoietic elements in bone marrow. Plasma amino acid analysis may reveal elevations of glycine and alanine. Elevations of C3-(propionyl)-acylcarnitines may result in a secondary free carnitine deficiency caused by conjugation of accumulating metabolites, and so carnitine supplementation should be initiated. Methylmalonic acid and other metabolites are detected in urine by organic acid analysis. The specific genetic diagnosis should be identified by DNA testing. Next-generation DNA sequencing panels are available, although results often take weeks to return. Most are inherited as autosomal recessive conditions, except for cobalamin X deficiency caused by a defect in *HCFC1*, a transcription factor that regulates the cobalamin C gene.[83]

Rapid institution of empiric therapy may improve outcome. Acute management protocols are available and should be administered in conjunction with a biochemical geneticist.[82] The treatment of acute metabolic decompensation includes cessation (for no more than 12 to 24 hours) of protein intake, empiric therapy with cobalamin, provision of high calories through protein-free enteral formula, and/or IV glucose at high concentration with IV intralipids, insulin, and carnitine. Some patients are responsive to pharmacologic doses of cobalamin, and 1 mg/day intramuscular hydroxocobalamin (preferred formulation) should be given as empiric therapy to a newborn presenting with hyperammonemia and acidosis.[82] Sodium bicarbonate should be used if indicated to correct acidosis. Dialysis is indicated for refractory hyperammonemia or acidosis. Additional therapies may include ammonia scavenger medications and carglumic acid, a stable analogue of the coactivator of the urea cycle that is depleted in MMA, causing the secondary hyperammonemia.[82] Chronic treatment includes a low natural-protein diet, an amino acid supplement lacking the MMA precursors, carnitine, and appropriate calories and fluid. Cobalamin is used chronically only when a specific and reproducible response is noted.

Most patients are now identified by NBS, although there are missed cases, and infants may become ill before the NBS result has been returned. False-positive NBS may result from maternal cobalamin deficiency. If the NBS and follow-up results are rapidly available, the outlook for the neonatal period may be improved. Episodes of decompensation will still occur after the newborn period associated typically with infection or other stressors. Patients who are particularly brittle or who develop renal failure receive either liver, kidney, or combined liver–kidney transplants. Long-term complications include basal ganglia stroke (which may occur in the newborn period), renal disease and failure, and pancreatitis.[82]

Propionic Acidemia

Propionic acidemia (PA) is due to a deficiency of propionyl-CoA carboxylase and is an autosomal recessive disorder. It was originally referred to as "ketotic hyperglycinemia," because patients may have elevations of glycine as well as ketosis. Propionyl-CoA carboxylase is the enzymatic reaction just upstream of methylmalonyl-CoA mutase, and the precursors are identical (see Fig. 29.4). The clinical presentation and therapy of PA in the newborn period are similar to that of MMA. A biochemical geneticist with experience in the management of inborn errors should guide care and help guide diagnosis and the differentiation of MMA and PA from other possible disorders. Empiric therapy should be administered as described for MMA, with the provision of calories, IV glucose at high concentration with IV intralipid (once a disorder of fat metabolism has been ruled out), insulin (if needed), carnitine, cobalamin (for possible cobalamin-responsive MMA), and temporary cessation (no more than 12 to 24 hours from last intake) of protein. Hyperammonemia may be marked, and dialysis may be indicated. Ammonia-scavenging medications and carglumic acid may also be used, as for MMA.[82]

Elevated ammonia levels may be associated with CNS injury. Metabolic stroke, because of cell death in the absence of thrombosis or ischemia, may be seen in both MMA and PA and may result in damage to the basal ganglia. In MMA the globus pallidus is classically affected, while in PA the caudate and putamen are typically affected, resulting in severe choreoathetosis.[82,84] Chronic therapy consists of a low natural-protein diet, supplementation with metabolic formula lacking the MMA/PA precursors, carnitine, and adequate calories and micronutrients.[82] Biotin is a cofactor for the enzyme, but no case of biotin-responsive PA has been described; an empiric trial can be considered. Intestinal bacteria (*Propionibacterium* species) contribute to propionate production. Antimicrobial therapy with metronidazole has been used to lower propionate metabolites in acute illness and may be given as intermittent therapy to lower metabolites as part of chronic therapy. While not curative of the disorder, liver transplantation is being performed in individuals with early-onset PA with the goal of ameliorating long-term complications and enhancing quality of life.[82] The efficacy of liver transplant is unclear.

Outside of the newborn period, or in late-presenting individuals, episodes of metabolic decompensation are characterized by hyperammonemia, acidosis, and ketosis precipitated by excessive protein intake or more commonly by infection resulting in poor oral intake and protein catabolism. Families may use home urine ketone strips for monitoring so that early decompensation can be recognized and aggressive measures instituted to avoid further decompensation, as episodes can result in permanent neurologic damage. Affected individuals may exhibit neurological symptoms including developmental delay, seizures, cerebral atrophy, and EEG abnormalities; other complications such as optic atrophy, cardiomyopathy, and pancreatitis; and late-onset presentations with only neurologic features.[84]

Diagnosis is made through urine organic acid analysis and confirmed by enzymatic and/or DNA testing. The acylcarnitine profile, on NBS or in plasma, will have elevated C3-(propionyl)-acylcarnitine, and urine organic acid analysis is required to distinguish between MMA and PA. In PA the urine has elevated 3-hydroxypropionic, propionyl glycine, and methylcitric acid but not methylmalonic acid. Both chronically and during acute decompensation, plasma ammonia may be mild to moderately elevated. The enzyme activity of propionyl-CoA carboxylase can be assayed in white blood cells or cultured skin fibroblasts. DNA testing may identify the numerous mutations that have been described in the two genes (*PCCA* and *PCCB*) that encode the subunits of this multimeric enzyme. Most patients are now identified by NBS, and the severe neonatal decompensation may be ameliorated if the results are rapidly available.

Isovaleric Acidemia

Isovaleric acidemia (IVA) is an autosomal recessive disorder caused by deficiency of the enzyme isovaleryl-CoA dehydrogenase. This is a defect in catabolism of the amino acid leucine. There are two major phenotypes, distinguished by the degree of enzyme deficiency and differing mutations. The acute form manifests as

catastrophic disease in the newborn period. The late-onset type is characterized by chronic, intermittent episodes of metabolic decompensation.[85] In the acute form the infants become ill in the first week of life, similar to MMA and PA. Marked acidosis and ketosis suggest an organic acidemia, and urine organic acid analysis is required for diagnosis and shows elevations of isovalerylglycine and 3-hydroxyisovaleric acid. An elevated C5-(isovaleryl)-acylcarnitine value on plasma acylcarnitine analysis or on NBS is strongly suggestive of this diagnosis, although urine organic acid analysis is still required. DNA mutation analysis of the *IVD* gene is available. Another diagnostic clue is the characteristic "sweaty-feet, rancid cheese, or dirty socks" odor caused by isovaleric acid, which is detectable in blood and urine, especially during acidosis. Dialysis may be necessary if there is marked hyperammonemia. As for other organic acid disorders, acute treatment consists of protein restriction (for 12 to 24 hours) with the provision of calories, IV glucose at high concentration with IV intralipids, insulin (if necessary), carnitine, and sodium bicarbonate if indicated, followed by a protein-free formula to provide additional calories via nasogastric tube if enteral nutrition is tolerated and oral glycine (150 to 250 mg/kg/day) for known or suspected IVA and oral or IV carnitine supplementation.[85] The excretion of isovaleric acid as the glycine conjugate is highly efficient, and improvement can occur rapidly with provision of glycine and other therapy.

In the chronic, intermittent form of IVA, patients have repeated episodes of metabolic decompensation precipitated by infection, other catabolic stress such as surgery, or excessive protein intake. These episodes may mimic Reye syndrome. The same therapeutic principles are applied as for the acute treatment of the neonatal disorder. Chronic therapy consists of a diet with low natural protein (limiting leucine to the amount required for growth), a leucine-free amino acid supplement to provide sufficient protein for growth, and glycine, which enhances isovalerylglycine production and reduces free isovaleric acid levels in body fluids while carnitine administration can augment the excretion of isovalerylcarnitine.[85] Some patients who remain largely asymptomatic are ascertained through NBS. This form of IVA is associated with a specific mutation (p.A282V) in the *IVD* gene.[86] NBS and early institution of treatment can alter the prognosis of affected individuals.[87]

Multiple Carboxylase Deficiency

Two enzymatic defects, holocarboxylase synthetase deficiency and biotinidase deficiency, lead to deficiency of the four carboxylase enzymes, propionyl-CoA carboxylase, pyruvate carboxylase (PC), 3-methylcrotonyl-CoA carboxylase, and acetyl-CoA carboxylase; these require covalent linkage of the cofactor biotin to a lysine residue of the enzyme for normal activity.[88] Holocarboxylase synthase is the enzyme that catalyzes this covalent linkage, and a severe defect of this enzyme causes early-onset multiple carboxylase deficiency. The biotinidase enzyme regenerates biotin from the amide biocytin, formed by biotin and lysine. Biotinidase deficiency causes late-onset multiple carboxylase deficiency because of the failure to regenerate biotin. This condition typically does not manifest in the newborn period. High-dose biotin therapy is extremely efficacious in both conditions.

Infants with severe deficiency of holocarboxylase synthase become ill in the newborn period. They develop a marked metabolic acidosis with severe lactic acidosis and become encephalopathic. Administration of enteral biotin is life-saving, and empiric therapy should be administered if there is a concern for this disorder. In an affected neonate, administration of this vitamin results in a dramatic improvement in clinical and laboratory findings. As described for MMA, PA, and IVA, empiric management should include initial protein restriction, provision of high calories, correction of acidosis, and empiric administration of cofactors and carnitine. After an initial period of empiric management, specific management should be based on the results of specialized biochemical testing. The goal is to have these results within 24 hours in order to provide the correct therapy. Evaluation and management should be guided by a biochemical geneticist familiar with the management of inborn errors.

The characteristic plasma acylcarnitine profile exhibits elevations of C3-(propionyl)-acylcarnitine and C5-OH-(3-hydroxyisovaleryl)-acylcarnitine and may be detected on NBS. Urine organic acid analysis identifies marked elevations of metabolites because of the deficiencies of pyruvate, propionyl-CoA, and 3-methylcrotonyl carboxylases. These include lactate, 3-hydroxypropionic acid and propionylglycine, and 3-methylcrotonylglycine, respectively. In most patients the defect is associated with decreased affinity of the holocarboxylase synthetase enzyme for biotin, and effective treatment requires between 10 mg and 100 mg of oral biotin per day. Long-term treatment may include dietary protein restriction and carnitine supplementation. The enzyme defect can be confirmed in cultured skin fibroblasts, or the diagnosis can be confirmed by DNA mutation analysis of the *BTD* or *HCLS* gene. Assay of individual carboxylases in white blood cells will show deficiency of all three carboxylases assayed, but this is not specific. The disorder is inherited as an autosomal recessive trait.

NBS also detects individuals with partial biotinidase deficiency (30% enzyme activity) who do not develop the severe symptoms of the profound deficiency. Enzyme analysis and mutation analysis distinguish these.[89,90]

Late-onset multiple carboxylase deficiency caused by profound biotinidase deficiency may present as seizures and lactic acidosis and episodic decompensation. Long-term sequelae include hearing loss and optic atrophy. This disorder is effectively treated with biotin (10 to 20 mg/day), which prevents these long-term complications.[88] Effective NBS requires a specific biotinidase enzyme assay as it is not reliably detected by tandem mass spectrometry.

Glutaric Aciduria Type 1

Glutaric aciduria type 1 (GA1) is due to a deficiency of glutaryl-CoA dehydrogenase, an enzyme in the catabolic pathway of the amino acids lysine, hydroxylysine, and tryptophan.[91] Unlike the organic acid disorders described previously that have systemic manifestations including hyperammonemia, lactic acidosis, ketosis, and bone marrow suppression, GA1 is termed a "cerebral" organic aciduria. In most instances the systemic manifestations of decompensation are not present, and GA1 does not have a catastrophic presentation in the newborn period. The classic presentation of GA1 is that of a child with normal growth and development in the first 6 to 18 months of life who experiences a metabolic stroke of the basal ganglia during illness, surgery, or another event that provokes catabolism.[92] Clinically affected children sustain irreversible bilateral damage to the caudate and putamen and, more rarely, the globus pallidus, structures of the basal ganglia that influence the control of voluntary movement, resulting in incapacitating dystonia and decreased life expectancy. Other affected individuals appear to have a slowly progressive course with hypotonia, dystonia, and dyskinesia in the first several years of life; these may have sustained early injury without a

recognized encephalopathic illness. Remarkably, there is a vulnerable period for neurologic injury, and affected individuals who do not have injury in the first 6 years of life may have normal growth and development. This disorder is identified on NBS with elevations of C5-DC-(glutaryl)-acylcarnitine, though some individuals termed "low excretors" may be missed.[91] Chronic therapy includes a low natural-protein diet balanced with supplementation of a lysine-free, tryptophan-reduced, and arginine-containing formula, carnitine, and riboflavin (the enzyme cofactor that may result in biochemical improvements in some cases). Acute emergency therapy includes protein restriction (for 12 to 24 hours) with the provision of calories as IV glucose at high concentrations with IV intralipids, insulin (if necessary), and carnitine, with supportive care from neurology or neurosurgery. These are critical to decreasing the incidence of devastating neurologic injury in affected individuals.[91,93]

GA1 may mimic nonaccidental trauma, as some individuals have subdural hematomata and retinal hemorrhages, independent of basal ganglia injury. Subdural hematomas may be identified in the investigation of crossing of percentiles for head circumference, a finding in GA1. Another finding that may prompt investigation for GA1 is increased fluid in the perisylvian fissures observed on brain computerized tomography or magnetic resonance imaging (MRI).[91] Manifestations in the newborn period include nonfamilial macrocephaly, increased fluid in the perisylvian fissures, and an abnormal NBS. Initial testing after an abnormal newborn screen, or if there is a clinical concern, should include a plasma acylcarnitine profile, measurement of total and free carnitine, and a urine organic acid analysis.

Urine organic acid analysis will identify elevated glutaric acid and 3-hydroxyglutaric acid, though the latter compound, considered diagnostic of GA1, may be normal or near normal in a non-decompensated state in some individuals. Critically, these individuals, termed low excretors, are at no less risk of severe neurologic injury. The diagnosis may be confirmed by enzyme assay in fibroblasts or by DNA mutation analysis of the *GCDH* gene. NBS has resulted in a decrease in the incidence of neurologic injury in affected individuals.[91,93]

Fatty Acid Oxidation Disorders

Fatty acid oxidation disorders (FAODs) are inborn errors of metabolism resulting in failure of β-oxidation within, or transport of fatty acids into, the mitochondria. There are at least 31 enzymes described. While short- and medium-chain fatty acids enter the mitochondria directly, carnitine is required for transport of long-chain fatty acids. Within the mitochondria the β-oxidation cycle will form acetyl-CoA and generate nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide ($FADH_2$), enabling electron transfer to the respiratory chain for adenosine triphosphate (ATP) generation. Acetyl-CoA is used as a substrate in the Krebs cycle to produce reducing equivalents for the electron transport chain (ETC) and ketone synthesis. FAODs lead to a deficit of energy production and produce a wide range of clinical presentations from mild hypotonia in adults to sudden death.[94]

FAODs present at all ages and spare the CNS when in good metabolic control. Life-threatening phenotypes present in the first few days of life (typically after 24 to 48 hours of life) because of the severe catabolic state and enhanced breakdown of free fatty acids from adipose stores, often before NBS results have been received.[95] Signs include hypoglycemia, liver disease and liver failure, cardiac and skeletal myopathy, rhabdomyolysis, and retinal degeneration.[94] The accumulation of long-chain acylcarnitine species has been suggested to be dysrhythmogenic and may be associated with cardiac dysfunction.[94]

Overall, the incidence of all FAODs ranges from about 0.9 to 15.2 per 100,000, with significant variability due to regional and genetic population differences, with founder populations having much higher incidences as would be expected, and the incidence on NBS has been higher than that from clinically identified symptoms.[95] Medium-chain acyl-CoA dehydrogenase deficiency (MCADD) is the most common FAOD, while very long chain acyl-CoA dehydrogenase deficiency (VLCADD) is the most common long-chain FAOD with an estimated incidence of 0.07 to 1.19 per 100,000, the other disorders being commonly less than 1 in 100,000.[95] There is significant variability in which FAODS are included in NBS panels around the world. The diagnosis is confirmed with a plasma acylcarnitine profile, total and free carnitine levels, and DNA testing. Urine organic acid analysis and measurement of urine acylglycines should also be performed. If variants of uncertain significance are identified on DNA sequencing, leukocyte, fibroblast, or liver enzyme assays may be implemented to determine whether an individual is affected. All FAODS are autosomal recessive and false-positive NBSs are commonly found due to carrier status. Many infants are now being identified with milder, and perhaps persistent, elevations of specific acylcarnitines but are not thought to be at significant risk for clinical disease, although long-term follow-up studies are needed to determine what, if any, risk is present for later-onset forms of these disorders, such as VLCADD. There is accumulating evidence from retrospective analyses that supports NBS identification in the neonatal period has improved outcomes compared with patients diagnosed clinically after development of symptoms.[95]

Treatment of FAOD shares many common features. Treatment primarily is targeted at prolonged fasting avoidance to maintain a constant energy supply and prevent the use of fat for energy, together with a higher-carbohydrate, low-fat diet. Safe fasting periods for different age groups have been proposed: neonates until about 3 months of age should fast no longer than 3 to 4 hours, with this time gradually increasing through infancy.[96,97] IV fluids with 10% dextrose at 150% maintenance rate should be given when the patient is fasting for surgery.

For long-chain FAOD, supplementation with medium-chain triglyceride (MCT) oil is used to provide a substrate for β-oxidation and should be prescribed to provide 20% to 25% of total calories.[97] Avoiding essential fatty acid deficiency may require supplementation with additional oils, depending on the degree of long-chain fat restriction. MCT supplementation and the recently approved anaplerotic treatment with triheptanoin (Dojolvi) has been associated with a reversal of cardiomyopathy in carnitine acylcarnitine translocase deficiency (CACT) and VLCADD, although compliant VLCADD patients still had significant muscle weakness, muscle pain, or myoglobinuria.[98–100] Carnitine supplementation is controversial although is supported if there is free carnitine deficiency.[97]

Medium-Chain Acyl-CoA Dehydrogenase Deficiency

Medium-chain acyl-CoA dehydrogenase deficiency (MCADD) is the most common FAOD with a frequency reaching approximately 1 in 20,000 births in northern European populations with the common mutation, c.985A>G. Classic presentations occur in

older infants during an infection, with poor oral intake, vomiting, dehydration, lethargy, hypoglycemia, seizures, and a presentation similar to Reye syndrome, leading to death from brain edema and hyperammonemia. Severe lethal presentations will develop during the first days of life before NBS, and MCADD is a recognized cause of sudden infant death syndrome.

The initial presenting episode historically has a high mortality rate, but this has improved with NBS.[101,102] Plasma acylcarnitine profiles demonstrate increased levels of C6, C8, C10, and C10:1 values. Urine acylglycine analysis shows elevations of suberylglycine and hexanoylglycine. Sequence analysis of *ACADM* confirms the diagnosis. Treatment focuses on avoiding prolonged fasting, especially during intercurrent illness, avoidance of MCT supplementation, and carnitine supplementation if necessary.[96]

Very Long-Chain Acyl-CoA Dehydrogenase Deficiency

VLCADD presents with variable phenotypes ranging from severe cardiomyopathy that may result in death in the first few days of life to recurrent hypoketotic hypoglycemia or to later-onset presentations with myopathy and/or rhabdomyolysis in adolescence or adulthood.[96,103] Cardiomyopathy and arrhythmias have been reported in a high proportion of cases presenting at less than 6 years in a country without newborn screening.[104]

Most patients with VLCADD are detected through NBS but present a significant challenge in that a large number of individuals appear to have mild or perhaps benign DNA variants and normal follow-up plasma acylcarnitine profiles. Many of these cases will have a single heterozygous mutation and so appear to be unaffected carriers. Others may have two compound heterozygous mutations but may have a reduced clinical risk of symptoms. Plasma acylcarnitines show a pattern of elevations of C14:1-, C14-, C16:1-, and C16-acylcarnitines levels with low secondary free carnitine levels in some infants. With an acute metabolic decompensation, urine organic acid analysis may demonstrate dicarboxylic aciduria. Confirmation by sequencing and deletion/duplication analysis of *ACADVL* is recommended. Functional enzyme assay or fibroblast acylcarnitine probe analysis may be helpful to determine treatment when a single heterozygous mutation is found, novel uncharacterized variants are found, and/or there are persistent elevations of acylcarnitines inconsistent with the genotype.[105]

Treatment follows the general principles for long-chain FAOD treatment, consisting of prolonged fasting avoidance, long-chain dietary fat restriction, medium-chain fat supplementation, and carnitine supplementation if necessary. Medium chain fat may be provided by MCT oil, powder, or as a component of formula (e.g., Enfaport) or as triheptanoin.[97] Triheptanoin (Dojolvi), which contains an odd-chain fat that is a source of additional anaplerotic energy to the TCA cycle was recently approved for infants with long-chain fat metabolism defects. Its use is associated with improved cardiac phenotype and decreased frequency of hospitalizations for rhabdomyolysis when compared to conventional MCT.[106] Severely affected infants should discontinue breastfeeding because of the high fat content in breast milk, with implementation of an MCT-containing formula or with formula and triheptanoin. Treatment of milder forms of VLCADD may include supplementation of breastfeeding with an MCT-containing formula.[97]

Short-Chain Acyl-CoA Dehydrogenase Deficiency

Short-chain acyl-CoA dehydrogenase deficiency (SCADD) is diagnosed through the detection of elevations of C4-acylcarnitine, urinary ethylmalonic acid, and butyrylglycine. Prior reports found decreased SCAD enzyme activity to be associated with failure to thrive, poor feeding, hypotonia, and seizures. Subsequently, up to 14% of the normal population has been found compound heterozygous or homozygous for two common polymorphisms (c.511 C>T and c.625 G>A) with the biochemical abnormalities of SCADD.[107] Currently, most infants with SCADD are detected on NBS and remain clinically asymptomatic, leading many to consider this a benign condition.[108,109] The need for treatment, carnitine or riboflavin supplementation, or management during illness is unclear.

Long-Chain 3-Hydroxy Acyl-CoA Dehydrogenase Deficiency and Trifunctional Protein Deficiency

The mitochondrial trifunctional protein complex of four alpha and four beta subunits comprises three enzymes: long-chain enoyl-CoA hydratase, long-chain 3-hydroxy acyl-CoA dehydrogenase, and 3-ketoacyl-CoA thiolase. Long-chain 3-hydroxy acyl-CoA dehydrogenase deficiency (LCHADD) occurs when there is only reduced dehydrogenase activity because of mutations in the *HADHA* gene, while trifunctional protein deficiency (TFP) results from mutations in either the *HADHA* or *HADHB* gene and in deficient activity of all three enzymes. The most severe forms of LCHADD or TFP present with a rapidly progressive neonatal cardiomyopathy.[110,111] Infants may later develop recurrent hypoketotic hypoglycemia with acute catabolic illness resulting in liver dysfunction (a Reye-like syndrome), cardiomyopathy, myopathy, and rhabdomyolysis. Sixty-five percent of surviving individuals with LCHADD or TFP experience skeletal myopathy, 21% develop a slowly progressing peripheral neuropathy, and 43% have pigmentary retinopathy.[111] Some patients may have severe liver disease with fibrosis in addition to necrosis and steatosis. Older children, adolescents, and adults may develop recurrent rhabdomyolysis during illness.

Heterozygous mothers may rarely develop either acute fatty liver of pregnancy or hemolysis, elevated liver enzymes, and low platelets (HELLP syndrome) when carrying a child with LCHADD.[112]

Diagnosis is made through the demonstration of elevations of C16:1-OH-, C16-OH-, C18:1-OH-, and C18-OH-acylcarnitines levels and the demonstration of longer-chain 3-hydroxydicarboxylic acids on urine organic acid analysis. Enzymatic diagnosis can be made in lymphocytes or in skin fibroblasts, but a combination of clinical biochemical abnormalities and *HADHA* or *HADHB* mutation analysis is often sufficient. The majority of moderate-to-severe cases are diagnosed by NBS. Follow-up of cases diagnosed on NBS demonstrates improved growth and development, but NBS does not completely prevent morbidity and mortality, especially in TFP, which has poorer survival.[110,111,113]

Treatment of LCHADD and TFP involves avoidance of prolonged fasting, dietary long-chain fat restriction, and MCT supplementation. A low long-chain fat diet and MCT or triheptanoin supplementation will decrease plasma hydroxyl acylcarnitine levels, and some LCHADD patients remain healthy without metabolic decompensation; many patients will have symptoms that

are refractory to therapy including pigmentary retinopathy and peripheral neuropathy.[114]

Primary Carnitine Transporter Deficiency

Carnitine is essential for long-chain fatty acid transport across the mitochondrial inner membrane. This is dependent upon a sodium-dependent plasma membrane carnitine transporter, two transferases that covalently link and then remove carnitine to the long-chain fatty acid, and a mitochondrial membrane translocase.

Carnitine transporter deficiency (CTD, primary carnitine deficiency, carnitine uptake defect) is characterized by hypoketotic hypoglycemia, hyperammonemia, liver dysfunction, cardiomyopathy, and skeletal hypotonia. Neonatal presentations are not common. In some older patients, cardiomyopathy may be the presenting sign. Profoundly low plasma total and free carnitine levels (typically <10 μmol/L in plasma) are found. False-positive NBS for low free carnitine levels may be due to neonatal nutritional deficiency or secondary to low maternal plasma carnitine levels from either a dietary restriction (e.g., vegan diet) or previously unrecognized maternal primary carnitine deficiency or organic acidemia. Measurement of maternal total and free carnitine levels is often necessary when performing follow-up testing of an abnormal NBS. Women with true maternal primary carnitine deficiency may be apparently asymptomatic at diagnosis. Diagnostic confirmation is through DNA sequencing of *SLC22A5* or analysis of fibroblast carnitine uptake.[115]

Treatment of primary carnitine deficiency involves supplementation of carnitine at 100 to 200 mg/kg/day to maintain normal or near-normal plasma carnitine levels. Treatment is successful in preventing or reversing symptoms but is dependent upon compliance.

Carnitine Palmitoyltransferase Type I Deficiency

Carnitine palmitoyltransferase type I (CPTI) covalently links long-chain fatty acids to carnitine prior to mitochondrial transport. CPTI deficiency will present in early childhood with hypoketotic hypoglycemia and liver dysfunction, less commonly as an adult-onset skeletal myopathy, and only with rare presentations of neonatal hypoglycemia.[116] Cardiomyopathy is typically not seen. Renal tubular acidosis has been reported.[117] Diagnostic laboratory findings include elevated total and free plasma carnitine levels, while NBS additionally uses a C0/(C16+C18) ratio to improve specificity. Confirmatory testing will demonstrate mutations in the *CPT1A* gene or through enzyme activity in skin fibroblasts. A common *CPTIA* variant, c.1436C>T (p.P479L), has been identified in the Arctic populations of the Inuit, Alaskan Native, Canadian First Nation, and Hutterite and has been associated with higher infant mortality and impaired fasting intolerance in these populations.[95,118,119] Treatment involves fasting avoidance, a low-fat diet, and MCT supplementation in some cases, and results in a normal outcome, although some suffer neurologic impairment from repeated episodes of metabolic decompensation.[117,120]

Carnitine Acylcarnitine Translocase Deficiency

Carnitine acylcarnitine translocase (CACT) transports fatty acids conjugated to carnitine across the mitochondrial membrane; CACT deficiency is one of the more severe FAODs, and the most common presentation is ventricular dysrhythmia and sudden neonatal death. Symptoms include hypoglycemia, vomiting, gastroesophageal reflux, and mild chronic hyperammonemia, as well as severe skeletal myopathy and mild hypertrophic cardiomyopathy. Early diagnosis and treatment can be beneficial, although significant morbidity includes profound developmental delay, seizures, and other complications despite NBS.[103,113,121,122] Milder disease is associated with higher residual enzyme activity.[123]

Individuals will have elevated C16-, C16:1-, C18-, and C18:1-acyclarnitine levels with low free carnitine levels on diagnostic testing and NBS. DNA sequencing of *SLC25A20* will confirm disease. Treatment includes prolonged fasting avoidance, a low long-chain fat, high-carbohydrate formula, MCT or triheptanoin supplementation, and carnitine.

Carnitine Palmitoyltransferase Type II Deficiency

Carnitine palmitoyltransferase type II deficiency (CPTII) results in identical elevations of C16- and C18:1-acylcarnitines in NBS as found with CACT deficiency; DNA testing of the gene *CPT2* is required for diagnosis; treatment depends on severity and is similar to that for CACT deficiency. Children with the severe form of CPTII deficiency may have congenital anomalies including renal cysts, dysmorphic facies, and brain malformations and may present with hypotonia, cardiomyopathy, arrhythmias, and seizures within the newborn period.[103,122] The later-onset form of CPTII deficiency is much more common and presents in the second or third decade of life with exercise intolerance or rhabdomyolysis.[122]

Multiple Acyl-CoA Dehydrogenase Deficiency

Multiple acyl-CoA dehydrogenase deficiency (MADD; also called *glutaric acidemia type 2*) is the result of a defect of electron transfer from multiple acyl-CoA dehydrogenases to the mitochondrial ETC. Each acyl-CoA dehydrogenase enzyme binds electron transfer flavoprotein (ETF); ETF accepts electrons from the $FADH_2$ cofactor in the oxidative dehydrogenation reactions and is made up of three subunits. Mutations in the genes for the three subunits, *ETFA, ETFB*, and *ETFDH*, will interfere with electron transfer from ETF to coenzyme Q10 within the mitochondria. Riboflavin (an $FADH_2$ component) deficiency or deficient riboflavin transport may show a similar presentation—a riboflavin-responsive form of MADD has been described. Because of the multiple dehydrogenase enzymes involved there will be elevations of metabolites from short-, medium-, and long-chain fatty acids and amino acid metabolism.[94,124]

There are three major ways MADD may present. Neonatal MADD presents with metabolic acidosis, hypoketotic hypoglycemia, and often hypertrophic cardiomyopathy. Neonatal presentations with congenital malformations may include enlarged polycystic kidneys, rocker-bottom feet, defects of the inferior abdominal musculature, and hypospadias and chordee. Hypotonia, cerebral cortical dysplasia, and gliosis have been reported, and dysmorphic facies may include telecanthus, malformed ears, macrocephaly, large anterior fontanel, high forehead, and a flat nasal bridge.[96] Later-onset MADD have a lifelong risk of acute intermittent episodes with vomiting, dehydration, hypoketotic hypoglycemia, acidosis and some with hepatomegaly and muscle disease including myopathy and rhabdomyolysis.

Many infants do not survive beyond the first few weeks or months of life because of rapidly progressing cardiomyopathy. In individuals identified through NBS and in whom treatment is initiated early, acute, life-threatening events or sudden death may still occur.[125,126]

Diagnosis of MADD is suspected due to the combination of increased anion gap lactic acidosis, hypoketotic hypoglycemia, and hyperammonemia. Patients may have an odor of isovaleric acid. Increased serum transaminases and prolongations of prothrombin time and partial thromboplastin time suggest liver dysfunction. Diagnostic testing should include plasma acylcarnitines and elevations of C4 and C5 acylcarnitines may be seen (which are the primary analytes on NBS), and elevated medium- and long-chain acylcarnitines may also be present (i.e., C8-, C10:1-, C12-, C14-, C14:1-, C16-, C16:1-, C18-, C18:1-, C16-OH-, C16:1-OH-, C18-OH-, and C18:1-OH-acylcarnitines). Urine organic acid analysis will show elevations of ethylmalonic acid, glutaric acid, 2-hydroxyglutaric acid, and 3-hydroxyisovalerate in addition to lactic acid, medium- and long-chain dicarboxylic acids, the glycine conjugates isovalerylglycine, isobutyrylglycine, and 2-methylbutyrylglycine. Ketone bodies, including acetoacetic acid and 3-hydroxybutyric acids, are minimal or undetectable. Generalized aminoaciduria will reflect impaired renal tubular function; DNA sequencing of *ETFA, ETFB*, and *ETFDH* will confirm the diagnosis.

Treatment of MADD includes a low-protein and low-fat diet, fasting avoidance, and supplementation with carnitine, riboflavin, and glycine. Individualized metabolic formulas often have to be designed to meet nutritional goals. Individuals at least heterozygous for common *ETFDH* mutations confer a milder riboflavin-responsive phenotype with some cases of complete correction of clinical and biochemical parameters after riboflavin treatment.[96,127] Acute decompensation should be treated with IV glucose and carnitine to restore anabolism with close monitoring of cardiac, hepatic, and renal function. Research is demonstrating that supplementation with D,L-3-HB treatment can be effective and safe in MADD patients.[128]

Ketone Metabolism Disorders

Disorders of ketone metabolism result from the inability to use ketone bodies, 3-hydroxybutyric acid and acetoacetic acid, for energy generation. Each cycle of fatty acid β-oxidation generates a single acetyl-CoA. This acetyl-CoA has to be converted to acetoacetic acid by mitochondrial acetoacetyl-CoA thiolase, 3-hydroxy-3-methylglutaryl CoA (HMG-CoA) synthase, and then HMG-CoA lyase. Acetoacetic acid and 3-hydroxybutyric acid are transported out of liver mitochondria and hepatocytes into blood to be used by other tissues, especially the brain and heart.

Patients with mitochondrial acetoacetyl-CoA thiolase deficiency (β-ketothiolase or 3-oxothiolase deficiency) have a metabolic acidosis associated with excess ketosis and most commonly presents in children around 15 months of age, but cases have been reported as early as 3 and 4 days of age.[129,130] The clinical presentation varies from severe, acute metabolic decompensation in infants to asymptomatic adults. The episodes are intermittent, are typically associated with a catabolic stressor or a high dietary protein intake, and have been associated with mild hyperketotic hypoglycemia and liver dysfunction, without hyperammonemia. Cardiomyopathy is rare. Plasma acylcarnitines and NBS will demonstrate elevations of C5-OH- and C5:1-acylcarnitines. Urine organic acid analysis demonstrates significant elevations of lactate and ketone bodies and a specific pattern of elevations of 2-methylacetoacetate, 2-methyl-3-hydroxybutyrate, and tiglylglycine. Diagnosis is confirmed by DNA sequence analysis of the *ACAT1* gene in this autosomal recessive disorder. Acute treatment involves IV glucose and bicarbonate to correct metabolic acidosis, which is often severe, while long-term therapy includes mild protein restriction, avoidance of prolonged fasting, and prompt attention to any intercurrent illness or catabolic stressor, as treatment and avoidance of severe ketoacidosis may lead to normal development.

HMG-CoA lyase deficiency is the most severe disorder of ketone metabolism and has been identified in only 211 patients.[131] It is the last step in synthesis of acetoacetate from HMG-CoA via the HMG-CoA lyase enzyme. Neonates present as early as 3 days of life with an often-catastrophic illness characterized by vomiting, lethargy, hypoketotic hypoglycemia, metabolic acidosis, hyperammonemia, elevated transaminases, hepatomegaly, seizures, and coma. Urine organic acid analysis reveals 3-hydroxy-3-methylglutaric acid, 3-methylglutaconic acid, and 3-hydroxyisovaleric acid in a specific diagnostic pattern with elevations of C5-OH- and C6-DC-acylcarnitines on plasma acylcarnitine analysis and NBS. Levels of acetoacetic acid and 3-hydroxybutyric acid may be unexpectedly low. Lactate values may be elevated during the acute metabolic decompensation. While nearly all patients are symptomatic, over 60% have normal development.[131] An autosomal recessive disorder, HMG-CoA lyase deficiency is confirmed by mutation analysis of the *HMGCL* gene. Acute treatment of an episode of decompensation consists of administration of IV rehydration with provision of glucose and bicarbonate to correct metabolic acidosis. Long-term treatment consists of avoiding prolonged fasting, a low-protein and high-carbohydrate diet, and carnitine supplementation.

Succinyl-CoA 3-ketoacid-CoA transferase deficiency results from the failure of extrahepatic tissues to convert acetoacetate back to acetoacetyl-CoA. This is required for hydrolysis to acetyl-CoA for final metabolism in the TCA cycle. Affected patients have a persistent ketosis with intermittent ketoacidosis that does not resolve, even postprandially. Affected newborns often present in the first week of life with severe ketosis, lactic acidosis, hypoglycemia, and coma, and many do not survive.[132] Elevated acetoacetate and 3-hydroxybutyrate levels are almost always present in blood and urine. Therapy focuses on fasting avoidance, which can cause profound acidosis and ketosis, and IV fluids, glucose, and sodium bicarbonate during crisis. Enzyme analysis in fibroblasts is available, but DNA sequencing of the *OXCT1* gene will likely confirm the diagnosis in this autosomal recessive disorder. Milder forms do exist, and patients may have nonketotic periods. In addition, there have been reports of heterozygotes with severe presentations.[133]

Mitochondrial Disorders

Primary Lactic Acidosis

Congenital lactic acidosis (CLA) is due to a severe disorder of energy metabolism. This can be caused by one of multiple diseases, including those in which lactate and pyruvate metabolism are impaired because of a primary defect in the mitochondrial electron transport chain (ETC) or the tricarboxylic acid (TCA) cycle. These disorders affect pyruvate metabolism, which, in turn, affects lactate. The majority of neonates presenting with CLA have defects of the mitochondrial ETC, of the pyruvate dehydrogenase (PDH) complex, or of the pyruvate carboxylase (PC) enzyme. Inborn errors of the TCA cycle are much rarer but should be considered in the differential diagnosis. An autosomal recessive

disorder of metabolism of the amino acid valine, short-chain enoyl-CoA hydratase deficiency due to mutations in the *ECHS1* gene, appears to cause a secondary inhibition of PDH and can present with refractory CLA with a Leigh disease-like presentation, neurodegeneration, and neonatal death.[134]

Some patients with CLA present with overwhelming lactic acidosis in the neonatal period. In others, lactate may be elevated only in CSF. This "cerebral" lactic acidosis may present more indolently. When blood lactate is elevated, the ratio of blood lactate to pyruvate can help narrow the differential diagnosis. The ratio is low to normal (10 to 20) in PDH deficiency, may be modestly elevated in PC type B deficiency (see later), but may be greatly elevated (>25) in an ETC defect. This ratio reflects the oxidation-reduction state of the mitochondria. When calculating this it is important to ensure the units of the two analytes are the same. These conditions are not identified through NBS, and patients will not be identified pre-symptomatically unless there has been a prior affected family member such as an older affected sibling.

Pyruvate Dehydrogenase Complex Deficiency

The PDH complex converts pyruvate, which is derived from the catabolism of glucose, to acetyl-CoA, which enters the TCA cycle at citrate synthase. Severe PDH deficiency may manifest in the neonatal period with profound lactic acidosis and a low to normal lactate-to-pyruvate ratio (<20). Patients may have moderately elevated plasma ammonia and congenital brain anomalies including an absent or underdeveloped corpus callosum and heterotopias. Patients are hypotonic and may require mechanical ventilation. Prognosis is poor, even with early recognition and intervention. Importantly, the high concentration dextrose-containing IV and enteral empiric therapy that may be life-saving for a child with a possible organic aciduria or urea cycle defect exacerbates the lactic acidosis in the neonate with PDH deficiency or other mitochondrial energy metabolism disorders. This worsening may suggest a primary energy metabolism disorder in the neonate. A possible diagnosis of PDH deficiency is inferred from the patient presentation, the clinical course, and the results of routine and specialized biochemical laboratory testing. Alanine will be elevated on plasma amino acid analysis, and TCA cycle intermediates may be elevated in the urine organic acid analysis. There is no specific diagnostic compound that identifies this disorder. The diagnosis is made through abnormal enzyme analysis in skin fibroblasts or white blood cells and/or by DNA testing. PDH is a large, multisubunit complex. It contains three enzymatic subunits and several regulatory subunits, including a phosphatase and a kinase. The first enzymatic step is a decarboxylation reaction catalyzed by a heterodimeric system consisting of the E1-α subunit and E1-β. The majority of individuals have a mutation in the *PDHA1* gene, which encodes the E1-α subunit of the PDH enzyme complex. This is an X-linked gene and this causes an X-linked dominant condition. Disorders associated with defects in all other subunits are autosomal recessive. Mutations in *PDHA1* account for 80% of cases of PDH deficiency.[135]

The majority of patients are indolent on clinical presentation with developmental delay and an MRI indicative of Leigh disease. This group of patients often responds well biochemically to a high-fat and low-carbohydrate, or ketogenic, diet. Fat, as acetyl-CoA, enters the energy pathway after the block, whereas glucose must traverse the defective PDH enzymatic reaction to provide energy.

Defects of the PDH complex due to defects in the two other enzyme subunits, the activating and deactivating enzymes and a structural protein that binds one subunit (E3 binding protein), are rarer. These usually result in chronic psychomotor retardation syndrome in late infancy and childhood. Deficiency of the E3 subunit of the PDH complex has pleiotropic biochemical effects because the subunit is a component of two other dehydrogenase complexes: the BCKAD complex and the α-ketoglutarate dehydrogenase complex of the TCA cycle. Therefore, these patients may have elevated BCAAs as in MSUD, which is due to BCKAD deficiency, as well as elevated TCA cycle metabolites because of α-ketoglutarate dehydrogenase complex deficiency. Most patients with E3 deficiency present after the newborn period and have progressive neurodegenerative disease. The key laboratory findings are the elevation of lactic acid in blood with elevated pyruvate and a low lactate-to-pyruvate ratio, elevated BCAAs on plasma amino acid analysis, and detection of α-ketoglutarate and BCAA metabolites in urine organic acid analysis. PDH phosphatase deficiency is a rare cause of CLA. Apart from E3 deficiency, defects in the PDH complex are responsive to the ketogenic diet.

In addition to E3 deficiency there is another class of defects that affects PDH and other enzyme complexes, including BCKAD and α-ketoglutarate dehydrogenase complex, because of the requirement for lipoate in these and the complex synthesis of lipoate. Defects in eight genes encoding proteins required for lipoate synthesis have been reported, and these may present as CLA. Symptoms are variable and may be characteristic of the specific genetic defect. In an early-onset presentation, symptoms can include seizures, encephalopathy, and cardiomyopathy.[136]

Pyruvate Carboxylase Deficiency

Pyruvate carboxylase (PC) is involved in gluconeogenesis. The enzyme adds bicarbonate to pyruvate to form oxaloacetate, a compound also involved in replenishing intermediates of the TCA cycle. PC is one of the four biotin requiring carboxylases. There are three main types of PC deficiency. Type A is characterized by lactic acidosis in the newborn period and delayed development, and the disease has a chronic course. Type B is the catastrophic form of the disorder. In type B, the neonate is acutely ill in the first week of life, is encephalopathic, and develops severe metabolic acidosis with lactic acidosis and hyperammonemia. The mortality rate from the type B form of PC deficiency is high. Type C is considered intermittent and benign.[137]

Most patients with the type B form of PC deficiency have been of French or English background. The blood lactate-to-pyruvate ratio is normal in type A as both lactate and pyruvate are comparably elevated, while patients with type B often have an elevated lactate-to-pyruvate ratio. Because oxaloacetate produces aspartate, which then combines with citrulline to create argininosuccinate through the urea cycle, PC deficiency leads to elevations of plasma citrulline and plasma ammonia. Although PC is an important enzyme in gluconeogenesis, hypoglycemia is not common. The liver may be enlarged. Prognosis is poor. Enzyme testing may be performed in white blood cells or fibroblasts. DNA mutational analysis may also be performed for diagnosis.

Electron Transport Chain Defects

Oxidative phosphorylation results in the generation of ATP and is the central process performed by mitochondria. Genetic defects

affecting the tightly coupled and regulated process of ATP generation may have profound effects on one or more organ systems. Derivatives of nutrients such as pyruvate and fatty acids are converted to carbon dioxide in mitochondria. The energy derived from this is harnessed by allowing the reducing equivalents (as NADH or $FADH_2$, which are derived from such metabolism) to combine with oxygen to form water and, in the process the synthesis of ATP is coupled to the flow of electrons down the ETC.

The important components in the mitochondrial respiratory chain are complex I (NADH dehydrogenase), complex II (succinate dehydrogenase), complex III (cytochromes *b*, c_1), and the terminal complex in this chain, complex IV (cytochrome *c* oxidase). In addition, there is a complex V (ATP synthetase), and an adenine nucleotide translocase that permit transport of adenosine diphosphate into, and ATP out of, the mitochondria. Complex II is involved primarily in fatty acid oxidation and oxidation of succinate derived from the TCA cycle, because the reducing equivalents extracted from fatty acids, glutaric acid, and succinate flow from ETF into complex II. Early understanding of the molecular mechanisms that produce ETC disturbances concerned those attributed to mutations of mitochondrial DNA (mtDNA—a small circular DNA molecule located within the mitochondria) genes, although multiple disorders that effect the ETC caused by nuclear (chromosomal) encoded genes have been identified through DNA sequencing. The mtDNA encodes at least one protein subunit of each respiratory chain complex—except for complex II. It also encodes components of the mitochondrial translational machinery required for mitochondrial protein synthesis (rRNA and tRNA). CLA caused by defects in ETC components can be due to either nuclear or mtDNA encoded subunits. This is important for genetic counseling of families of affected children, as mitochondrial DNA is maternally inherited. In addition to defects of the structural components of a respiratory chain complex, ETC deficiency can be due to defects in genes encoding proteins responsible for the assembly of the protein subunits into functional complexes. Nuclear DNA encodes these assembly proteins, and, taken together, defects in more than 1000 nuclear and mitochondrially encoded genes can result in ETC deficiency.[138]

The relationship between phenotype and a specific mtDNA mutation is not straightforward, due, at least in part, to heteroplasmy (the proportion of mutant and nonmutant mtDNA molecules within each cell, each of which contain many mitochondria that also each contain multiple copies of mtDNA). Mitochondria and the mtDNA are inherited solely from the mother. Random segregation of mitochondria—having greater or fewer mutant mtDNA molecules as oocytes are formed—can lead to a "bottleneck effect" in which the fetus has a higher concentration of cells with mutant mtDNA than the mother, who may have little or no mutant mtDNA detectable in blood. Often there is a different proportion of defective mitochondria in different cells and different tissues, and, crucially, there is a tissue-specific "threshold effect"; that is, a certain proportion of mutant mtDNA molecules must be present to have clinical consequences in a given tissue.[138,139]

The disorders caused by nuclear DNA gene mutations are most often autosomal recessive, and they comprise the majority of ETC disease presenting in neonates and infants, with a minority resulting from mtDNA gene mutations with a maternal inheritance pattern in this age group. With the exception of *n*eurogenic muscle weakness, *a*taxia, and *r*etinitis *p*igmentosa (NARP, caused by a mutation at position 8993 of the mtDNA), only a minority of diseases caused by mtDNA mutations manifest in the newborn period.[140] Other examples of syndromes caused by mtDNA mutations are MELAS (*m*itochondrial *e*ncephalopathy, *l*actic *a*cidosis, and *s*troke-like episodes, mtDNA position 3243) and MERRF (*m*yoclonic *e*pilepsy with *r*agged-*r*ed *f*ibers, position 8344) syndromes, Leber hereditary optic neuropathy,[139] and sporadic deletion–duplication syndromes such as Pearson marrow–pancreas syndrome.[141] Important mitochondrial energy metabolism disorders that affect infants include CLA, the most dramatic form, with presentation often in the first few days of life during which there is marked lactic acidosis[142,143]; subacute necrotizing encephalomyopathy or Leigh disease that classically presents at later than 3 months of age but can present earlier; Alpers disease presenting with seizures and liver disease; benign infantile mitochondrial myopathy, cardiomyopathy, or both; lethal infantile mitochondrial disease; lethal infantile cardiomyopathy; and Pearson syndrome. The hallmarks of mitochondrial disease are often multisystem involvement and lactic acidosis, but involvement of only one organ system and the absence of lactic acidosis do not exclude mitochondrial disease.

Important classes of mitochondrial disorders include nuclear encoded mtDNA depletion syndromes resulting from defects in mtDNA replication component proteins or in the availability of nucleotides for DNA replication, which may include fatal hepatopathy.[144,145] Another class is the defects in lipoate synthesis, including those of iron–sulfur protein synthesis.[136] A potentially treatable class is that of coenzyme Q10 biosynthesis diagnosed by DNA testing of nuclear genes or by coenzyme Q10 levels in muscle. This includes a severe infantile multisystem disease, and it is important to identify these defects as they are one of the few classes of mitochondrial disorders in which administration of coenzyme Q10 may be effective.[146]

The diagnostic tests for electron transport chain defects include standard measurements of plasma lactate and pyruvate and analysis of urine organic and plasma amino acids. CSF lactate may be measured by lumbar puncture and by magnetic resonance spectroscopy during MRI. The current availability of mtDNA sequencing panels and whole exome sequencing with full sequencing of the mitochondrial genome has decreased the use of muscle biopsy. However, histologic analysis of muscle tissue by light and electron microscopy and mitochondrial respiratory chain complex assay on either fresh (preferred, but infrequently available) or flash-frozen tissue may be of aid in diagnosis. In many cases however, DNA sequencing has replaced muscle biopsy as a first-line test for suspected mitochondrial disease.[138] In fatal cases, rapid (metabolic) autopsy and proper preservation of tissue specimens are essential if functional assays are to be performed. DNA testing can also be performed on tissues.

Leigh Disease: Subacute Necrotizing Encephalomyelopathy

Leigh disease is a progressive neurodegenerative disorder with severe hypotonia, seizures, extrapyramidal movement disorder, optic atrophy, and defects in automatic ventilation or respiratory control.[147] Generally, disease onset is outside of the neonatal period, but symptoms may be evident in the first months of life. There are more than 75 genetic defects associated with Leigh disease.[148] These include defects in the PDH complex, ETC structural proteins, assembly factors of individual ETC complexes, coenzyme Q10 biosynthesis, biotinidase, and others. MRI

characteristically shows bilateral symmetrical T2-weighted hyperintense lesions in the basal ganglia. One disorder with features of Leigh disease is biotin–thiamine-responsive basal ganglia disease caused by *SLC19A3* deficiency, and empiric biotin and thiamine should be trialed.[147]

Clinical findings in infants with Leigh disease include optic atrophy, ophthalmoplegia, nystagmus, respiratory abnormalities, ataxia, hypotonia, spasticity, seizures, developmental delay, psychomotor retardation, myopathy, and renal tubular dysfunction. Some patients may manifest hypertrophic cardiomyopathy, liver dysfunction, and microcephaly. The neuropathologic lesions include demyelination, gliosis, necrosis, relative neuronal sparing, and capillary proliferation in specific brain lesions.

Pearson Syndrome

One class of mitochondrial disorders that is genetic but not familial is caused by a spontaneous mtDNA deletion or duplication. The disorders include Kearns–Sayre syndrome and chronic progressive external ophthalmoplegia, which manifest in older individuals. The manifestation in early infancy is Pearson syndrome. This disorder manifests with anemia, ringed sideroblasts, and exocrine pancreatic dysfunction. This disease of the bone marrow can lead to death in infancy. However, patients able to recover or who benefit from aggressive therapy may demonstrate other signs of this systemic disorder in late infancy or childhood, such as poor growth, pancreas dysfunction, mitochondrial myopathy, lactic acidosis, and progressive neurologic damage, and develop Kearns–Sayre syndrome.[149]

Barth Syndrome

Barth syndrome is an X-linked disorder associated with cardiomyopathy, skeletal muscle disease, and neutropenia.[150] Skeletal muscle shows abnormal mitochondrial morphology and deficiencies of respiratory chain complexes III and IV. The *TAZ* gene encodes tafazzin, a cardiolipin acyltransferase. Deficiency of this leads to disruption of the inner mitochondrial membrane and disruption of ETC function. The accumulation of NADH and $FADH_2$ inhibit the TCA cycle, causing lactic acidosis, the development of muscle weakness, and cardiomyopathy. Urine organic acid analysis demonstrates increased urinary excretion of 3-methylglutaconic acid. Barth syndrome is also known as *3-methylglutaconic aciduria type II* and is genetically distinct from primary 3-methylglutaconic aciduria type I, caused by mutations in *AUH* involved in leucine metabolism, and other forms of 3-methylglutaconic aciduria.[151] Patients must be supported from birth to early infancy as acute neonatal presentations may have lactic acidosis, hypoglycemia, hyperammonemia, and liver dysfunction. Fetal loss and stillbirth have also been reported. Treatment includes standard support for heart failure and neutropenia as well as nutrition support and physical therapy.[150]

Early Lethal Lactic Acidosis

In some patients with primary disturbances of mitochondrial ETC, massive lactic acidosis develops within 24 to 72 hours of birth. Commonly the condition is untreatable, because it is relentless and unresponsive to buffer therapy.[152] Dialysis may be helpful but is not a cure and is not feasible in all patients. Often, affected infants have no obvious organ damage early in the course or evidence of malformations. In addition, acidemia per se can easily cause the coma or impaired cardiac contractility that may be encountered. Some infants have survived with aggressive therapy, and specific treatments can include thiamine, biotin, riboflavin, coenzyme Q10, or carnitine.[152] Overall prognosis is likely poor, and the care of neonates with severe lactic acidosis is difficult as the prognosis is unclear. Decisions regarding management must be individualized, because the mitochondrial dysfunction and resultant pathophysiology can vary among infants, and expedited nuclear and mitochondrial DNA testing, to facilitate diagnosis and therefore guide prognosis, should be considered.

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30

Lysosomal Storage Disorders Presenting in the Neonate

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KEY POINTS

- Lysosomal storage diseases are a genetically and phenotypically heterogeneous group of metabolic disorders caused by multisystemic accumulation of complex substrates.
- Clinical manifestations of lysosomal storage diseases in the neonatal period are myriad, including nonimmune hydrops fetalis, respiratory distress, sepsis, macular cherry-red spot, dysmorphic facial features, dysostosis multiplex, and hepatosplenomegaly.
- Newborn screening for certain lysosomal storage diseases such as mucopolysaccharidosis type I and Pompe disease is included on the Recommended Uniform Screening Panel and has been implemented in most states.
- Growing recognition of neonatal symptoms and improved analytic methods has led to early diagnosis and treatment of lysosomal storage diseases in the newborn period.
- Treatment options for lysosomal storage diseases are advancing rapidly, including enzyme replacement therapy, substrate reduction therapy, and hematopoietic stem cell transplantation. Gene therapy trials are ongoing.

Introduction

Lysosomes are single membrane–bound intracellular organelles that contain enzymes called hydrolases. These lysosomal enzymes are responsible for splitting large molecules into simple, low-molecular-weight compounds, which can be recycled. The endogenous materials digested by lysosomes are derived from endocytosis and phagocytosis, and separated from other intracellular materials and delivered to lysosomes through the process of autophagy.

Lysosomal storage diseases (LSDs) are a genetically and phenotypically diverse group of metabolic disorders caused by enzyme deficiencies that result in the pathologic accumulation of complex substrates within lysosomes throughout various tissues in the body.[1] LSDs are classified according to the stored compound. The common element of all compounds digested by lysosomal enzymes is a carbohydrate portion attached to a protein or lipid. These glycoconjugates include glycolipids, glycoproteins, and glycosaminoglycans (GAGs).

Glycolipids are large molecules with carbohydrates attached to a lipid moiety. Sphingolipids, globosides, gangliosides, cerebrosides, and lipid sulfates all are glycolipids. The different classes of glycolipids are distinguished from one another primarily by different polar groups at C-1. Sphingolipids are complex membrane lipids composed of one molecule of each of the amino alcohol sphingosine, a long-chain fatty acid, and various polar head groups attached by a β-glycosidic linkage. Sphingolipids occur in the blood and nearly all tissues of the body, the highest concentration being found in white matter of the central nervous system (CNS). In addition, various sphingolipids are components of the plasma membrane of practically all cells. The core structure of natural sphingolipids is ceramide, a long-chain fatty acid amide derivative of sphingosine. Free ceramide, an intermediate in the biosynthesis and catabolism of glycosphingolipids and sphingomyelin, composes 16% to 20% of normal lipid content of stratum corneum of the skin. Sphingomyelin, a ceramide phosphocholine, is one of the principal structural lipids of membranes of nervous tissue.

Cerebrosides are a group of ceramide monohexosides with a single sugar, either glucose or galactose, and an additional sulfate group on galactose. The two most common cerebrosides are galactocerebroside and glucocerebroside. The largest concentration of galactocerebroside is found in the brain. Glucocerebroside is an intermediate in the synthesis and degradation of more complex glycosphingolipids. Gangliosides, the most complex class of glycolipids, contain several sugar units and one or more sialic acid residues. Gangliosides are normal components of cell membranes and are found in high concentrations in ganglion cells of the CNS, particularly in nerve endings and dendrites. GM1 is the major ganglioside in the brain of vertebrates.

GAGs, also called mucopolysaccharides, are complex heterosaccharides consisting of long sugar chains rich in sulfate groups. The polymeric chains are bound to specific proteins (core proteins). Glycoproteins contain oligosaccharide chains (long sugar molecules) attached covalently to a peptide core. Glycosylation occurs in the endoplasmic reticulum and Golgi apparatus. Most glycoproteins are secreted from cells and include transport proteins, glycoprotein hormones, complement factors, enzymes, and enzyme inhibitors. There is extensive diversity in the composition and structure of oligosaccharides.

The degradation of glycolipids, GAGs, and glycoproteins occurs within lysosomes of phagocytic cells, related to histiocytes and macrophages, in any tissue or organ. A series of hydrolytic enzymes cleaves specific bonds, resulting in stepwise removal of constituents such as sugars and sulfate as well as degrading

complex glycoconjugates to their basic building blocks. LSDs most commonly result when an inherited defect causes significantly decreased activity in one of these hydrolases.

Our understanding of the pathogenesis of LSDs continues to evolve. In addition to catabolic enzyme deficiencies, abnormal autophagy, aberrant vesicular trafficking, cellular signaling, and homeostasis have also been implicated in the multifaceted pathomechanism of LSDs.[2–4] Regardless of etiology, incompletely metabolized molecules accumulate, especially within the tissue responsible for catabolism of the glycoconjugate. Excess storage material may be excreted in urine.

While individually rare, there are over 70 different LSDs, with a collective incidence of approximately 1 in 5000 live births. Approximately 20 of these LSDs may present in the newborn period, of which 14 are reviewed in this chapter.[5]

The neonatal clinical presentation of LSDs includes nonimmune hydrops fetalis, respiratory distress, seizures, cherry-red spot in the macula, dysmorphic facial features, sepsis, dysostosis multiplex, and/or hepatosplenomegaly. However, symptoms may be nonspecific and physical findings such as skeletal abnormalities may not be apparent in the neonatal period. Diagnosis is typically confirmed with enzyme assay in conjunction with molecular testing.

Clinical Presentations

Table 30.1 summarizes the clinical characteristics of the neonatal presentations of LSDs.

Acid Sphingomyelinase Deficiency (Niemann–Pick Disease Types A and B)

Etiology

Acid sphingomyelinase deficiency (ASMD; also known as Niemann-Pick disease types A and B [NPD-A and NPD-B]) is caused by deficiency of the enzyme sphingomyelinase. Historically, ASMD was grouped with Niemann-Pick disease type C (NPC) due to similar pathology findings of accumulated foamy sea-blue histiocytes,[6] but they are now understood to be genetically distinct disorders. Sphingomyelinase normally catalyzes the breakdown of sphingomyelin to form ceramide and phosphocholine. Sphingomyelinase deficiency results in the pathologic accumulation of sphingomyelin within lysosomes particularly in the nervous system, spleen, liver, and lungs. Cholesterol is also stored, suggesting that its metabolism is tied to that of sphingomyelin. Sphingomyelin normally composes 5% to 20% of phospholipids in the liver, spleen, and brain, but in ASMD it can compose up to 70% of phospholipids. Patients with severe ASMD usually have less than 5% of normal enzyme activity.

Clinical Features

ASMD was historically classified into NPD-A and NPD-B based on severity, clinical manifestations, and age of onset. NPD-A presents as severe, infantile-onset neurovisceral disease, whereas NPD-B presents later in childhood. Patients with NPD-A typically present with massive hepatosplenomegaly by 3 months of age.[7,8] Other clinical features include constipation, feeding difficulties, and vomiting, with consequent failure to thrive, and respiratory failure. Patients eventually appear strikingly emaciated with a protuberant abdomen and thin extremities. Neurologic disease is evident by 6 months of age, with hypotonia, decrease or absence of deep tendon reflexes, and weakness. Loss of motor skills, spasticity, rigidity, irritability, and loss of vision and hearing occur later. Seizures are rare. A macular cherry-red spot is present in about half of cases, and the electroretinographic findings are abnormal. Respiratory infections are common. The skin may have an ochre or brownish-yellow color, and xanthomas have been observed. Radiographic findings consist of widening of medullary cavities, cortical thinning of long bones, and osteoporosis. In the brain and spinal cord, neuronal accumulation of sphingomyelin is widespread, leading to cytoplasmic swelling together with atrophy of cerebellum. Bone marrow and tissue biopsy samples may show foam cells or sea-blue histiocytes, which represent lipid-laden cells of the monocyte–macrophage system. Similarly, vacuolated lymphocytes or monocytes may be present in peripheral blood. Tissue cholesterol levels may be threefold to tenfold that of normal, and patients may have a microcytic anemia and thrombocytopenia. Death occurs by 2 to 3 years of age after a rapid neurodegenerative course.

Niemann-Pick Disease Type C

Etiology

NPC is caused by a defect in the intracellular transport of exogenous low-density lipoprotein (LDL)-derived cholesterol, which leads to impaired esterification of cholesterol and trapping of unesterified cholesterol in lysosomes.[9,10] The incidence is roughly 1 in 100,000 births.[11] NPC is caused by a defect of either the NPC1 or NPC2 protein. The latter binds cholesterol liberated by acid lipase and shuttles it to NPC1, which facilitates egress of cholesterol from late endosomes/lysosomes to the endoplasmic reticulum. There is secondary storage of sphingomyelin. Sphingomyelinase activity appears normal or elevated in most tissues but is partially deficient in fibroblasts from most patients with this disorder. Storage of sphingomyelin in tissues is much less than in ASMD and is accompanied by additional storage of unesterified cholesterol, phospholipids, and glycolipids in the liver and spleen. Only glycolipids levels are increased in the brain.

Clinical Features

The age of onset, clinical features, and natural history of NPC are highly variable. Onset can occur from birth to adulthood. Findings on prenatal ultrasound include fetal ascites or hydrops, hepatosplenomegaly, intrauterine growth restriction, and oligohydramnios or polyhydramnios.[12] In the neonatal period, 50% of infants have conjugated hyperbilirubinemia, which usually resolves spontaneously. Liver failure, sometimes misdiagnosed as fetal hepatitis, and respiratory failure can occur, with neurologic symptoms appearing later in childhood in those who survive. In the severe infantile form, hepatosplenomegaly is common and often present at birth, accompanied by hypotonia and delayed motor development. Further neurologic regression is usually evident by the age of 1 to 1.5 years, in association with vertical supranuclear ophthalmoplegia, progressive ataxia, dystonia, spasticity, drooling, dysphagia, and dysarthria. Seizures are rare. Foam cells and sea-blue histiocytes may be found in many tissues. Neuronal accumulation of glycolipids with cytoplasmic ballooning, inclusions, meganeurites, and axonal spheroids is also seen. The average survival is 5 years or less. Patients with mutations in the *NPC2* gene have pronounced pulmonary involvement as accumulation of cholesterol in alveolar macrophages leads to abnormal surfactant composition, resulting in early death due to respiratory failure.[13]

TABLE 30.1 Lysosomal Storage Disorders in the Newborn Period: Genetic and Clinical Characteristics of Neonatal Presentation

Disorder	Onset	Facies	Neurologic Findings	Distinctive Features	Eye Findings	Cardiovascular Findings	Dysostosis Multiplex	Hepatomegaly/ Splenomegaly	Defect	Gene Location/ Molecular Findings	Ethnic Predilection
Acid sphingomyelinase deficiency (Niemann–Pick disease type A)	Early infancy	Frontal bossing	Difficulty feeding, apathy, deafness, blindness, hypotonia	Brownish-yellow skin, xanthomas	Cherry-red spot (50%)	—	—	++/+	Sphingomyelinase deficiency	*SMPD1* gene at 11p15.4; three of 18 mutations account for approximately 92% of mutant alleles in the Ashkenazi population	1:40,000 in Ashkenazi Jews with carrier frequency of 1:60
Niemann–Pick C disease	Birth–3 months	Normal	Developmental delay, vertical gaze paralysis, hypotonia, later spasticity	—	—	—	—	+/++	Abnormal cholesterol esterification	*NPC1* gene at 18q11 accounts for >95% of cases; *HE1* gene mutations may account for remaining cases	Increased in French Canadians of Nova Scotia and Spanish Americans in the southwest United States
Gaucher disease type 2	In utero–6 months	Normal	Poor suck and swallow, weak cry, squint, trismus, strabismus, opsoclonus, hypertonic, later flaccidity	Congenital ichthyosis, collodion skin	—	—	—	+/++	Glucocerebrosidase deficiency	1q21; large number of mutations known; five mutations account for approximately 97% of mutant alleles in the Ashkenazi population but approximately 75% in the non-Jewish population	Panethnic
Krabbe disease	3–6 months	Normal	Irritability, tonic spasms with light or noise stimulation, seizures, hypertonia, later flaccidity	Increased CSF protein level	Optic atrophy	—	—	–/–	Galactocerebrosidase deficiency	14q 24.3–q32.1; >60 mutations with some common mutations in specific populations	Increased in Scandinavian countries and in a large Druze kindred in Israel

GM1 gangliosidosis	Birth	Coarse	Poor suck, weak cry, lethargy, exaggerated startle, blindness, hypotonia, later spasticity	Gingival hypertrophy, edema, rashes	Cherry-red spot (50%)	—	+	+/+	β-Galactosidase deficiency	3pter–3p21; heterogeneous mutations; common mutations in specific populations	Panethnic
Mucopolysaccharidosis type I	Childhood	Variable coarseness	Mild to severe mental retardation	Gibbus deformity	Variable corneal clouding	Variable	++	Variable	α-L-iduronidase deficiency	*IDUA* gene at 4p16.3; heterogeneous mutations	Panethnic
Mucopolysaccharidosis type VII	In utero–childhood	Variable coarseness	Mild to severe mental retardation	Nonimmune fetal hydrops	Variable corneal clouding	Variable	++	Variable	β-Glucuronidase deficiency	*GUSB* gene at 7q21.2–q22; heterogeneous mutations	Panethnic
Wolman disease	First weeks of life	Normal	Mental deterioration	Vomiting, diarrhea, steatorrhea, abdominal distention, failure to thrive, anemia, adrenal calcifications	—	—	—	+/+	Lysosomal acid lipase deficiency	10q23.2–q23.3; variety of mutations identified	Increased in Iranian Jews and in non-Jewish and Arab populations of Galilee
Farber disease	Birth-infancy	Normal		Joint swelling with nodules, hoarseness	Normal macula, corneal opacities	—	—	++/++	Acid ceramidase deficiency	*ASAH1* gene at 8p21.3–p22	Panethnic
Congenital sialidosis	In utero–birth	Coarse, edema	Mental retardation, hypotonia	Neonatal ascites, inguinal hernias, renal disease	Corneal clouding	—	+	+/+	Neuraminidase (sialidase) deficiency	*NEU1* gene at 6p21	Panethnic

Continued

TABLE 30.1 Lysosomal Storage Disorders in the Newborn Period: Genetic and Clinical Characteristics of Neonatal Presentation—cont'd

Disorder	Onset	Facies	Neurologic Findings	Distinctive Features	Eye Findings	Cardiovascular Findings	Dysostosis Multiplex	Hepatomegaly/ Splenomegaly	Defect	Gene Location/ Molecular Findings	Ethnic Predilection
Galactosialidosis	In utero–birth	Coarse	Mental retardation, occasional deafness, hypotonia	Ascites, edema, inguinal hernias, renal disease, telangiectasias	Cherry-red spot, corneal clouding	Cardiomegaly progressing to failure	+	+/+	Absence of a protective protein that safeguards neuraminidase and β-galactosidase from premature degradation	*CTSA* gene at 20q13.12	Panethnic
Infantile sialic acid storage disease	In utero–birth	Coarse, dysmorphic	Mental retardation, hypotonia	Ascites, anemia, diarrhea, failure to thrive	—	Congestive heart failure	+	+/+	Defective transport of sialic acid out of the lysosome	*SLC17A5* gene at 6q13	Panethnic
I-cell disease	In utero–birth	Coarse	Mental retardation, deafness	Gingival hyperplasia, restricted joint mobility, hernias	Corneal clouding	Valvular disease, congestive heart failure, cor pulmonale	++	+++/+++	Lysosomal enzymes lack mannose 6-phosphate recognition marker and fail to enter the lysosome (phosphotransferase deficiency, 3-subunit complex [α2 β2 γ2])	Enzyme encoded by two genes; α and β subunits encoded by *GNPTAB* gene at 12p23.2; γ subunit encoded by *GNPTAG* gene at 16p13.3	Panethnic
Mucolipidosis type IV	Birth–3 months	Normal	Mental retardation, hypotonia	—	Severe corneal clouding, retinal degeneration, blindness	—	—	—/—	Unknown; some patients with partial deficiency of ganglioside sialidase	*MCOLN1* gene at 19p13.2–13.3 encoding mucolipin 1; two founder mutations accounting for 95% of mutant alleles in the Ashkenazi population	Increased in Ashkenazi Jews

—, Not seen; +, typically present, usually not severe; ++, usually present and moderately severe; +++, always present, usually severe; *CSF*, cerebrospinal fluid; *HSM*, hepatosplenomegaly.

Gaucher Disease Type 2 (Acute Neuronopathic)

Etiology

Gaucher disease is caused by deficiency of lysosomal glucocerebrosidase and results in storage of glucocerebroside in mononuclear phagocytes. Glucocerebrosidase splits glucose from cerebroside, yielding ceramide and glucose. Three types of Gaucher disease have been defined. Type 1, the non-neuronopathic form, is the most common and is distinguished from types 2 and 3 by the lack of CNS involvement. Type 1 disease most commonly manifests itself in early childhood but may do so in adulthood. Type 2 disease, the acute neuronopathic form, is the rarest form and is characterized by infantile onset of severe CNS involvement. Type 3 disease, the subacute neuronopathic form, presents in childhood with slower neurologic progression. Although there is significant variability in clinical presentation among individuals with the same mutations in *GBA*, there are some correlations between certain mutations and clinical symptoms involving the CNS.[14] A few patients with Gaucher disease type 2 have a deficiency of saposin C, a cohydrolase required by glucocerebrosidase.

Clinical Features

The age of onset of Gaucher disease type 2 is approximately 3 months. Clinical features include early hepatosplenomegaly (splenomegaly predominates) with later neurologic deterioration including bulbar signs, hyperextension of the neck, spasticity, and horizontal gaze palsy. Hydrops fetalis, congenital ichthyosis, and collodion skin are well-described presentations.[5] In a review of 18 cases of Gaucher disease manifesting in the newborn period, Sidransky et al. found that eight patients had associated dermatologic findings, and six patients had hydrops.[15] The cause of the association of such findings in Gaucher disease is unclear, although the enzyme deficiency appears to be directly responsible. Ceramides have been shown to be major components of intracellular bilayers in epidermal stratum corneum, and they have an important role in skin homeostasis.[16] Therefore Gaucher disease should be considered in the differential diagnosis for infants with hydrops fetalis and congenital ichthyosis. For the subset of patients who present in the prenatal period or at birth, death commonly occurs within 2 to 3 months. In other infants, splenomegaly, cachexia, and chronic pulmonary disease are progressive, and death follows within 2 years.

Krabbe Disease (Globoid Cell Leukodystrophy)

Etiology

The synonym for Krabbe disease, globoid cell leukodystrophy, is derived from the finding of large numbers of multinuclear macrophages in cerebral white matter that contain undigested galactocerebroside. Krabbe disease is caused by a deficiency of lysosomal galactocerebroside β-galactosidase, which degrades galactocerebroside to ceramide and galactose, resulting in storage of galactocerebroside. Galactocerebroside is present almost exclusively in myelin sheaths. Accumulation of the toxic metabolite psychosine, another substrate for the enzyme, leads to early destruction of oligodendroglia. Impaired catabolism of galactosylceramide is also important in the pathogenesis of the disease. Saposin A is an activator protein that aids the galactocerebroside β-galactosidase enzyme. Saposin A deficiency presents with a Krabbe-like phenotype.

Clinical Features

The age of onset ranges from the first weeks of life to adulthood. The typical age of onset of infantile Krabbe disease is between 3 and 6 months, but there are cases in which neurologic symptoms are evident within weeks after birth. Signs and symptoms are confined to the nervous system; no visceral involvement is present. The clinical course has been divided into three stages. In stage I, patients who appeared relatively normal after birth begin to exhibit hyperirritability, vomiting, episodic fevers, hyperesthesia, tonic spasms with light or noise stimulation, stiffness, and seizures. Peripheral neuropathy is present, but reflexes are increased. Stage II is marked by CNS deterioration and hypertonia that progresses to hypotonia and flaccidity. Deep tendon reflexes are eventually lost. Patients with stage III disease are decerebrate, deaf, and blind with hyperpyrexia, hypersalivation, and frequent seizures. Routine laboratory findings are unremarkable except for an elevation of the level of cerebrospinal fluid protein. Cerebral atrophy and demyelination become evident in the CNS, and segmental demyelination, axonal degeneration, fibrosis, and macrophage infiltration are common in the peripheral nervous system. The segmental demyelination of peripheral nerves is demonstrated by the finding of decreased motor nerve conduction. The white matter is severely depleted of all lipids, especially glycolipids, and nerve and brain biopsies show globoid cells. Death from hyperpyrexia, respiratory complications, or aspiration occurs at a median age of 13 months.

GM1 Gangliosidosis

Etiology

Infantile GM1 gangliosidosis is caused by a deficiency of lysosomal β-galactosidase. The enzyme cleaves the terminal galactose in a β linkage from oligosaccharides, keratan sulfate, and GM1 ganglioside. Deficiency of the enzyme results in storage of GM1 ganglioside and oligosaccharides. Clinical severity correlates with the extent of residual enzyme activity and substrate storage. The same enzyme is deficient in mucopolysaccharidosis (MPS) type IVB, but in MPS-type IVB the breakdown of keratan sulfate is primarily affected.

Clinical Features

The age of onset ranges from prenatal to adult. Infantile or type 1 GM1 gangliosidosis may be evident at birth as coarse and thick skin, hirsutism on the forehead and neck, and coarse facial features consisting of a puffy face, frontal bossing, depressed nasal bridge, maxillary hyperplasia, large and low-set ears, macroglossia, and gingival hypertrophy. These dysmorphic features, however, are not always obvious in the neonate. A retinal cherry-red spot is seen in 50% of patients, and corneal clouding is often observed. Shortly after birth, or by 3 to 6 months of age, failure to thrive and hepatosplenomegaly become evident, as does neurologic involvement with developmental delay, hyperreflexia, hypotonia, and seizures. Cardiomyopathy can develop in the first few months of life. Cranial imaging shows diffuse atrophy of the brain, enlargement of the ventricular system, and evidence of myelin loss in white matter. The neurologic deterioration is progressive, resulting in generalized spasticity, vision impairment, hypersensitive startle response, and psychomotor regression. By 6 months of age, skeletal features are present, including kyphoscoliosis, stiff joints with generalized contractures, and striking bone changes including vertebral beaking in the thoracolumbar region, broadening of

shafts of the long bones with distal and proximal tapering, and widening of the metacarpal shafts with proximal pinching of four lateral metacarpals. Tissue biopsy samples demonstrate neurons filled with membranous cytoplasmic bodies and various types of inclusions as well as foam cells in the bone marrow. Death generally occurs before 2 years of age.

Mucopolysaccharidosis Type I

Etiology

Mucopolysaccharidosis (MPS) type I, also known as Hurler syndrome, is caused by deficiency of the enzyme alpha-L-iduronidase, which results in accumulation of dermatan and heparan sulfate in various organs including the brain, eye, heart, liver, spleen, and bone. The alpha-L-iduronidase enzyme is encoded by the *IDUA* gene. Pathogenic variants that critically disrupt the protein result in severe clinical disease, whereas milder variants give rise to an attenuated phenotype.[17] The incidence is 1 in 100,000 births.[18]

Clinical Features

Infants with MPS type I appear normal at birth. However, this condition was added to the Recommended Uniform Screening Panel for newborn screening in 2016, so the diagnosis is now made shortly after birth when the infant is presymptomatic. Therefore, the neonatologist must be prepared to counsel families appropriately and work with a metabolic specialist to manage the newborn's care. As with other LSDs, a spectrum of clinical severity exists in MPS type I. In infants with severe disease, developmental delay is evident in the first 6 to 12 months of life. Respiratory tract infections are common. A gibbus deformity (kyphosis in the mid-back region) may be an early sign of severe disease. With time, signs of storage emerge including coarse facial features, hepatosplenomegaly, upper airway obstruction, short stature, and specific skeletal changes termed dysostosis multiplex. Ultimately, neurologic regression occurs, leading to early death, although treatment can extend lifespan. In the mildest form of MPS type I, cognition and lifespan can be normal, although somatic manifestations can lead to significant morbidity.

Mucopolysaccharidosis Type VII (Sly Disease)

Etiology

Sly disease is a member of a group of LSDs called MPSs that are caused by deficiencies of enzymes catalyzing the stepwise degradation of complex sugar molecules called GAGs. There is a wide spectrum of clinical severity and variable skeletal and neurologic involvement among the MPSs, even within a single enzyme deficiency. Most of these disorders manifest in childhood, but type VII is included in this chapter because of its well-recognized neonatal and infantile presentations. Sly disease is caused by β-glucuronidase deficiency and results in lysosomal accumulation of GAGs, including dermatan sulfate, heparan sulfate, and chondroitin sulfate, causing cell, tissue, and organ dysfunction.

Clinical Features

Sly disease has a wide spectrum of severity. One unique and distinguishing symptom in up to 41% of patients with the neonatal form of disease is nonimmune fetal hydrops.[19] Other clinical features in infantile-onset disease may include coarse facies, hepatosplenomegaly, moderate dysostosis multiplex, hernias, cardiac valvular disease and cardiomyopathy, pulmonary insufficiency, and cognitive impairment. Corneal clouding is variably present. Frequent episodes of pneumonia during the first year of life are common. Short stature becomes evident. Causes of death include cardiopulmonary and renal failure. There are also milder forms of the disease with later onset.

Wolman Disease

Etiology

Wolman disease is caused by deficiency of lysosomal acid lipase, which is an enzyme involved in cellular cholesterol homeostasis and responsible for hydrolysis of cholesterol esters and triglycerides. Lysosomal acid lipase deficiency causes defective release of free cholesterol from lysosomes, leading to upregulation of endogenous LDL and cholesterol synthesis and further deposition of lipids in lysosomes. As a result, nonhydrolyzed cholesterol esters and triglycerides accumulate in most tissues of the body, including the liver, spleen, lymph nodes, heart, blood vessels, and brain. An extreme level of lipid accumulation occurs in cells of the small intestine, particularly in the mucosa. In addition, neurons of the myenteric plexus demonstrate a high level of lipid accumulation, with evidence of neuronal cell death, which may account for the prominence of gastrointestinal (GI) symptoms.[20]

Clinical Features

Clinical presentation of Wolman disease is within weeks of birth, with evidence of malnutrition and malabsorption, including symptoms of vomiting, diarrhea, steatorrhea, faltering growth, abdominal distention, and hepatosplenomegaly. Adrenal calcifications may be seen on radiographs, and adrenal insufficiency occurs. The presence of adrenal calcifications in association with hepatosplenomegaly and GI symptoms is strongly suggestive of Wolman disease. Later, mental deterioration becomes apparent. Laboratory findings include anemia secondary to foam cell infiltration of the bone marrow and evidence of adrenal insufficiency. The serum cholesterol level is normal. Death usually occurs before 1 year of age.

Farber Lipogranulomatosis

Etiology

Farber lipogranulomatosis results from a deficiency of lysosomal acid ceramidase. Ceramidase catalyzes the degradation of ceramide into its long-chain base, sphingosine, and a fatty acid. Clinical disease is a consequence of storage of ceramide and other glycolipids, particularly gangliosides, in various organs and body fluids. In severely affected cases, ceramide content of subcutaneous nodules, liver, kidney, and brain can be quite high. Mildly affected cases may have normal levels. In all types of Farber lipogranulomatosis, tissue biopsy samples show granulomatous infiltration, foam cells, and lysosomes with comma-shaped, curvilinear tubular structures called *Farber bodies*.

Clinical Features

Farber lipogranulomatosis is classified into five types based on age of onset, severity, and systems affected, but the disease is now understood to lie along a phenotypic continuum.[21,22] Types 1 and 4 present in the neonatal period. Type 1, classic disease, presents in the first weeks of life with the clinical triad of painful, progressive joint swelling and deformity, palpable subcutaneous nodules at pressure points and over joints, and hoarse cry developing into aphonia from granulomas of the larynx and epiglottis.

Patients also exhibit feeding and respiratory difficulties, poor weight gain, and intermittent fever caused by granuloma formation and swelling.[21,23] Later, joint contractures and pulmonary disease appear. Liver and cardiac involvement can occur, and patients can have a subtle macular cherry-red spot. Severe and progressive psychomotor impairment can occur, as can seizures, decreased deep tendon reflexes, hypotonia, and muscle atrophy. Affected patients die in early infancy prior to 2 years of age, usually of infiltrative pulmonary disease. Cerebrospinal fluid protein level may be elevated in patients with type 1 disease. Type 4, neonatal visceral disease, manifests at birth as hepatosplenomegaly caused by massive histiocyte infiltration of the liver and spleen, and also in the lungs, thymus, and lymphocytes.[23] Subcutaneous nodules and laryngeal involvement may be subtle. Death occurs by 6 months of age.

Sialidosis

Etiology

Sialidosis is caused by a deficiency of neuraminidase, which is responsible for the cleavage of terminal sialyl linkages of several oligosaccharides and glycopeptides. The defect results in multisystem lysosomal accumulation of sugars rich in sialic acid. In sialidosis, vacuolated cells can be seen in almost all tissues, and in bone marrow foam cells are present. The activities of the sialidase 1 and β-galactosidase enzymes are reduced, respectively, to less than 1% and ~15% of normal values in fibroblasts. The activity of cathepsin A, which is the lysosomal protective protein (PPCA), is markedly reduced in leukocytes and fibroblasts. Urine-bound sialic acid may be elevated, while the free sialic acid is normal.

Clinical Features

Sialidosis is divided into two types: type I and type II. Type I sialidosis is characterized by retinal cherry-red spots and generalized myoclonus with onset generally in the second decade of life. Type II is distinguished from type I by the early onset of a progressive, severe phenotype with somatic features. Type II is often subdivided into juvenile, infantile, and congenital forms. Congenital sialidosis begins in utero and manifests at birth as coarse features, facial edema, hepatosplenomegaly, ascites, hernias, and hypotonia and occasionally frank hydrops fetalis. Radiographs demonstrate dysostosis multiplex and epiphyseal stippling. Individuals with sialidosis type II may have a brain MRI with cerebellar atrophy and spinal cord lesions. They may have anemia. A bull's eye maculopathy has been noted. Delayed mental development is often apparent. The patient may have recurrent infections. Severely dilated coronary arteries, excessive retinal vascular tortuosity, and an erythematous macular rash may also be features of this disease.[24] Most patients with congenital type II sialidosis are stillborn or die before 1 year of age.

Galactosialidosis

Etiology

Galactosialidosis results from a deficiency of two lysosomal enzymes: neuraminidase and β-galactosidase. The primary defect in galactosialidosis is a defect in the protective protein cathepsin A, an intralysosomal protein that protects the two enzymes from premature proteolytic processing. The protective protein has catalytic and protective functions, and the two functions appear to be distinct. Deficiency of the enzymes results in the accumulation of sialyloligosaccharides in tissue lysosomes and in excreted body fluids.

Clinical Features

Galactosialidosis has been divided into three phenotypic subtypes based on age of onset and severity of clinical manifestations. Most cases occur in adolescence and adulthood, but early infantile and late infantile presentations occur. Galactosialidosis is one of the most important causes of nonimmune hydrops fetalis. Patients develop early infantile galactosialidosis between birth and 3 months of age, with ascites, edema, coarse facial features, inguinal hernias, proteinuria, hypotonia, and telangiectasias. Patients subsequently demonstrate organomegaly, including cardiomegaly progressing to cardiac failure, psychomotor delay, and skeletal changes, particularly in the spine. Ocular abnormalities can occur, including corneal clouding and retinal cherry-red spots. Death occurs at an average age of 8 months, usually from cardiac and renal failure. Galactosialidosis can be a cause of recurrent fetal loss or recurrent hydrops fetalis.

Late infantile galactosialidosis manifests itself in the first months of life as coarse facial features, hepatosplenomegaly, and skeletal changes consistent with dysostosis multiplex. Cherry-red spots and corneal clouding may also be present. Neurologic involvement may be absent or mild. Valvular heart disease is a common feature, as is growth retardation, partially because of spinal involvement and often in association with muscular atrophy. Early death is not a feature of the late infantile form. Vacuolated cells in blood smears and foam cells in bone marrow are present in all forms of galactosialidosis.

Infantile Sialic Acid Storage Disease

Etiology

Infantile sialic acid storage disease is caused by a defective lysosomal sialic acid transporter that is responsible for efflux of sialic acid and other acidic monosaccharides from the lysosomal compartment. The defective transporter results in greater storage of free sialic acid and glucuronic acid within lysosomes and increased sialic acid excretion.

Clinical Features

Infantile sialic acid storage disease often manifests at birth as mildly coarse features, hepatosplenomegaly, ascites, hypopigmentation, and generalized hypotonia. Mild dysostosis multiplex may be seen on radiographs. Failure to thrive and severe mental and motor retardation soon appear. Cardiomegaly may be present. Corneas are clear, but albinoid fundi have been reported.[25] Vacuolated cells are seen in a tissue biopsy sample, and electron microscopy demonstrates swollen lysosomes filled with finely granular material. CNS changes include myelin loss, axonal spheroids, gliosis, and neuronal storage. Death occurs in early childhood. Infantile sialic acid storage disease can also manifest itself as fetal ascites, nonimmune fetal hydrops, or infantile nephrotic syndrome.[25]

I-Cell Disease

Etiology

In normal cells, targeting of enzymes to lysosomes is mediated by receptors that bind a mannose 6-phosphate recognition marker on the enzyme. The recognition marker is synthesized in a two-step reaction in the Golgi complex. It is the enzyme that catalyzes the first step of this process, uridine diphosphate–N-acetylglucosamine:lysosomal enzyme N-acetylglucosaminyl-1-phosphotransferase, that is defective in I-cell disease. As a

result, the enzymes lack the mannose 6-phosphate recognition signal, and the newly synthesized lysosomal enzymes are secreted into the extracellular matrix instead of being targeted to the lysosome. Consequently, multiple lysosomal enzymes are found in plasma at 10 to 20 times their normal concentrations. Affected cells, especially fibroblasts, show dense inclusions of storage material that probably consists of oligosaccharides, GAGs, and lipids; these are the inclusion bodies from which the disease name is derived. This disorder is found more frequently in Ashkenazi Jews, because of a putative founder effect.

Clinical Features

I-cell disease can manifest itself at birth as coarse features, corneal clouding, organomegaly, hypotonia, and gingival hyperplasia. Birthweight and length are often below normal. Kyphoscoliosis, lumbar gibbus, and restricted joint movement are often present, and there may be hip dislocation, fractures, hernias, or bilateral talipes equinovarus. Dysostosis multiplex may be seen on radiographs. Severe psychomotor retardation, evident by 6 months of age, and progressive failure to thrive occur. The facial features become progressively coarser, with a high forehead, puffy eyelids, epicanthal folds, flat nasal bridge, anteverted nares, and macroglossia. Linear growth slows during the first year of life and halts completely thereafter. The skeletal involvement is also progressive, with development of increasing joint immobility and claw-hand deformities. Respiratory infections, otitis media, and cardiac involvement are common complications. Death usually occurs in the first decade of life because of cardiorespiratory complications.

Mucolipidosis Type IV

Etiology

Although mucolipidosis type IV (ML4) is associated with a partial deficiency of the lysosomal enzyme ganglioside sialidase, a deficiency of mucolipin 1, a member of the transient receptor potential mucolipin subfamily of channel proteins, is the cause of the disorder.[26,27] Mutations in the *MCOLN1* gene result in lysosomal storage of lipids such as gangliosides, plus water-soluble materials such as GAGs and glycoproteins in cells from almost all tissues.

Clinical Features

The age of onset for ML4 ranges from infancy to 5 years. Presenting features include corneal clouding (may be congenital), retinal degeneration, blindness, hypotonia, and mental retardation. An usual feature of ML4 is the presence of achlorhydria. Cytoplasmic inclusions are noted in many cells, including those in conjunctiva, liver, and spleen, as well as fibroblasts. Survival of affected patients into the fourth decade of life has been reported.[28]

Diagnosis, Management, and Prognosis of Lysosomal Storage Diseases

Growing recognition of LSDs in the neonate has led to expansion of the spectrum of possible clinical presentations in the newborn period. Diagnostic tools and options for treatment also continue to advance. Newborn screening for LSDs has been implemented in almost every state, with the goal to offer treatment with enzyme replacement therapy (ERT) or hematopoietic stem cell transplantation (HSCT) for affected babies. Currently, a federal advisory committee actively reviews and makes recommendations to the US Secretary of Health and Human Services for the introduction of new newborn screening tests in the United States, with the aim of vetting proposed tests for need, cost-effectiveness, and availability of effective and timely therapy. The Recommended Uniform Screening Panel now includes MPS type I and Pompe disease. Larger panels of multiplex testing in some states include Gaucher disease, Fabry disease, and Krabbe disease. Depending on the screening assay, false-positive results can be an issue. For example, Krabbe disease, MPS type I, and Pompe disease are associated with well-known pseudodeficiency alleles, which result in enzyme deficiency in vitro but do not cause clinical disease. In some cases, molecular testing identifies variants of uncertain significance, which may require time to establish pathogenicity. The neonatologist must work closely with appropriate experts to determine which infant with a positive newborn screen may be truly affected and require treatment.

Recognizing LSDs in the newborn period can be difficult because they often mimic more common causes of illness in newborns, such as respiratory distress, nonimmune hydrops fetalis, liver disease, and sepsis. The initial step in the diagnosis of these disorders is to consider them in the differential diagnosis of a sick or unusual-appearing newborn. At times, the phenotype may suggest a specific diagnosis, such as respiratory distress and painful, swollen joints in Farber lipogranulomatosis; neonatal cholestasis in NPC; or GI symptoms, hepatosplenomegaly, and adrenal calcifications in Wolman disease. Subtle dysmorphic features, coarse facies, and radiographic evidence of dysostosis multiplex are also strong indications that a patient may have an LSD. Routine laboratory findings are often normal or nonspecific. Affected infants do not have episodes of acute metabolic decompensation. Anemia and thrombocytopenia may be seen because of bone marrow involvement. Vacuolated cells may be found in peripheral blood, but the absence of this finding does not exclude LSDs. Elevated cerebrospinal fluid protein level is seen in Krabbe disease and Farber lipogranulomatosis type I.

Nonimmune hydrops fetalis deserves special mention. The physician must consider LSDs as the cause of nonimmune hydrops fetalis or unexplained ascites in the affected newborn. The following LSDs are potential causes: sialidosis type II, MPS types IV and VII, infantile sialic acid storage disease, Salla disease, galactosialidosis, Gaucher disease type 2, GM1 gangliosidosis, I-cell disease, NPC, ASMD, Wolman disease, and Farber disease.[5] The mechanisms of fetal edema in these diseases are unclear. Furthermore, not all of the above LSDs routinely appear in the neonatal period.

Various clinical samples can be used to diagnose LSD, including blood, urine, amniotic fluid, skin fibroblasts, and tissue biopsies. Directed analysis of urine is helpful for conditions in which characteristic metabolites are excreted in urine. One- or two-dimensional electrophoresis or thin-layer chromatography can detect excess excretion of urine GAGs, oligosaccharides, or free sialic acid, but all urinary tests for the diagnosis of LSDs can have false-negative results. Examination of bone marrow or other tissues may demonstrate storage macrophages in Gaucher disease, ASMD, and NPC. Small skin or conjunctival biopsy specimens may demonstrate storage within lysosomes in most of these disorders.

Definitive diagnosis for almost all LSDs is confirmed by genetic testing and enzymatic assays in serum, leukocytes, or fibroblasts. Biochemical testing for ASMD and NPC is based on measurement of plasma oxysterol concentrations. In addition, prenatal diagnosis is available for most LSDs using enzyme assays

performed on amniocytes or chorionic villus cells or measurements stored at substrate levels in cultured cells or amniotic fluid. If the responsible gene mutations are known, genetic testing can be performed prenatally. Identifying the specific genetic variants can be helpful as there are well-established genotype-phenotype correlations for some LSDs.

LSDs must also be considered in the dying infant. The neonatologist must be prepared to obtain appropriate samples for diagnosis at the time of death. Samples may include urine, blood for DNA banking, fibroblasts, liver tissue, and others. In surviving patients, treatment and management must be considered. All LSDs are chronic and progressive conditions for which treatments slow disease progression and symptoms but are not curative. Patients must be continually reassessed for evidence of disease progression and associated complications.

ERT is available for Gaucher disease, most of the mucopolysaccharidoses, Pompe disease, Wolman disease, and other disorders which are not covered in this chapter. ERT slows disease progression, and the clinical benefits are greater if treatment is started at a younger age, hence, the importance of newborn screening despite the diagnostic challenges. ERT has minimal impact on CNS disease since little protein crosses the blood-brain barrier. Thus, in some instances (e.g., Gaucher disease type 2), ERT is not indicated.[29]

Substrate reduction therapy is another treatment modality for LSDs. As the name implies, these agents reduce the production of storage material by inhibiting an upstream enzyme. In the case of Gaucher disease, the drugs miglustat and eliglustat inhibit glucosylceramide synthase, the first step in glycosphingolipid synthesis, thereby decreasing glucocerebroside production. These medications are approved for adults only, but off-label use in infants and children has been tried in some cases. Miglustat is approved in Europe for children with NPC.

HSCT is indicated for a small number of LSDs. The rationale for the procedure is that circulating blood cells derived from the transplanted marrow become a source of the lacking enzyme. Results of HSCT in MPS type I show that after successful engraftment, leukocyte and liver tissue enzyme activity normalizes and organomegaly decreases, although significant morbidity remains, including skeletal disease, cardiac valve disease, and corneal clouding.[30] Because early HSCT, ideally in the first few months of life, can stabilize neurocognitive function in MPS type I, it is currently the standard of care for patients with severe disease.[18] HSCT appears to be beneficial for MPS type II,[31,32] but fewer outcome data are available.

HSCT is used for patients with infantile Krabbe disease, but outcomes are not as encouraging as for MPS type I. Because infants become symptomatic much earlier in life, and treatment must be initiated before neurologic deterioration occurs, the window of opportunity for treatment is narrower. In a cohort of 18 children with early infantile Krabbe disease who received HSCT in the first 7 weeks of life, 5 (28%) died (3 from peritransplant complications, 1 from surgical complications, and 1 from disease progression).[33] Of the surviving children, all but one (92%) had cognitive impairment, and most could not walk independently.

Gene therapy holds promise, and clinical trials for many LSDs are under way. Gene therapy employs viral vectors to incorporate a correct copy of the gene into cells and may be administered by various routes including intravenous, intrathecal, and intracerebroventricular. It is thought that gene therapy may provide more a durable therapeutic effect compared to ERT which must be administered weekly or biweekly.

Other therapies for LSDs are not discussed in this chapter as they are only approved in the adult population or are still under investigation. In the future, it is likely that the use of combination therapies will become more prevalent. Regardless of the treatment modality, early initiation of treatment results in better clinical outcomes. Thus, the neonatologist plays an important role in the early diagnosis and treatment of neonates with LSDs.

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31

Congenital Disorders of Glycosylation, Peroxisomal Disorders, and Smith-Lemli-Opitz Syndrome

JANET A. THOMAS AND CHRISTINA LAM

KEY POINTS

- The phenotypic spectrum of glycosylation disorders is broad and ranges from mild to severe and from single-organ system to multisystem disease; glycosylation defects should be considered in any unexplained clinical condition, but especially in multiorgan disease with neurologic involvement.
- Diagnosis of congenital disorders of glycosylation mainly relies on next-generation sequencing techniques. Treatment is largely supportive except for rare exceptions where nutritional supplements are effective.
- Peroxisomal disorders are a broad and heterogeneous group of inherited diseases with most often multisystem features, including craniofacial dysmorphism, neurologic dysfunction, including hearing and vision dysfunction, hepatodigestive dysfunction, renal cysts, and skeletal abnormalities seen in the newborn period.
- Diagnosis of peroxisomal disorders is best made by next-generation sequencing techniques following abnormal biochemical screening test findings. Treatment is supportive.
- Smith-Lemli-Opitz syndrome (SLOS) is a multisystemic, developmental, and dysmorphic disorder with a wide clinical spectrum caused by a defect in cholesterol biosynthesis.
- Diagnosis of SLOS is based on elevated 7-dehydrocholesterol and 8-dehydrocholesterol levels in the blood and treatment is largely supportive.

Congenital Disorders of Glycosylation

Epidemiology of Congenital Disorders of Glycosylation

Congenital disorders of glycosylation (CDGs) are a group of more than 140 genetic diseases which involve various defects in the process of modifying proteins, lipids, or other biomolecules with glycans (sugar molecules or chains).[1,2] Glycosylation, the addition of glycans to biomolecules, is essential to many biologic processes, such as aiding with correct folding, protecting against premature destruction, directing intracellular localization and transport, and modifying the biologic function of these biomolecules.

The first discovered CDG (PMM2-CDG) was described by Professor Jaak Jaeken in 1980 and was initially termed "carbohydrate-deficient glycoprotein syndrome" due to abnormalities seen in multiple serum glycoproteins in the affected individuals.[3,4] When several more human glycosylation disorders were identified, this group of disorders was renamed "congenital disorders of glycosylation." The decision was made to designate types of CDG into either a group I or II disorder based on the transferrin pattern obtained by isoelectro-focusing with specific diagnoses alphabetized consecutively as they were identified (i.e., CDG Ia, Ib, Ic, IIa, IIb, etc.).[5] Improved molecular diagnostics expanded the definition of CDGs to include genetic diseases that primarily disrupt the process of formation of any glycoconjugate (i.e., glycoproteins, glycolipids, glycosaminoglycan [GAG], etc.), resulting in an exponential growth of the number of pathways and individual disorders.[6] In 2009, the nomenclature was updated, and currently specific CDG types are named starting with the affected gene symbol (not in italics) followed by -CDG (e.g., CDG-Ia is now PMM2-CDG).[7]

It is estimated that approximately 3% to 4% (~700) of our genes encode for proteins involved with the glycosylation process. The glycosylation process takes place in a variety of locations within the cell including the cytosol, endoplasmic reticulum (ER), and Golgi apparatus.[8] The underlying mechanism for the clinical manifestations of most of CDGs is still unclear. Given the complexity of glycosylation, there are multiple methods to group these disorders. One classification schema groups CDGs into protein N-linked glycosylation defects, protein O-linked glycosylation defects, glycosylphosphatidylinositol (GPI) anchor glycosylation defects, lipid glycosylation defects, and defects in multiple glycosylation and other pathways.[9] In this chapter, we use this classification method to help organize our discussion of CDGs that manifest in the neonatal period.

Clinical Presentation of Congenital Disorders of Glycosylation

Because so many biologic functions are dependent on the correct glycosylation, the phenotypic spectrum of CDG defects is extremely broad and ranges from mild to severe disease and from a single-organ system to multisystem disease. Clinical features alone are insufficient to define the CDG type. A CDG should be considered in any unexplained clinical condition, but especially in multiorgan disease with neurologic involvement (Table 31.1).

TABLE 31.1 Common Clinical Features of Congenital Disorders of Glycosylation by Pathway

		KEY FEATURES BY SYSTEM									
Pathway	Example Disorders	Neurologic	Ophthalmologic	Cardiologic	Gastroenterological	Hematologic	Renal	Endocrine	Dermatologic	Musculoskeletal/ Other	Diagnostic Screen
N-linked glycosylation	PMM2-CDG, MPI-CDG, ALGx-CDG, MOGS-CDG	ID (except MPI), DD, seizures (50%), hypotonia, ataxia, dysmetria, dysarthria, peripheral neuropathy, cerebral and cerebellar atrophy, myasthenic syndrome	Strabismus, nystagmus, optic hypoplasia, retinal pigmentary changes, alacrima, congenital cataracts	Pericardial effusion, cardiomyopathy, fetal hydrops	Protein-losing enteropathy, diarrhea, failure to thrive, gastroesophageal reflux, hepatopathy with elevated AST & ALT, edema and hypoalbuminemia, low cholesterol	Factors II, V, VII, VIII, IX, X, XI, Antithrombin III, Protein C, Protein S deficiency, increased bleeding tendency, thrombotic events, hypogammopathy, coagulopathy and thrombosis	Hyperechoic kidneys, microcystic changes, proteinuria	Abnormal thyroid function test, short stature, IGF1 deficiency, hypogonadotropic hypogonadism, hyperinsulemic hypoglycemia	Lipodystrophy, hypohidrosis	Osteopenia, kyphoscoliosis, dysmorphic features, skeletal dysplasia	Transferrin profiling, N-glycan profiling, urine oligosaccharide analysis (MOGS-CDG only)
O-linked glycosylation	GALNT3-CDG, B3GLCT-CDG, POMK-CDG, EXT1-CDG, CHST-CDG	ID (not universal), DD, congenital and later onset muscular dystrophy, hypotonia, polymicrogyria lissencephaly	Peters-plus syndrome, other structural eye abnormalities, glaucoma, isolated macular corneal dystrophy, corneal opacity, cataracts		Failure to thrive			Tumoral calcinosis with phosphatemia	Loose skin, Dowling Degos disease	Skeletal dysplasia, short stature, Ehlers-Danlos syndrome, hypermobility, exostoses, elevated creatine kinase, dysmorphic facies	
Mixed glycosylation	COGx-CDG, TMEMx-CDG,	Seizures, ID (not universal), DD, microcephaly, hypotonia, cortical and cerebellar atrophy	All findings seen in N-linked pathway possible	Cardiomyopathy, congenital structural heart defects	All findings seen in N-linked pathway possible, isolated polycystic liver disease, high cholesterol, cholestatic liver disease, prenatal growth retardation	Isolated leukocyte adhesion deficiency, isolated congenital dyserythropoietic anemia type II, immunodeficiency	Obstructive uropathy, micropenis, hypospadias		Ichthyosis, cutis laxa, hypohidrosis, hyperkeratosis	Skeletal dysplasia, dysmorphic features, elevated creatine kinase	Transferrin profiling, N-glycan profiling, O-glycan profiling, APO-CIII profiling

Continued

TABLE 31.1 Common Clinical Features of Congenital Disorders of Glycosylation by Pathway—cont'd

		KEY FEATURES BY SYSTEM									
Pathway	Example Disorders	Neurologic	Ophthalmologic	Cardiologic	Gastroenterological	Hematologic	Renal	Endocrine	Dermatologic	Musculoskeletal/ Other	Diagnostic Screen
GPI Anchor disorder	PIGx-CDG, PGAPx-CDG	Seizures, ID, DD, macrocephaly, hypotonia		Congenital heart defects, cardiomyopathy				Accelerated linear growth, advanced bone age, +/− hyperphosphatasia, hypophosphatasia		Dysmorphic features, multiple congenital anomalies	Flow cytometry studies using cell surface markers like FLAER and CD59 on granulocytes, lymphocytes, etc.
Lipid glycosylation	ST3GAL5-CDG (Amish Infantile Epilepsy syndrome)	Seizures, ID, DD, hypotonia, diffuse brain atrophy, irritability, microcephaly	Optic atrophy, cortical visual impairment		Failure to thrive				Dyspigmentation, "salt and pepper" pattern on skin macules		

ALT, Alanine transaminase; *AST*, aspartate transaminase; *DD*, developmental disability; *GPI*, glycosylphosphatidylinositol; *ID*, intellectual disability; *IGF1*, insulin-like growth factor 1.

N-Linked Protein Glycosylation Defects

Etiology

N-linked protein glycosylation, the process involved with attaching glycans to the asparagine residue of target proteins, was the first discovered and is the best understood glycosylation pathway in humans. Classically, these disorders were divided into two categories: type I which results in defects in N-glycan assembly, and type II which results from defects in N-glycan processing.

The initial assembly steps of N-glycosylation take place on the ER membrane, where sugars are attached in a stepwise manner to Dolichol-P to form a lipid-linked oligosaccharide (LLO). Sugars are donated by an activated nucleotide-sugar (UDP-GlcNAc and GDP-Man), with the attached nucleotide providing the necessary energy for the transfer of the sugar to the LLO. This oligosaccharide is then transferred to the nascent protein cotranslationally. Once the oligosaccharide chain has been transferred to the protein, further processing takes place. The oligosaccharide is then transported to the Golgi apparatus, where further processing occurs. Different types of CDGs have been found in affected individuals who have defective enzymes in individual steps of this complex pathway including the enzymes that form the dolichol backbone, transfer single sugars to the growing chain, interconvert activated monosaccharides, and transfer the oligosaccharide from dolichol to protein.[10,11]

Clinical Features

N-linked glycosylation defects encompass many disorders. Taken together these several dozen disorders are typically multisystemic with significant neurologic involvement with the notable exception of MPI-CDG, in which development can be normal.[12] The most common perinatal findings include hypotonia, nonspecific dysmorphic features (inverted nipples or abnormal fat pads occasionally present), feeding problems with growth delay, hepatopathy with elevated transaminases, and abnormal coagulation profiles. Discriminating findings include neonatal hemorrhages (including cerebral hemorrhage) and thrombotic events, pericardial effusion, strabismus, nystagmus, and other ophthalmologic findings, neonatal seizures, abnormal thyroid function screening results, and nonimmune hydrops.[13,14] Transferrin glycosylation analysis previously performed using isoelectric-profiling and now performed using mass spectrometry methods show an abnormal glycosylation pattern in many, but not all, of these disorders.[15]

Two disorders warrant special mention: PMM2-CDG (CDG-Ia) and MPI-CDG (CDG-Ib). PMM2-CDG (CDG-Ia) is the classic and most common presentation, and many other N-linked CDGs mirror its presentation. Most affected infants appear normal at birth, although a subset of individuals present with nonimmune hydrops with and without hypertrophic cardiomyopathy. In infancy, patients with PMM2-CDG can exhibit dysmorphic features, strabismus, nystagmus, hypoglycemia, and feeding difficulties; subsequently patients may exhibit growth failure, hypotonia, lipocutaneous abnormalities (including prominent fat pads on the buttocks), coagulopathy with thrombosis and bleeding, pericardial effusion, and mild to moderate hepatomegaly and hepatopathy.[16] Approximately 20% of patients with PMM2-CDG die during the first year of life after a course of severe fluid imbalance and sometimes anasarca in response to infection or their underlying glycosylation disorder.[17] Having survived infancy, patients with PMM2-CDG can live into their seventh and eighth decades. Later manifestations include intellectual disability, retinitis pigmentosa or retinal degeneration, renal cysts, coagulopathy, stroke-like episodes, thrombotic disease, cerebral and olivopontocerebellar hypoplasia, ataxia, dysarthria, peripheral neuropathy, followed by lower extremity atrophy, kyphoscoliosis, and hypogonadism.[16]

MPI-CDG (CDG-Ib) stands out in this group of disorders because patients with MPI-CDG can have normal development and mannose is a known targeted therapy. Mortality rate during infancy is 23.5%, and the causes of death, when known, included hepatic failure and sepsis. The major manifestations of MPI-CDG are liver fibrosis, hepatomegaly, hypoglycemia, growth restriction, hypoalbuminemia, diarrhea, protein-losing enteropathy, edema, faltering growth, and coagulopathy. Mannose supplementation is not associated with improvement hepatopathy but improves most of the other manifestations. Thus, early diagnosis and treatment of MPI-CDG are imperative.[18]

O-Linked Protein Glycosylation Defects

Etiology

O-glycosylation consists of attachment of a monosaccharide (mannose, fructose, or xylose), or the assembly of a glycan and its attachment to a serine or threonine residue of a target protein. O-glycosylation differs from N-glycosylation in that it does not take place at the same time as the protein is being translated, but occurs posttranslationally, exclusively in the Golgi apparatus, without further processing.[19] O-glycosylation can be classified according to which type of sugar is attached to the serine or threonine. Examples of O-glycosylation include O-mannosylation, O-xylosylation, and O-fucosylation.[8]

Clinical Features

Clinical features vary significantly depending on which type of O-glycosylation is defective. Deficiency of O-N-acetylgalatosamine linkage can lead to familial tumoral calcinosis with phosphatemia and massive calcium deposits in the skin and subcutaneous tissues.[20] A defect in O-fucosylation has been shown to lead to Peters-plus syndrome characterized by anterior eye chamber defects, disproportionate short stature, developmental delay, and cleft lip and/or palate.[21] O-fucosylation defects also lead to Dowling-Degos disease 2 characterized by abnormal skin pigmentation,[22] and spondylocostal dysostosis type 3 characterized by short stature and vertebral abnormalities.[23] Defects in O-xylosylation will lead to defective anchoring of GAGs to proteins and thus impaired proteoglycan formation. Defective O-xylosylation can lead to progeroid-type Ehlers-Danlos syndrome characterized by failure to thrive, loose skin, skeletal abnormalities, hypotonia, and hypermobile joints. Defects in forming heparin sulfate, also attached to proteins via O-xylosylation, cause congenital exostosis, an autosomal dominant disorder where patients have bony outgrowths usually at the growth plate of the long bones. Defective cartilage proteoglycan sulfation leads to achondrogenesis, diastrophic dystrophy, atelosteogenesis that manifest symptoms in cartilage and bone like cleft palate, club feet, and in the most severe cases lead to perinatal death from respiratory insufficiency.[20]

Additionally, there are close to 20 different genetic disorders that lead to a defect in O-mannosylation.[24] O-mannosylation defects lead to hypoglycosylation of α-dystroglycan, an important glycoprotein needed to link the intracellular cytoskeleton of

muscle to the extracellular matrix. These disorders, collectively referred to as α-dystroglycanopathies, have a wide spectrum of clinical severity and encompass previously described disorders, ranging from Walker-Warburg syndrome, muscle-eye-brain disease, Fukuyama congenital muscular dystrophy, to limb-girdle muscular dystrophy.[25,26]

In the neonate, clinical features of O-linked protein glycosylation defects involve the triad of muscle, eye, and brain and may include hypotonia; muscle weakness; microcornea; microphthalmia; pale, hypoplastic or absent optic nerves; colobomas; cataracts; iris hypoplasia; glaucoma; retinal dysplasia or detachment; and brain structural abnormalities including hydrocephalus, brainstem hypoplasia, cerebellar cysts, cobblestone lissencephaly, polymicrogyria, cerebellar vermis and hemisphere atrophy, hypoplasia of the pyramidal tracts, and absence of the corpus callosum. There is no specific blood or urine biochemical marker available for this group of disorders. Elevated creatine kinase is frequently noted. Muscle biopsy with specialized immunohistochemical staining may show deficient glycosylated alpha-dystroglycan and normal beta-dystroglycan. Molecular testing is needed to confirm the specific type.[27]

Combined Glycosylation Defects

Etiology

Combined N- and O- and other glycosylation defects are important because they appear to affect trafficking in the glycosylation machinery.[28] Several of these disorders involve defects in channels involved in activated sugar-nucleotide transport (SLCx-CDG). Some affect vesicular transport (COGx-CDG) in general. Others affect the process of sugar activation (attaching nucleotides to monosaccharides so that they can be used for glycosylation). And yet others cause abnormalities in the Golgi apparatus structure (TMEMx-CDG) that is needed to be intact for glycosylation to proceed.[29]

Clinical Features

In the neonate, the most frequent presenting symptoms of combined glycosylation defects include neonatal microcephaly; neonatal seizures; strabismus; hypotonia; dysmorphic features, especially cutis laxa; feeding problems with growth delay; and hepatic involvement.[13] Neonatal cholestatic hepatic failure can be the presenting or predominant symptom of a neonate with a combined defect.[30] Encompassing a very large group of disorders, presentations are heterogeneous and include not only multisystemic diseases, but also single-system disorders such as congenital dyserythropoietic anemia type II due to SEC23B-CDG.

Glycosylphosphatidylinositol Anchor Glycosylation Defects

Etiology

The biosynthesis and attachment of GPI anchors to proteins occur in the ER and Golgi and involve 11 steps and at least 27 genes.[31] To date, inherited loss of function mutations in over a dozen of these genes have been implicated in human disease. GPI anchors are attached during posttranslational modification and allow these proteins to attach to the outer leaflet of the cell membrane and face the extracellular environment. This permits these proteins to participate in processes such as signal transduction and immune response.[32,33]

Clinical Features

Typically, individuals affected with GPI anchor disorders have a severe phenotype and present in infancy with epilepsy; intellectual disability; and multiple congenital anomalies including heart, skeletal (especially abnormalities in phalanges), endocrine, ophthalmologic, and facial anomalies (dysmorphic features), with possible abnormalities in alkaline phosphatase levels depending on the specific diagnosis.[34,35] Although there is no standard blood or urine biomarker, flow cytometry markers show promise to be effective biomarkers in many of these disorders.[36]

Lipid Glycosylation Defects

To date, three disorders of lipid glycosylation have been described: SIAT9-CDG, ST3GAL-CDG, and B4GALNT1-CDG. SIAT9-CDG, also known as Amish infantile epilepsy was the first identified and is caused by a defect of lactosylceramide α-2,3 sialyltransferase (GM3 synthase).[37] This enzyme catalyzes the initial step in the biosynthesis of most complex gangliosides from lactosylceramide.[19] The defect causes accumulation of lactosylceramide associated with decreased gangliosides.[37] Individuals with this disorder present with infantile-onset epilepsy with developmental stagnation, blindness, poor feeding, vomiting, failure to thrive, later onset "salt and pepper" macules, and variable survival.[38] ST3GAL-CDG is a cause of West syndrome.[39] B4GALNT1-CDG, also known as spastic paraplegia-26, is also a defect in ganglioside biosynthesis. However, onset of symptoms including gait abnormalities, as well as central and peripheral nervous system involvement typically occurs after the neonatal period, in the first two decades of life.[40]

Diagnosis of Congenital Disorders of Glycosylation

CDG should be considered in young infants with a combination of clinical features as shown in Table 31.1. Serum transferrin isoform analysis is the most available screening method for CDGs, but it is only able to detect N-glycosylation and some mixed glycosylation defects. Until about 2000, transferrin screening was achieved by isoelectric focusing of transferrin; failure to correctly synthesize the N-linked glycans alters the charge on serum transferrin and consequently its migration in an electrophoretic field. In recent years, however, mass spectrometric methods, capable of identifying individual oligosaccharides and complete glycans by mass and charge, have replaced transferrin isoelectric focusing as the standard method for screening for CDGs.[15] Transferrin and glycan analysis may yield false-positive results in galactosemia, inborn errors of fructose metabolism, alcohol consumption, certain bacterial (neuraminidase-producing) infections, and in cases of mutations in transferrin itself. False-negatives can occur in the first 3 weeks of life.[36] There are reported cases where initially abnormal transferrin glycosylation normalizes without improvement in symptoms. There are also N-linked defects known to not show transferrin isoform abnormalities (i.e., MOGS-CDG, TUSC3-CDG, SLC35A1-CDG, SLC35C1-CDG).[41]

Apo CIII glycan analysis has been used in the screening of some mixed and O-glycosylation disorders. Urine oligosaccharide screening is useful in detecting a MOGS-CDG. Many defects in GPI synthesis can be identified using flow cytometry of GPI-anchored proteins, such as FLAER or CD59 on leukocytes. Not all subtypes of CDGs have convenient biochemical markers; for example, screening for congenital muscular dystrophies caused by

defective O-mannosylation requires a muscle biopsy with the use of monoclonal antibodies directed against the glycan.[41] There are also no available markers for defects in GAG biosynthesis.

Since the advent of next-generation genome sequencing and exome analysis, the majority of CDGs have been diagnosed molecularly.[42] Once variants are identified in the specific gene, if novel, the functional consequence of the mutation can be confirmed using enzymatic assays in peripheral blood leukocytes or cultured fibroblasts for PMM2-CDG and MPI-CDG as well as on a research basis for other types. Prenatal diagnosis is possible in all types of CDG for which the molecular defect is known.[28] The vast majority of CDGs are autosomal recessive disorders; POGLUT1-CDG and POFUT1-CDG (Dowling-Degos disease), EXT1&2-CDG (hereditary multiple exostoses syndrome), GANAB-CDG (polycystic kidney disease 3), and SEC63-CDG and PRKCSH-CDG (polycystic liver disease), GNE-CDG (sialuria form), DHDDS-CDG (Retinitis pigmentosa 59), NUS1-CDG, COG4-CDG (Saul-Wilson syndrome), COPA-CDG (autoimmune interstitial disease), ARCN1-CDG (Rhizomelic short stature with microcephaly, micrognathia, and developmental delay), SEC23B-CDG (Cowden syndrome), and HS6ST1-CDG, can be autosomal dominant; PIGA-CDG, HS6ST2-CDG, OGT-CDG, SSR4-CDG, SLC35A2-CDG, SLC9A7-CDG, TRAPPC2-CDG, ATP6APT1-CDG, ATP6AP2-CDG, VMA21-CDG, ALG13-CDG, and MAGT1-CDG are X-linked; C1GALT1C1-CDG is somatic.

Management of Congenital Disorders of Glycosylation

Only a minority of CDGs have a specific treatment available. MPI-CDG can be treated with oral mannose,[43] which ameliorates protein losing enteropathy, coagulopathy, and hyperinsulinism, but does not necessarily halt the progression of the liver disease. Heparin has also been used for protein-losing enteropathy in MPI-CDG.[44] In PGM1-CDG, D-galactose supplementation improves hypoglycemia, coagulopathy, rhabdomyolysis, and endocrinopathy, but not muscle weakness and cardiomyopathy.[45,46] Similarly, D-galactose supplementation shows promise in improving similar symptoms in TMEM165-CDG, as well as seizures in SLC39A8-CDG and SLC35A2-CDG. Manganese has also been used in SLC39A8-CDG.[46] In SLC35C1-CDG, some patients respond to oral fucose supplementation; this treatment is only effective with regard to the typical recurrent infections with hyperleukocytosis, and does not correct the neurodevelopmental aspects.[47] In PIGM-CDG, butyrate has been shown to control the seizures in some cases,[48] and in PIGO-CDG, vitamin B6 supplementation has also aided in seizure control.[49,50] Uridine and uridine triacetate have been reported to improve seizures and anemia in CAD-CDG.[51] Sialic acid and ManNAc are also promising therapies for GNE myopathy.[52] Organ transplantation has also been beneficial in several CDGs including liver for MPI-CDG, CCDC115-CDG, ATP6AP1-CDG; heart for DOLK-CDG; and stem cell for PGM3-CDG.[46]

The treatment and management for other types of CDGs are primarily supportive and palliative. In infancy, evidence of multisystem involvement and the resulting complications must be treated promptly. There is substantial mortality in the first years of life because of severe infection or vital organ failure.[28,37] Fresh frozen plasma and/or protein C concentrate has been used to prevent bleeding episodes in multiple CDGs, as well as improving capillary leakage and edema, especially during times of infections.[53]

The future is promising with multiple targeted therapies on the horizon currently being studied and developed including acetazolamide, aldose reductase inhibitors, chaperone therapy, and liposomal mannose-1-phosphate for PMM2-CDG; and gene therapy preclinically in several CDGs.[46]

Peroxisomal Disorders

Epidemiology of Peroxisomal Disorders

Peroxisomes are small, evolutionarily conserved, single membrane–bound cellular organelles that contain no internal structure or DNA. Peroxisomes are characterized by an electron-dense core and a homogeneous matrix. Peroxisomes are found in all cells and tissues except mature erythrocytes and are in highest concentration in the liver and kidneys. They are formed predominantly by growth and division of preexisting peroxisomes, but they can also arise de novo from peroxisomal vesicles that originate from specialized compartments of the ER.[54–56] Their half-life is 1.5 to 2 days before they are randomly destroyed by autophagy. All peroxisomal proteins are encoded by nuclear genes, synthesized in cytosol, and imported posttranslationally into the peroxisome.[54] The import of proteins into the peroxisome is mediated by specific targeting sequences known as peroxisomal targeting sequences (PTS1 and PTS2).[56,57] Peroxisomes have significant interaction with other subcellular organelles, including mitochondria, ER, lysosomes, and lipid bodies, mediated by "tethering proteins".[57,58] Defects related to disturbed contact between two subcellular organelles are increasingly described (e.g., acyl-CoA binding protein 5 [ACBD5] deficiency).[57]

Peroxisomes contain enzymes that use oxygen to oxidize a variety of substrates, thereby forming peroxide. The peroxide is decomposed within the organelle by the enzyme catalase to water. This process protects the cell against peroxide damage through compartmentalization of peroxide metabolism within the organelle. Peroxisomes can also function to dispose of excess reducing equivalents and may contribute to thermogenesis, producing heat from cellular respiration.[59] Peroxisomes also fulfill crucial nonmetabolic roles including participation in the cellular stress response, defense of pathogens and viruses, as signaling platforms, and in healthy aging.[58]

More than 70 enzymes have been found within peroxisomes.[55] The proteins have multiple functions, both synthetic and degradative.[55,56] The primary synthetic functions are plasmalogen synthesis, bile acid, and docosahexanoic acid formation. Plasmalogens constitute 5% to 20% of phospholipids in cell membranes and 80% to 90% of phospholipids in myelin. They are involved in platelet activation and may also protect cells against oxidative stress. Degradative functions include (1) β-oxidation of very-long-chain fatty acids (VLCFAs) ($\geq C_{23}$), fatty acids (down to C_8 to C_6), long-chain dicarboxylic acids, pristanic acid, prostaglandins, and polyunsaturated fatty acids; (2) alpha-oxidation of bile acid intermediates, pipecolic acid and glutaric acid (intermediates in lysine metabolism), and phytanic acid; (3) deamination of D-amino acids and L-amino acids; (4) metabolism of glycolate to glyoxylate; (5) polyamine degradation (spermine and spermidine); and (6) ethanol clearance. At least 16 conditions caused by peroxisomal enzyme deficiencies have been confirmed.[55,56,60]

Peroxisomal disorders constitute a clinically and biochemically heterogeneous group of inherited diseases that result from the absence or dysfunction of one or more peroxisomal enzymes.

Disorders in which more than one enzyme is affected are collectively termed *peroxisomal biogenesis disorders* (PBDs). Disorders in which only one enzyme is affected encompass the remaining known disorders. All but one are inherited in an autosomal recessive manner.

Clinical Presentation of Peroxisomal Disorders

The pathophysiologic features apparently involve either deficiency of necessary products of peroxisomal metabolism or excess of unmetabolized substrates. Disorders with similar biochemical defects may have markedly different clinical features, and disorders with similar clinical features may be associated with different biochemical findings. General features of peroxisomal disorders evident in the newborn period are shown in Table 31.2.

Disorders of Peroxisomal Biogenesis

Conditions in which multiple peroxisomal enzymes are affected can result from a disturbance of biogenesis of the organelle. Peroxisomal assembly includes matrix protein import, synthesis of new organelles, and fusion of existing organelles. The coordinated activity of 16 PEX proteins, or peroxins, encoded by their corresponding genes is required for this process.[61] Peroxins are classified into three groups: (1) those essential for peroxisomal membrane assembly (PEX3, PEX 16, PEX19); (2) those required for matrix protein import (10 different peroxins); and (3) different forms of PEX11 (α, β, γ) involved in peroxisomal division.[62] The PEX genes responsible for disease in most human patients are known, with more than 60% of patients with PBD having mutations in *PEX1*; the second most commonly involved gene is *PEX6*.[54,55,61] The overall incidence of PBD is estimated to be approximately 1 in 50,000 newborns.[55,60]

Zellweger syndrome is the prototype of neonatal peroxisomal disease. It is a disorder of peroxisome biogenesis caused by failure to import newly synthesized peroxisomal proteins into the peroxisome. The proteins remain in the cytosol, where they are rapidly degraded. In this condition, peroxisomes are absent from liver hepatocytes or exist as "ghosts." Neonatal adrenoleukodystrophy and infantile Refsum disease are also disorders of peroxisome biogenesis in which, as in Zellweger syndrome, disruption of function of more than one peroxisomal enzyme is demonstrable. A few residual peroxisomes, however, may be seen in the liver. These disorders represent a continuum of clinical severity, and the term *Zellweger spectrum disorders* (ZSDs) is now preferred.[55,61]

Features common across the spectrum of ZSDs include liver disease, variable neurologic dysfunction, developmental delay, retinopathy, neurosensory hearing loss, and adrenocortical dysfunction.[60,63] Rhizomelic chondrodysplasia punctata (RCDP), types 1 and 5, are caused by a defect in a subset of peroxisomal enzymes resulting from mutations in the *PEX7* gene and the *PEX5L* isoform, respectively. PEX5L is required for PEX7-mediated protein import.[57] In these disorders, impaired alpha-oxidation of phytanic acid and impaired synthesis of plasmalogens result in the accumulation of phytanic acid and deficiency of plasmalogens. Liver peroxisomes are demonstrable and normal in number, but their distribution and structure are abnormal.

A relatively new category of disorders, referred to as *peroxisomal fission defects*, has also been recognized. Peroxisomal fission defects are disorders caused by defects in proteins known to be involved with membrane dynamics and the proliferation and division of peroxisomes rather than by loss of metabolic function.[54,56,64–66] Peroxisomal growth and division involves the coordinated interplay of key membrane-shaping and fission proteins such as PEX11β, FIS1 (mitochondrial fission protein 1), MFF (mitochondrial fission factor), and DRP1 (dynamin-like protein 1 encoded by the *DNML1* gene).[66] With the exception of PEX11β, these proteins are also key mitochondrial division factors.[66] Finally, Heimler syndrome, a rare recessive disorder, typically presenting in young childhood with sensorineural hearing loss, amelogenesis imperfecta, nail abnormalities, and retinal pigmentation, was recognized as a mild PBD involving mutations in *PEX1* and *PEX6*.[67,68] With the advent of next-generation sequencing, broader, often atypical, phenotypes are being increasingly recognized, often presenting in later childhood with nonclassical signs and symptoms and longer survival.[57,62,69,70]

TABLE 31.2 Common Clinical Features of Peroxisomal Disorders Evident in the Newborn Period

- Dysmorphic craniofacial features
- Neurologic dysfunction, including severe hypotonia, possibly associated with hypertonia of extremities, seizures, vision difficulties, hearing loss, and abnormalities in neuronal migration
- Hepato-digestive dysfunction, including hepatomegaly, cholestasis, prolonged hyperbilirubinemia, coagulopathy secondary to vitamin K malabsorption, and feeding difficulties
- Rhizomelic shortening of the limbs, stippled calcifications of epiphyses
- Renal cysts

Zellweger Syndrome

Zellweger syndrome is most often evident at birth, with affected newborns having dysmorphic facial features including large fontanels, high forehead, flat occiput, epicanthus, hypertelorism, upward-slanting palpebral fissures, hypoplastic supraorbital ridges, abnormal ears, severe weakness and hypotonia, hepatomegaly, multicystic kidneys, and congenital heart disease. Seizures, feeding difficulties, and postnatal growth failure soon manifest themselves. Ophthalmologic examination may detect cataracts, corneal clouding, glaucoma, optic atrophy, retinitis pigmentosa, and Brushfield spots. Somatosensory evoked responses and electroretinograms are abnormal. Hearing assessment often shows an abnormal brainstem auditory evoked response consistent with sensorineural hearing loss. Skeletal radiographs demonstrate epiphyseal stippling, and cranial imaging may show brain atrophy, germinolytic cysts, corpus callosum dysgenesis, disorders of myelination, leukodystrophy, and/or neuronal migration abnormalities (typically perisylvian polymicrogyria).[71] Progressive hepatic fibrosis, cirrhosis, and liver failure, and severe psychomotor retardation occur later. Laboratory analysis often demonstrates abnormal liver function values, hyperbilirubinemia, hypoprothrombinemia, coagulopathy, and/or adrenal insufficiency. Death usually occurs within the first year of life with the average life span being 12.5 weeks.

Neonatal Adrenoleukodystrophy

Clinically, neonatal adrenoleukodystrophy is similar to, but less severe than, Zellweger syndrome. Differences include less dysmorphology and absence of chondrodysplasia punctata and renal cysts. Patients with neonatal adrenoleukodystrophy may have striking white matter signal abnormalities with early-onset progressive

leukoencephalopathy with or without perisylvian polymicrogyria.[71] They often show degenerative changes in the adrenal glands. They have slow psychomotor development followed by neurodegeneration that usually begins before the end of the first year of life. Disease progression is slower than that observed in Zellweger syndrome, and longer survival is usual, to an average of approximately 15 months of age or into the teen years.[56]

Infantile Refsum Disease

Patients with infantile Refsum disease also have relatively mild dysmorphic features, such as epicanthic folds, midface hypoplasia with low-set ears, and mild hypotonia. Early neurodevelopment is normal, possibly up to 6 months of age, but then slow deterioration begins. Later, sensorineural hearing loss (100%), anosmia, retinitis pigmentosa, hepatomegaly with impaired function, and severe cognitive impairment are evident. Patients learn to walk, although their gait may be ataxic and broad-based. Diarrhea and failure to thrive may also be seen. Chondrodysplasia punctata and renal cysts are absent. Neuroimaging findings range from normal to progressive leukodystrophy.[71] Adrenal hypoplasia occurs. The life span of patients with infantile Refsum disease ranges from 3 to 11 years or into adulthood.

Rhizomelic Chondrodysplasia Punctata

Patients with defects in the biosynthesis of ether phospholipids present with RCDP. Five genetically distinct, but clinically indistinguishable, groups exist, three of which are single-enzyme defects (types 2, 3, and 4) and the other two of which are peroxisomal biogenesis defects (types 1 and 5).[56] RCDP, type 1 accounts for greater than 90% of all reported cases with an incidence of 1 per 10,000 live births.[72] At birth, patients with RCDP have facial dysmorphia, microcephaly, cataracts, hearing loss, rhizomelic shortening of extremities with prominent stippling, and coronal clefting of vertebral bodies. The chondrodysplasia punctata is more widespread than in Zellweger syndrome and may involve extraskeletal tissues. Neuroimaging may show delayed myelination, white matter signal abnormalities, generalized cerebral atrophy, or progressive cerebellar atrophy.[71] Infants with this disorder have severe psychomotor retardation from birth onward and severe failure to thrive. In addition, patients may have joint contractures, and 25% experience ichthyosis. The life span is usually less than 1 year.

Peroxisomal Fission Defects

The first described patient with a peroxisomal fission defect was a severely affected female patient with mitochondrial encephalopathy who died at 1 month of age.[64] She was noted to have microcephaly, mild dysmorphic features, truncal hypotonia, absent deep tendon reflexes, optic atrophy, failure to thrive, abnormal brain development, and severe developmental delay. She had elevated peripheral and central lactic acid and alanine levels, mildly elevated VLCFA levels, and abnormal-appearing peroxisomes and mitochondria in fibroblasts but normal oxidative phosphorylation values in fibroblasts and skeletal muscle specimens.[64] Evaluation revealed a peroxisomal and mitochondrial fission defect with a heterozygous, dominant-negative mutation in the dynamin-like protein 1 gene (*DLP1*).[64] Additional patients with *DLP1* mutations have now been described with variable clinical phenotypes ranging from severe neonatal presentations to later, more mild presentations.[73–78] In addition, multiple patients with MFF deficiency have been described.[58,79–82] These individuals present with Leigh-like encephalopathy associated with global developmental delay and/or developmental regression, acquired microcephaly, early-onset seizures, optic atrophy, and peripheral neuropathy typically within the first year of life.[58,79,80,82] Patients may not show significant peroxisomal or biochemical abnormalities.

Single Peroxisomal Enzyme Defects

Of patients with suspected ZSD and elevated VLCFA levels, approximately 10% to 15% will have a single enzyme defect.[55,61] To date, three childhood disorders of peroxisomal fatty acid β-oxidation have been defined: D-bifunctional protein deficiency (DBPD), acyl-CoA oxidase deficiency, and 2-methylacyl-CoA racemase deficiency.[56] The clinical presentation resembles that of biogenesis disorders. Previously, an isolated case of a fourth disorder, peroxisomal thiolase deficiency, was described.[83] On reinvestigation, however, this case was identified as DBPD.[84]

D-Bifunctional Protein Deficiency

DBPD is a rare single peroxisomal enzyme defect that results in a phenotype similar to Zellweger syndrome. It is caused by mutations in the *HSD17B4* gene encoding 17β-estradiol dehydrogenase, an enzyme involved in β-oxidation of VLCFAs and branched-chain fatty acids, including pristanic acid and bile acid intermediates, resulting in accumulation of VLCFAs, pristanic acid, dihydroxycholestanoic acid, and trihydroxycholestanoic acid.[56,85] DBPD can be divided into three subtypes according to the functional subunit involved. Deficiency of both the 2-enoyl-CoA hydratase and 3-hydroxyacyl-CoA dehydrogenase functional subunits represent the type 1 subgroup and individuals have the most severe phenotype with early onset of symptoms and early death (<2 years of age).[71] Isolated deficiency of either subunit results in type II and III disease (hydratase and dehydrogenase, respectively) typically with less severe phenotype and longer survival (>10 years).[71] In general, children have severe neurologic involvement consisting of profound hypotonia, uncontrolled seizures, and failure to acquire any significant developmental milestones. Children are usually born at term without evidence of intrauterine growth restriction. Dysmorphic features, similar to those seen in Zellweger syndrome, are notable in most children. In most cases, neuronal migration is disturbed, with areas of polymicrogyria and heterotopic neurons in the cerebrum and cerebellum. Periventricular white matter signal abnormalities and thinning of the corpus callosum can also been seen.[71] Death generally occurs before 1 year of age, but survival to at least 3 years of age is possible.

Acyl-Coenzyme A Oxidase Deficiency

Acyl-CoA oxidase deficiency, also called *pseudoneonatal adrenoleukodystrophy*, is a rare, neuroinflammatory, neurodegenerative disorder.[86,87] It is caused by mutations in *ACOX1* exclusively involved in the β-oxidation of straight-chain fatty acids resulting in only the accumulation of VLCFAs.[56,86] Patients exhibit global hypotonia, early onset seizures, deafness, failure to thrive, hepatomegaly, areflexia, and delayed developmental milestones with or without facial dysmorphic features as well as retinopathy with extinguished electroretinograms, nystagmus, and optic atrophy.[86,88,89] Patients often demonstrate early developmental skills and have a normal brain MRI, but then show regression of skills typically

between 24 and 48 months of age.[71,87] Eventually, brain MRI shows diffuse and progressive leukodystrophy and white matter demyelination.[71]

2-Methylacyl-Coenzyme A Racemase Deficiency

2-Methylacyl-CoA racemase (AMACR) deficiency is a rare disorder caused by mutations in the *AMACR* gene encoding the enzyme 2-methylacyl-CoA racemase. The enzyme catalyzes the isomerization of fatty acids with a methyl group in the *R* configuration to the corresponding *S* configuration, an obligatory reaction in the steps leading to peroxisomal β-oxidation. This results in impaired bile acid synthesis and pristanic acid metabolism and subsequent accumulation of pristanic acid, (25 *R*)-trihydroxycholestanoic acid, and (25 *R*)-dihydroxycholestanoic acid.[90] Most patients present with an adult-onset ataxia and sensory neuropathy; however, an infantile presentation with cholestatic liver disease, coagulopathy, and fat-soluble vitamin deficiency has been reported.[56,90]

X-Linked Adrenoleukodystrophy

X-linked adrenoleukodystrophy (X-ALD) is the most common peroxisomal disorder, with an estimated incidence of ~1 in 15,000.[56,91,92] It is caused by the altered function of the peroxisomal transmembrane protein ABCD1, which predominantly catalyzes the import of straight-chain VLCFAs into peroxisomes.[56] Defects in ABCD1 lead to an inability to transport VLCFAs across the peroxisomal membrane resulting in accumulation of VLCFA in plasma and tissues, preferentially in the central nervous system, adrenal cortex, and Leydig cells of the testes. X-ALD does not usually present in the newborn period; however, contiguous *ABCD1 DXS1357E* deletion syndrome, caused by a contiguous gene deletion of *ABCD1* and its upstream gene *DXS1357E*, may. Four male patients have been reported with profound neonatal hypotonia, severe growth and developmental retardation, cholestatic liver disease, accumulation of VLCFAs, and death within the first year of life.[85,93,94] Increasingly, as more and more states adopt newborn screening (NBS) for X-ALD, this diagnosis will be made in the newborn period. Notably, adrenal insufficiency, a common feature of this disorder, has been found in infants as young as 4.5 months of age with biochemical evidence of adrenal insufficiency as young as 5 weeks of age.[92,95] Early screening for adrenal insufficiency is, thus, recommended.[92]

Diagnosis of Peroxisomal Disorders

The key to diagnosing peroxisomal disorders is a high index of suspicion. Peroxisomal disorders should be considered in newborns with dysmorphic facial features, skeletal abnormalities, shortened proximal limbs, neurologic abnormalities (including hypotonia or hypertonia), ocular abnormalities, and hepatic and renal abnormalities. Babies with abnormal vision, hearing, or somatosensory evoked potentials should also be considered for these diagnoses.

Peroxisomal disorders are not associated with acute metabolic derangements or abnormal routine laboratory test findings. Measurements of the levels of VLCFAs, phytanic acid, pristanic acid, pipecolic acid, bile acid intermediates, and plasmalogens are required for diagnosis and remain the screening studies of choice. Zellweger syndrome is associated with elevations of the levels of VLCFAs, phytanic acid, pipecolic acid, and bile acid intermediates as well as a decrease in plasmalogen synthesis. Neonatal adrenoleukodystrophy and infantile Refsum disease have similar biochemical findings; however, the defect in plasmalogen synthesis and the degree of VLCFA accumulation are less severe. Laboratory findings in RCDP include elevations of the levels of phytanic and pipecolic acids, a decrease in the levels of plasmalogens, and normal levels of VLCFAs and bile acid intermediates. Hence, biochemical screening using only levels of VLCFAs will fail to detect RCDP. Also, a small number of patients with mutations in PEX genes have been identified with mild or absent elevations in VLCFA levels.[55,57] DBPD is associated with deficient oxidation of C23:0 and pristanic acid, leading to elevations of the levels of pristanic acid and, to a lesser extent, phytanic acid. This deficiency results in an elevated pristanic acid to phytanic acid ratio, which is generally not elevated in PBD. Abnormal VLCFA levels and elevations of the levels of varanic acid, an intermediate metabolite in β-oxidation, are also seen. Accumulation of bile acid intermediates is a variable finding. Recently, C26:0-lysophosphatidylcholine (C26:0 lyso PC) measurement in dried blood spots using liquid chromatography tandem mass spectrometry (LC-MS/MS) has been introduced as a screening method, but may be of limited practical use due to its limited clinical availability.[57,96] Another possible biomarker for VLCFA accumulation is C26-carnitine. This, too, can be measured in dried blood spots, but also has limited clinical availability.[57,96]

Abnormalities in the levels of phytanic acid and plasmalogens are age-dependent. The elevation of the levels of phytanic and pristanic acids might not be demonstrable in newborns not consuming dairy products or other dietary sources of these fatty acids, and reduction in red blood cell plasmalogen levels may not be evident in children older than 20 weeks.[55,59,97] Pipecolic acid levels are more likely to be abnormal in the urine of newborns and more abnormal in plasma at later ages.[55] A ketogenic diet may elevate VLCFA levels as will excess dietary intake of peanuts.[55,96,97] A liver biopsy may be a useful adjunct diagnostic tool to assess the presence or absence and structure of peroxisomes. Definitive diagnosis for all types may require cultured skin fibroblasts for measurement of the levels of VLCFAs and their β-oxidation and, as needed, assay of the peroxisomal steps of plasmalogen synthesis, phytanic acid oxidation, subcellular localization of catalase, enzyme assays, and immunocytochemistry studies. Increasingly, next-generation sequencing panels for the PEX genes and whole exome/genome sequencing are being used for both diagnostic and confirmatory testing.[55,96] DNA study for deletions also has a role in diagnostic evaluation in some cases as demonstrated by the neonatal presentation of cases with deletion of the *ABCD1* gene on the X chromosome.[85,93,94] Diagnostic flow diagrams have been published by Shimozawa et al. and Klouwer et al.[60,85]

Prenatal diagnosis with a variety of methods is available. It can be accomplished in the first or second trimester by biochemical or genetic testing in chorionic villi cells or cultured amniocytes.[54,55,60] Preimplantation genetic diagnosis can be performed when the PEX mutations are known. Carriers cannot be identified by biochemical testing.[54]

One of the more interesting recent developments in peroxisomal diseases is consideration of NBS. The combination of liquid chromatography and tandem mass spectrometry to detect elevated levels of VLCFAs (C26:0-lysophosphatidylcholine) in newborn dried blood spots has been validated as a diagnostic approach for X-ALD.[55] Screening for X-ALD was approved for addition to the Recommended Uniform Screening Panel in 2016 and an increasing number of states are implementing NBS for X-ALD. NBS for X-ALD should also detect the majority of ZSDs, permitting early diagnosis and intervention.[55,57,60]

Management of Peroxisomal Disorders

Treatment for all peroxisomal disorders in the newborn period remains symptomatic and supportive. These disorders are chronic, progressive diseases with no currently available curative therapy. In patients with severe disease, seizure control, feeding, and respiratory support are the main focus of management.[55] Feeding difficulties, including malabsorption, are prominent and may require the use of elemental formulas and/or gastrostomy tube placement. Dietary reduction in VLCFAs has not been shown to reduce plasma VLCFA levels as most VLCFAs are produced endogenously.[55] In patients with X-ALD, dietary reduction of VLCFAs in combination with supplementation with Lorenzo's oil (a 4:1 mixture of glyceryl trioleate and glyceryl trierucate) can reduce plasma VLCFA levels but does not affect progression of already present leukodystrophy.[55,98] Use of Lorenzo's oil has not been studied in ZSDs but may be contraindicated because of the presence of increased levels of dietary monounsaturated fatty acids in patients who already accumulate large amounts of C26:1.[55,60] Because of impaired synthesis of docosahexanoic acid, supplementation with docosahexanoic acid was previously recommended. A placebo-controlled study, however, showed no clinical benefit with supplementation.[99] Also, because of defective bile acid synthesis, supplementation with the fat-soluble vitamins, A, D, E, and K, is recommended.[55] Studies evaluating the effectiveness of bile acid supplementation (cholic acid and chenodeoxycholic acid) are limited, but bile acid supplementation may improve liver function, urine bile acids, and growth, but does not appear to impact extra-hepatic disease manifestations or disease progression.[55,57,90,100] Caution is warranted in individuals with preexisting advanced liver disease due to possible hepatoxic effects.[57] Bezafibrate has been suggested as a possible therapeutic option in DNML1 deficiency.[81] Betaine and arginine have been recognized to be molecular chaperones that can improve peroxisomal assembly and may have a future therapeutic role.[54]

Further supportive care includes use of antiepileptic medications for seizure control, oxygen supplementation as needed for respiratory difficulties, use of hearing aids or cochlear implants for hearing loss, use of glasses for vision difficulties, routine dental care, and routine immunizations. Screening for adrenal insufficiency should occur regularly, and replacement therapy should be started as indicated with stress doses when necessary. Citrate therapy may help prevent renal oxalate stones. Bone density and vitamin D status should be monitored. Comprehensive developmental services should be provided. Treatment guidelines have been proposed and published by Braverman et al.[55]

Hematopoietic stem cell transplantation (HSCT) is the established therapy for the cerebral childhood form of X-ALD, but there are no reports describing HSCT in ZSDs.[60] Use of HSCT was reported in a young child with acyl-CoA oxidase deficiency. It was considered as a possible disease-arresting therapeutic intervention following recognition that the neuropathologic features of acyl-CoA oxidase deficiency resemble those of X-ALD.[87] Despite full engraftment, the child experienced neurodegeneration and died in childhood.[87] Hepatocyte transplantation and orthotopic and living donor liver transplantation have been described in patients with infantile Refsum disease with improvement in biochemical parameters and clinical course.[101–104] Gene therapy may provide future hope.[105,106]

Outcomes of Peroxisomal Disorders

The prognosis for patients with a neonatal-onset peroxisomal disease remains poor, and patients frequently die within the first year of life. Patients with later presentation have a better prognosis but still have progressive disease. Plasma levels of metabolites do not correlate well with disease severity.[60] There is, however, a generally good correlation between the defective PEX gene, the type of mutation, and the impact on peroxisomal assembly and function and the clinical severity.[54]

Smith-Lemli-Opitz Syndrome

Etiology of Smith-Lemli-Opitz Syndrome

Smith-Lemli-Opitz syndrome (SLOS) is a well-recognized autosomal recessive malformation syndrome, with an estimated incidence ranging from 1 in 10,000 to 1 in 70,000 in various populations.[107–109] In 1993, it was discovered that SLOS is caused by a defect in cholesterol biosynthesis that results in low levels of cholesterol and elevated levels of 7-dehydrocholesterol (7DHC) and its isomer 8-dehydrocholesterol (8DHC).[110,111] Patients with SLOS have markedly reduced activity of 7DHC reductase,[112] the enzyme responsible for conversion of 7DHC to cholesterol encoded by the gene *DHCR7*.[113] The cause of the clinical phenotype of SLOS may be related to deficient cholesterol, deficient total sterols, and toxic effects of either 7DHC or compounds derived from it, or a combination of these factors.[114]

Cholesterol is a major lipid component of cellular membranes such as myelin, and it is an important structural component of lipid rafts, which play a major role in intracellular signaling. In animal and in vitro models of SLOS, altered ratios of cholesterol, its dehydrocholesterol precursors, and its derivatives have been noted to alter membrane rigidity, alter electrostatic properties of biologic membranes that can change the activity of ion-dependent adenosine triphosphatases and channels, decrease the stability of lipid rafts leading to increases in degranulation of mast cells, and reduce ligand binding to receptors such as the serotonin 1 A receptor. In addition, bile acids, steroid hormones, neuroactive steroids, and oxysterols are all synthesized from cholesterol, and dehydrocholesterols can also serve as precursors of related steroids, bile acids, and oxysterols that may be antagonists or agonists of the ones derived from cholesterol.[114] Cholesterol is also involved in hedgehog signaling by acting as a cofactor and covalent adduct to hedgehog members.[115] Hedgehog is a family of signaling proteins that are critical to pattern formation through interactions with the homeobox genes during embryonic development, and altered hedgehog signaling could explain some malformations seen in SLOS, such as holoprosencephaly and postaxial polydactyly.[115–117] In addition, 7DHC is highly sensitive to oxidation, and thus increased free radical generation may be a possible contributor to certain aspects of the disease, such as retinal degeneration.[118,119]

Clinical Features of Smith-Lemli-Opitz Syndrome

Classic SLOS is often evident at or before birth; affected patients have prenatal and postnatal growth retardation, microcephaly, and facial dysmorphism, including bitemporal narrowing,

ptosis, epicanthic folds, anteverted nares, broad nasal tip, prominent lateral palatine ridges, retromicrognathia, and low-set ears. Other features include two-three toe syndactyly (found in 95% of patients), small proximally placed thumbs, equinovarus foot deformity, postaxial polydactyly, and cataracts. Males usually have hypospadias, cryptorchidism, and a hypoplastic scrotum but may have ambiguous or female genitalia. Females may have a bicornuate uterus and/or septate vagina. Pyloric stenosis, cleft palate, bifid uvula, pancreatic anomalies, constipation, Hirschsprung disease, renal anomalies, renal calculi, congenital heart defects, lung segmentation defects, anomalies of thymus development, hypothyroidism, and hypoparathyroidism have also been reported. Hypotonia progressing to hypertonia is also present. Feeding difficulties and vomiting are common problems in infancy. Irritable behavior and shrill screaming may also pose problems during infancy. Additional features reported in infancy include pulmonary vein stenosis, adrenal insufficiency with hyperkalemia and hyponatremia, hypocalcemia and hypercalcemia, necrotizing enterocolitis, sepsis-like episodes, and cholestasis with frequent early death.[120–122] Hydrops fetalis has also been reported.[121] Older children frequently have hyperactivity, self-injurious behavior, sleep difficulties, and autistic characteristics. Acute adrenal crisis has been reported as a rare initial presentation of SLOS.[123] Neuroimaging studies and autopsies show defects in brain morphogenesis, including holoprosencephaly, frontal lobes, cerebellum, and brainstem hypoplasia, irregular gyral patterns, and irregular neuronal organization.[114,124]

Historically, approximately 20% of patients die within the first year of life, although others may survive for more than 30 years. The clinical severity in SLOS correlates best with either reduction in absolute cholesterol levels or the sum of 7DHC and 8DHC levels expressed as a fraction of total sterol levels.[125] In one report, a plasma cholesterol level of less than 0.35 mmol/L was associated with early death, but a direct correlation between cholesterol levels and severity of symptoms could not be firmly established.[121] Life expectancy appears to correlate inversely with the number and severity of organ defects and with the kinds and numbers of limb, facial, and genital abnormalities.[126] Developmental outcomes are also highly variable, ranging from severe mental retardation to normal intelligence.[127] Intelligence quotient does not, however, correlate with genotype.[127] Growth is typically lower than in unaffected individuals, and specific growth charts have been developed.[128] In adults, depression and anxiety may manifest themselves, and there has at least been one mildly affected female who has undergone a pregnancy with a good outcome.[129] Testing for SLOS has been suggested for all patients with idiopathic intellectual impairment, behavioral anomalies, or both when associated with nonfamilial two-three toe syndactyly and failure to thrive.[130]

Diagnosis of Smith-Lemli-Opitz Syndrome

Recognition of the biochemical defect in SLOS provided the diagnostic test required to recognize the mildest and severest cases, substantially expanding the clinical spectrum of the condition. Biochemical testing is thus the first-tier choice for the diagnosis of SLOS. The diagnosis is based on findings of elevated levels of 7DHC and 8DHC. False-positive elevations of 7DHC levels occur in patients taking psychoactive medications such as aripiprazole, trazodone, and haloperidol as well as in patients with increased cholesterol synthesis because of bile acid loss after ileal resection.[114] Plasma cholesterol levels are usually low, but cholesterol is a poor diagnostic marker since, and as many as 10% of patients with SLOS have normal cholesterol levels. Also, in many laboratories, measured cholesterol levels include cholesterol as well as 7DHC and 8DHC.[117]

Confirmation of diagnosis through molecular analysis of *DHCR7* is possible and recommended in cases where the serum concentration of 7DHC is difficult to interpret or prenatal or preimplantation genetic diagnosis is desired. Rapid whole genome sequencing is quickly becoming the test of choice for an ill neonate. Phenotype-genotype correlation is poor and patients with the same genotype can have markedly different severity.[131] Modifier genes are likely present, and maternal *APOE* and *ABCA1* genotypes that alter maternoplacental cholesterol transfer appear to modify disease severity.[132,133] If the genotype is unknown but prenatal testing is desired, abnormal levels of 7DHC from amniotic fluid or tissue from chorionic villus samples can be used for prenatal diagnosis, although false-negatives can occur in mild cases. Prenatal sonographic findings of intrauterine growth retardation, increased nuchal translucency, nonimmune hydrops, unusual facial features, cystic hygroma, or major malformations in brain, heart, kidneys, limbs, genitalia, and palate are consistent with SLOS but have low sensitivity and specificity. Maternal serum screening showing low levels of unconjugated estriol, human chorionic gonadotropin, and alpha-fetoprotein is also consistent with SLOS.[124]

Management of Smith-Lemli-Opitz Syndrome

Because of the underlying biochemical defect in SLOS, targeted treatment strategies to date have mainly focused on supplying exogenous cholesterol with the goal of raising cholesterol levels and secondarily lowering 7DHC and 8DHC levels by downregulating the patient's endogenous cholesterol synthesis. Cholesterol is typically given as a dietary modification (egg yolk, breast milk in infants), as a crystalline cholesterol suspension, or as a microencapsulated cholesterol powder with dosing dependent on the formulation ranging from 20 to 300 mg/kg.[117,134,135] Unfortunately, dietary studies on cholesterol supplementation have not been conducted in a randomized fashion except for one short-term study that found no difference in short-term behavior in patients treated with cholesterol supplementation.[136] Case series have reported that cholesterol supplementation in SLOS has improved growth, development, and behavior, increased nerve conduction velocity, and decreased skin photosensitivity, susceptibility to infection, and cholestatic liver disease of infancy when used with and without bile acid replacement. Cholesterol supplementation in SLOS has minimal side effects.[114,124] However, given that dietary cholesterol does not cross the blood–brain barrier and that SLOS cells have impaired intracellular cholesterol transport, the efficacy of cholesterol supplementation is likely limited.[137,138]

Other targeted therapies have also been attempted, but none have been validated by controlled studies. Bile acid replacement has been used with cholestatic liver disease in infancy. Fresh frozen plasma, which contains high levels of cholesterol-rich lipoproteins such as low density lipoprotein (LDL), has been used in acutely ill or severely stressed patients and in the setting of fetal intravenous and intraperitoneal transfusion. Stress steroid dosing has been used when there is evidence of adrenal insufficiency. A 3-hydroxy-3-methyl-glutaryl-CoA reductase inhibitor (simvastatin) has been used to improve cholesterol profiles, but its use had to be stopped in one individual who experienced liver dysfunction.[114] A more recent randomized, double-blind, placebo controlled, crossover

trial of simvastatin in 23 individuals with SLOS was well tolerated and appeared to improve serum DHC/total sterol ratio and significantly improve irritability symptoms.[139] Additionally, there may be a role for antioxidants in SLOS since 7DHC is highly reactive and gives rise to biologically active oxysterols.[140] Direct delivery of cholesterol to the CNS by low-pressure catheter infusions has been proposed but not tested.[141] Gene therapy, the use of neuroactive steroids, and inhibition of glycosphingolipids are also being investigated as possible therapeutic options in SLOS.[142]

Even without proven targeted treatments, appropriate supportive management is important. Following the initial diagnosis, to establish the extent of disease and the needs of the individual, recommended evaluations include the following lengthy list: developmental assessment, ophthalmologic evaluation, cardiac evaluation including ECG and echocardiogram, musculoskeletal evaluation especially for the need for orthotics, genital urinary examination, nutritional assessment, renal ultrasonography, brain magnetic resonance imaging, hearing evaluation, GI evaluation with special effort to evaluate the patient for pyloric stenosis, gastroesophageal reflux, and Hirschsprung disease if indicated, evaluation of immune status, medical genetics consultation, and laboratory studies to evaluate the patient for adrenal insufficiency, thyroid dysfunction, electrolytes abnormalities, and cholestatic liver disease. Referral to early intervention and physical, occupational, and speech therapies is needed in the majority of cases. Surgical interventions, such as gastrectomy tube insertion, surgical repair of cataracts, ptosis, or strabismus, pyloromyotomy, surgical repair of syndactyly or polydactyly, tendon release surgery in cases with significant hypertonia, and tympanostomy may be required in individuals with SLOS. Anesthetic complications of malignant hyperthermia have been reported. Treatment with medications with high affinity for the 7DHC reductase substrate may worsen the biochemical abnormalities so when medications such as haloperidol, trazodone, or aripiprazole are being used, potential benefits need to be weighed against the theoretical risk of worsening the underlying disease. Some infants with severe feeding problems benefit from use of hypoallergenic, elemental formulas. Patients also need to avoid extended periods of sun exposure and use appropriate sun protection measures given the issue with photosensitivity.[124]

Suggested Readings

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32 Immunology of the Fetus and Newborn

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KEY POINTS

- The fetus and newborn express a distinct and evolving immune system that mediates transition from intrauterine life to the microbe- and antigen-rich world.
- Multiple mechanisms including regulatory T cells help ensure maternofetal immune compatibility.
- Newborns are highly reliant on soluble and cellular innate immune mechanisms whose ontogeny depends on gestational and postnatal age.
- Adaptive immunity in newborns features distinct ontogeny and functionality of T and B cells.
- Primary immunodeficiencies that present in early life include genetic defects in innate and adaptive immunity.

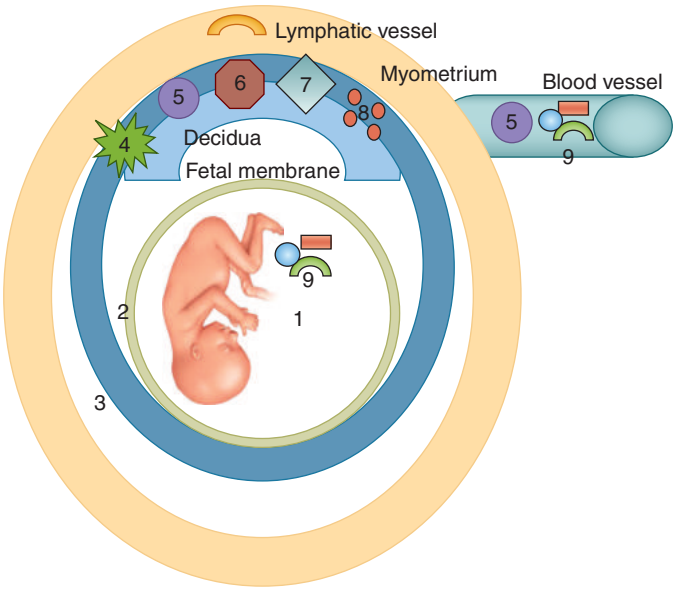
Newborns are at high risk of infection-induced morbidity and death. Understanding the contribution of the newborn's distinct immune response to age-dependent susceptibility to infectious diseases has important implications for efforts to protect newborns from infection and entails review of the immunologic environment of pregnancy and the ontogeny of fetal and neonatal immunity.[1] The distinct functions of fetal, neonatal, and maternal immunity reflect adaptation to developmental challenges such as preservation of fetal well-being as an allogeneic graft versus adequate immunologic protection in the extrauterine environment. These immunologic transitions are regulated by a number of incompletely understood developmental and genetic mechanisms. The diversity and importance of these mechanisms are suggested by the heterogeneity and frequency of neonatal infections. Differences in immunologic responsiveness between newborns and adults are not defects or abnormalities but rather highly regulated ontogenic differences that facilitate transitions between distinct age-specific challenges. Just as the ductus arteriosus, a cardiopulmonary necessity in the intrauterine environment, closes at different rates in different infants, there is variability in the pace at which developmentally and genetically programmed human fetal and newborn immunity changes from graft preservation to identification and destruction of invading pathogens.

Maternal and Placental Immunology

Immunologic tolerance to the growing fetus is a prerequisite for a successful pregnancy. The maternal–fetal interface is a dynamic site that encompasses multiple cellular interactions in an environment rich in cytokines and hormones. While immune mechanisms need to be in place to defend against microbial invasion,[2,3] the placenta is typically programmed to protect the fetus from rejection by the maternal immune system.[3,4]

Several distinct but complementary innate and adaptive immune mechanisms contribute to the commensal immunologic relationship between the mother and the fetus throughout pregnancy (Fig. 32.1 and Table 32.1). Local (placental) and systemic (circulatory) factors mediate maternal tolerance to the fetus.[4] For example, human trophoblasts do not express conventional major histocompatibility complex (MHC) class I human leukocyte antigen (HLA)-A or HLA-B molecules, likely contributing to reduced alloantigenic recognition at the fetal–maternal interface. Human trophoblasts express HLA-C, principally during the first trimester of pregnancy,[5] and two nonclassical HLA molecules, HLA-E and HLA-G. HLA-G class Ib is expressed on extravillous cytotrophoblast and endothelial cells of fetal vessels in the chorionic villi as well as in amnion cells and amniotic fluid.[6,7] Unlike classical MHC molecules, HLA-G does not have a significant role in stimulating $CD8^+$ T cells via the T-cell receptor (TCR) complex. Rather, the principal function of HLA-G molecules expressed by the trophoblast appears to be modulation of the activity of natural killer (NK) cells. HLA-G has additional immunomodulatory properties, including inhibition of activity of cytotoxic T cells, inhibition of alloproliferative responses by $CD4^+$ T cells, and modulation of dendritic cell (DC) maturation and function.[8] These data reveal that the unique MHC class I molecule expression pattern on fetal trophoblast constitutes an intricate mechanism for orchestrating the activity of immune cells.

Other local factors contributing to maternal–fetal tolerance include selective degradation of tryptophan by the inducible enzyme indoleamine 2,3-dioxygenase inhibiting T-cell proliferation[9] and engagement of the proapoptotic molecule Fas on maternal lymphocytes by its ligand (FasL) on interstitial trophoblast cells.[10] FasL is expressed in both maternal and fetal components of the uteroplacental unit throughout gestation. Activated T cells express the Fas receptor, which delivers an apoptotic (death) signal when bound by FasL. Therefore, expression of FasL limits the reciprocal migration of activated fetal and maternal T cells. Mice with a nonfunctional FasL demonstrate leukocyte infiltration and necrosis at the decidual–placental border, with many resorption sites and small litters.[11] Progesterone-induced blocking factor is an immunomodulatory molecule released in response to progesterone by trophoblasts.[12] Its properties include indirect suppression of NK-cell function and inducement of bias of $CD4^+$ T cells toward T_h2-type cytokine secretion.[13]



• **Fig. 32.1** Immunology of the Fetomaternal Interphase. While competent immune cells and bacteria can enter the fetomaternal interphase, multiple complex immune interactions are in place during healthy pregnancy to balance immune defense with immune tolerance between the mother and the allogeneic fetus. Several distinct but complementary innate and adaptive immune mechanisms contribute to the commensal immunologic relationship that exists between the mother and the fetus throughout pregnancy. In humans the decidua represents the uterine implantation site, where maternal blood flows into the intervillous space, "bathing" fetus-derived villous trees composed primarily of cytotrophoblasts. The surface of these villi consists of multinucleate syncytiotrophoblasts mediating nutrient and gas exchange between maternal and fetal tissues. The villi are directly exposed to circulating maternal immune cells. However, these cells do not recognize fetal tissue as "foreign" mainly because human trophoblasts lack classical class I and class II antigens and instead express human leukocyte antigen G (HLA-G). HLA-G and pregnancy hormones suppress natural killer (*NK*) cell and macrophage function and induce a bias of $CD4^+$ T cells toward T_h2-type cytokine secretion. DCs are present in small numbers during pregnancy in decidual tissue but show impaired migration to draining uterine lymph nodes (entrapment). Regulatory T (*Treg*) cells specific to the fetus increase in number in the mother during gestation, and cells maintaining tolerance to fetal antigen can rapidly expand during a subsequent pregnancy. The role of exposure to microbial antigens in fetal immune priming seems important, but the exact mechanism is still being explored.

TABLE 32.1 Local and Systemic Factors Mediating Maternal Tolerance to the Fetus

Factor	Function
Expression of nonclassical HLA molecules (e.g., HLA-G)	Inhibition of NK cells, $CD4^+$ T cells, and cytotoxic T cells, and modulation of dendritic cell maturation and function
Indoleamine 2,3-dioxygenase	Depletes tryptophan and prevents T-cell proliferation
Fas ligand	Apoptosis of activated fetal and maternal T cells
Programmed death 1 and its ligand	Negative regulator of T-cell responses
Galectins	Apoptosis of activated fetal and maternal T cells
Decay-accelerating factor	Control of complement activation
Cytokines	T_h2 bias prevents immune activation
Decidual macrophages	Suppressing immune activation
Decidual NK cells	Suppressing immune activation
$FoxP3^+$ regulatory T cells	Suppressing immune effector cells (e.g., in response to paternal antigens)
Microbiome	Balanced immune response

FoxP3, Forkhead box P3; *HLA*, human leukocyte antigen, *NK*, natural killer.

Galectins are expressed in human placenta primarily by the syncytiotrophoblast early in pregnancy.[14] On cell surface contact, galectins downregulate the cellular immune response, in part by inducing programmed cell death (apoptosis) of T lymphocytes.[15]

On a cellular level, DCs, an important type of antigen-presenting cell (APC) critical for cellular and humoral immune responses, play a prominent role in organ transplant rejection. The potential deleterious actions of DCs against the fetus may be curtailed by at least two factors: the progressive decline of decidual DC tissue densities shortly after implantation and impaired migration from decidual tissue to draining uterine lymph nodes. This entrapment of DCs during pregnancy may reflect both disappearance of lymphatic vessels during decidualization[16] and stromal cell-based processes limiting chemokine-directed cell migration.[17] Other important cellular factors that may limit potential anti-fetal immune responses include the immunosuppressive phenotype of decidual macrophages[18] and decidual NK cells.

Complement inhibition is essential for normal pregnancy in a murine model of antiphospholipid syndrome, an autoimmune condition characterized by thrombosis, thrombocytopenia, and recurrent fetal loss. In this model, fetal injury results from placental inflammation initiated by local dysregulation of complement proteins. Both complement activation and fetal loss can be prevented by administration of anticoagulants with complement-inhibitory properties such as heparin but not by anticoagulants lacking complement-binding properties.[19,20] Some but not all human clinical interventional studies using anticoagulants with complement-binding properties to prevent fetal loss in antiphospholipid syndrome have suggested benefit.[21] Control of complement activation during human pregnancy is achieved by expression of decay-accelerating factor, membrane cofactor protein, and CD59 (protectin) on the trophoblast membrane,[22] as well as high reproductive tract and systemic prostaglandin E_2 levels contributing to maternal immune tolerance to the fetus.[23]

In addition to local components, systemic elements are in place to maintain immune tolerance at the maternal–fetal interface. Abatement of certain autoimmune diseases during pregnancy[24] and an increased risk of infections[25] provide evidence for a broadly immunosuppressive state during pregnancy.[26] Although the precise mechanisms underlying this phenomenon are incompletely characterized, several reproductive hormones may play critical roles. For example, a relatively large study demonstrated that 48% of

patients with at least moderate rheumatoid arthritis showed signs of remission in the first trimester of pregnancy, while ~40% had a disease flare-up in the postpartum period.[24] The rate of relapse in multiple sclerosis declines during pregnancy,[27] and treatment with pregnancy levels of estriol significantly reduced enhancing lesions on brain imaging.[28] In addition, estrogen (17β-estradiol) in concentrations typically expressed during normal pregnancy augments forkhead box P3 (FoxP3) expression and expansion of T regulatory (Treg) cells in vitro and in vivo.[29,30]

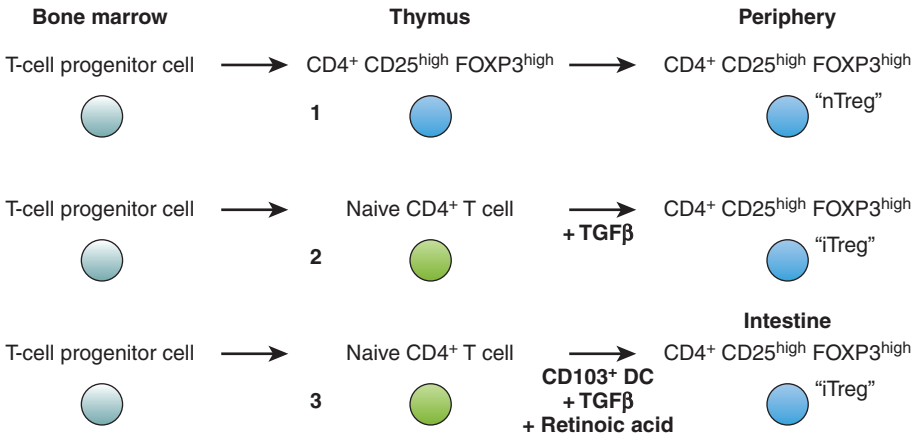
T cells and T-cell–derived cytokines play a central role in immune regulation and inflammation. T_h1 cells are involved in cellular immunity and transplant rejection and are characterized by production of interleukin (IL)-2, interferon gamma (IFN-γ), and tumor necrosis factor alpha (TNF-α). In contrast, T_h2 cells are mediators of humoral immunity and produce IL-4, IL-5, and IL-13. Traditional dogma holds that a bias in T-cell cytokine secretion toward T_h2-type cytokines and away from T_h1-type cytokines is an immunologic condition necessary for maintaining healthy pregnancy.[31] For example, IL-10 is a pregnancy-compatible cytokine that plays a vital role in maintaining immune tolerance.[32] For example, IL-10 induces expression of HLA-G on trophoblasts, which has direct and indirect immune suppressive effects as described above.[33] In vitro, IL-10 suppresses macrophage activity and $CD4^+$ T-cell proliferation and can convert DCs and conventional T cells into a tolerogenic state.[34] In human pregnancy, lower levels of serum IL-10 are associated with pregnancy complications such as spontaneous miscarriage, preeclampsia, and fetal growth restriction.[35]

Significant shifts in the T_h1/T_h2 balance have been associated with various immune-mediated pregnancy complications. However, the emerging role of NK cells and cytokines such as IL-12, IL-15, IL-18, IL-19, and IL-20 suggest that the T_h1/T_h2 paradigm may be oversimplified.[36,37] For example, a subset of CD4 T cells that produce IL-17A and IL-17F, called *T_h17 cells*, may be involved in rejection of the fetus.[38,39] Another molecule with an immunomodulatory effect on multiple cell types present in the decidua is TGF-β. TGF-β suppresses APCs and decidual NK cells, while promoting the development of regulatory T cells.[34] Serum concentrations of TGF-β are elevated in pregnant women and may protect them from spontaneous abortion.[40]

Role of Regulatory T Cells in Pregnancy

The discovery of a distinct lineage of T lymphocytes with dominant immunosuppressive properties[41] provided a significant breakthrough in understanding the mechanisms whereby adaptive allogeneic responses against paternal antigens are actively suppressed during pregnancy. This type of Treg cell is characterized by expression of a lineage-specific transcription factor, FoxP3 (Fig. 32.2). Upon activation via their antigen-specific TCR, Treg cells suppress immune effector cells, including DCs and effector T cells via a variety of mechanisms,[42] thereby preventing a fatal form of autoimmune disease throughout life.[43] Treg cells and T_h17 cells are two distinct lymphocyte subsets with opposing actions and share a complex relationship. Treg cells have a role in suppressing autoimmune responses and preventing the rejection of the fetus, and a decrease in Treg cell number is associated with miscarriage. In contrast, T_h17 cells promote inflammation, transplant rejection, and autoimmunity, and increases in T_h17 cell numbers and decreases in Treg cell numbers are associated with recurrent miscarriage.[38,39,44] The numbers of Treg cells specific to the fetus increase in the mother during gestation, and cells maintaining tolerance to fetal antigen can rapidly expand during subsequent pregnancy.[45] Paternal antigens may induce expansion of Treg cells, likely contributing to maternal tolerance to the allogeneic fetus.[46] Maternal and fetal Treg cells are essential in promoting fetal survival by avoiding the recognition of paternal semi-allogeneic tissues by the maternal immune system.[44,47]

In addition to $FOXP3^+$ Treg cells, $FoxP3^-$ $HLA\text{-}G^+$, Tr1, and T_h3 Tregs, $CD8^+$ Treg cells, nitric oxide (NO) induced FoxP3– Tregs, $TIGIT^+$ Tregs, $FoxP3^{dim}$ Tregs, and gamma/delta T cells have been reviewed.[34] Similar to $FOXP3^+$ Tregs, these cells via



• **Fig. 32.2** Forkhead Box P3–Positive Regulatory T-Cell Subpopulations and Their Development. Most human regulatory T (*Treg*) cells in peripheral blood originate from the thymus and are typically called *natural Treg* (*nTreg*) or *thymus-derived Treg cells* (*1*). Treg cells can originate from naive T cells in the periphery and are induced to express the identifying transcription factor forkhead box P3 (*FOXP3*) under the influence of transforming growth factor beta (*TGFβ*), at least in vitro (*2*). The Treg cells are often called *induced Treg* (*iTreg*) *cells*. Most Treg cells in the intestinal mucosa are thought to be iTreg cells originating from naive T cells in the mucosal immune system under the influence of specific antiinflammatory dendritic cells ($CD103^+$ dendritic cells), TGFβ, and retinoic acid (*3*). The iTreg cells play an important role in pregnancy maintenance. *DC*, Dendritic cell.

production of soluble factors such as soluble HLA-G, IL-10, or TGF-β or costimulatory molecules, such as programmed death 1 (PD1). The interaction between PD1 and its ligand (PDL1) plays important roles in maintaining tolerance at the fetomaternal interface. PDL1 is expressed on the trophoblasts of the placenta, and PD1 is expressed on the maternal effector T cells and Treg cells. PDL1 expression maintains Treg cell/effector T-cell ratios and suppresses increases in the number of T_h17 cells.[48] Blockade of PDL1 signaling in animal models results in fetal rejection.[49] The role and ontogeny of fetal Treg cells is discussed more fully in the section entitled Adaptive Immunity.

Role of the Microbiome

The paradigm of a sterile uterus postulates that the fetus develops free of bacteria and antigenic agents.[50] However, bacteria can be cultured from amniotic fluid and fetal tissues in pregnancies complicated by preterm labor even without rupture of membranes.[51] Bacterial DNA was detected in the intestines and placentas of fetal mice at late gestation (day 17).[52] Analysis of human fetal tissues in the second trimester detected microbial signals in fetal gut, skin, placenta, and lungs as well as live bacteria that were able to induce the activation of memory T cells in the fetal mesenteric lymph node.[53] Of note, no microbial signatures were detected in fetal meconium, in contrast to postnatal first-pass meconium, suggesting that microbial colonization of the intestine occurs predominantly during and/or immediately after birth.[54] While fetal intestinal samples did not contain detectable bacterial DNA, a metabolomic intestinal profile was noted with an abundance of bacterial metabolites and aryl hydrocarbon receptor (AHR) ligands implicated in mucosal immune regulation.[55] While not yet widely accepted in humans, maternal microbial transmission to the fetus is a universal phenomenon in animals and even plants, likely constituting an essential evolutionary act of symbiosis.[56,57] The exact mechanisms by which bacterial antigens may pass from the mother to the fetus are being investigated. Placental bacteria resemble most closely the human oral microbiome,[2] suggesting hematogenous bacterial transfer. In mice, the majority of fetal gut genera overlapped with placental, maternal oral, and vaginal taxa but not with maternal or newborn feces.[52]

Globally, these studies imply a possibly critical role of maternal bacteria to inform normal immune development of the developing fetus. The importance of fetal programming has been well described for cardiovascular and metabolic diseases and is now also considered in the context of environmentally influenced immune-mediated diseases. Initial exposure of bacterial molecular patterns to the fetus in utero may prime the immune system and/or the epithelium to respond appropriately to pathogens and commensals after birth.[58,59] For example, maternal exposure to farm animals during pregnancy was associated with greater Toll-like receptor (TLR) gene expression and lower risk of atopic sensitization in children.[60] Similar protective effects against atopic sensitization were observed after dietary interventions during pregnancy, such as maternal supplementation with fish oil[61] or probiotics.[62] In contrast, antibiotic use in pregnancy was associated with asthma during the fifth year of life.[63]

The mechanism underlying prenatal immune priming is unknown. In an experimental asthma model, microbial exposure to pregnant mice resulted in epigenetic changes in promoter regions of cytokines associated with an allergic phenotype-increased expression of IFN-γ and reduced expression of IL-4, IL-5, and IL-13.[64] This concept has been confirmed in human studies where exposure to farms during pregnancy has been associated with increased DNA demethylation of the FoxP3 locus and increased number and function of Treg cells in UCB cells.[65]

Maternal dietary factors may play an important role in microbiome-associated fetal immune education. For example, short chain fatty acids derived from fermentation of dietary fiber by intestinal microbes of the pregnant mother increased fetal Treg cells and protected offspring from inflammatory diseases, including asthma and metabolic syndrome.[66–68] Maternal gut colonization during pregnancy programs transcriptional profiles of the offspring that not only decrease susceptibility to inflammation but can also strengthen innate immune defenses, such as the integrity of the intestinal epithelial barrier. Some of these effects seem dependent on maternal antibodies that potentially retain microbial molecules and transmit them to the offspring during pregnancy.[69]

Despite growing evidence of the importance of the maternal microbiome on fetal immune regulation, much remains to be learned regarding the molecular mechanisms strengthening fetal immunity while at the same time promoting tolerance and anti-inflammatory acceptance of the antigen-rich postnatal environment.

Effect of Chorioamnionitis on the Developing Fetal Immune System

To enable initiation and maintenance of pregnancy, the intrauterine environment significantly shapes the developing immune system as is evident from the anti-inflammatory cytokine profile and protection from atopic sensitization in offspring after maternal exposure to farming activities and farm dairy products during pregnancy.[70,71] This effect is at least in part mediated through an increase in the number of fetal Treg cells.[65]

In contrast, fetal exposure to inflammation during critical developmental windows can influence immune programming to augment inflammatory neonatal responses. Histologic chorioamnionitis (HCA) is a common complication of pregnancy, typically caused by intrauterine bacterial infection and defined by inflammation of the fetal membranes. Fetal exposure to HCA induces immune activation, resulting in fetal inflammatory response syndrome (FIRS), and shapes the neonatal transcriptomic immune response.[72] The clinical characteristics of FIRS consist of systemic inflammation and elevation of fetal plasma IL-6 and other proinflammatory cytokine levels.[73] Long-term sequelae of the sustained systemic inflammation precipitated by fetal exposure to HCA include blindness,[74] cerebral palsy,[75] impaired cardiac function,[76] lung disease,[77] and disruption of normal fetal immune development.[78–82] In humans, placental infection, chorioamnionitis, or villitis together with a fetal inflammatory response appear to increase the risk of surgical necrotizing enterocolitis (NEC).[83]

Studies of fetal sheep and human UCB have demonstrated activation of the adaptive immune system following exposure to HCA. In a model of chorioamnionitis and FIRS caused by administration of intra-amniotic lipopolysaccharide (LPS) in rhesus monkeys at approximately 80% of gestation, fetal Treg cell generation in the thymus was inhibited, while the concentration of proinflammatory cells in the spleen increased.[84] The immunologic changes associated with endotoxin-induced systemic and organ-specific immune priming in the fetus can be mimicked by administration of IL-1α or IL-1β, suggesting a possibly important role of IL-1 receptor signaling in FIRS. In a similar model using intra-amniotic injection of LPS 7 or 14 days before preterm delivery in fetal sheep, involution and activation

of the fetal thymus with structural organ changes was observed.[85] Furthermore, UCB derived from human neonates with clinical evidence of perinatal infection exhibited a higher proportion of T_h1 cells than UCB from uninfected neonates.[86] There is evidence that fetal immune activation persists at least for weeks after birth. $CD4^+$ T cells isolated from preterm blood 10 days post-partum showed altered metabolomic activity and a strong T_h1-biased immune profile.[87]

Overall, epidemiologic and experimental data point to the central role of maternal immune activation and/or FIRS in the pathogenesis of many immune-mediated complications of prematurity such as chronic lung disease, brain damage, retinopathy of prematurity, gut injury, and behavior abnormalities. On the other hand, prenatal immune activation may improve vaccine responses and render the newborn more resistant to infectious challenges later in life.[88]

Developmental Fetal–Neonatal Immunology

Newborn and young infants, especially those born preterm, are at increased risk of developing a range of bacterial and viral opportunistic infections. This age-dependent susceptibility is in part based on immune ontogeny.[1,89] In the past few decades research has focused on the molecular, cellular, and functional bases for immunologic differences between newborns and older individuals, which we discuss as they relate to innate and adaptive immunity.

Innate Immunity

During fetal/newborn adaptation from the intrauterine environment to the colonization of skin and mucosal surfaces following birth, the innate immune system shields the newborn from infection while orchestrating the acquisition of protective adaptive immune responses.[90] These innate mechanisms evolve across gestation and postnatal age (Fig. 32.3) and include protective barriers such as the vernix caseosa, which contains antimicrobial proteins and peptides (APPs) and microbicidal fatty acids,[91] developmentally controlled functional regulation of TLR signaling,[92] expression of acute-phase reactants (Fig. 32.4)[90] and complement proteins, and alterations in neutrophil and monocyte function.[93,94] Importantly, functional maturation of innate immunity enables colonization with commensal organisms while limiting potentially dangerous inflammatory responses.[1] Herein we discuss key features of innate immunity in early life beginning with soluble-based defense systems that progress to leukocyte-based defense systems.

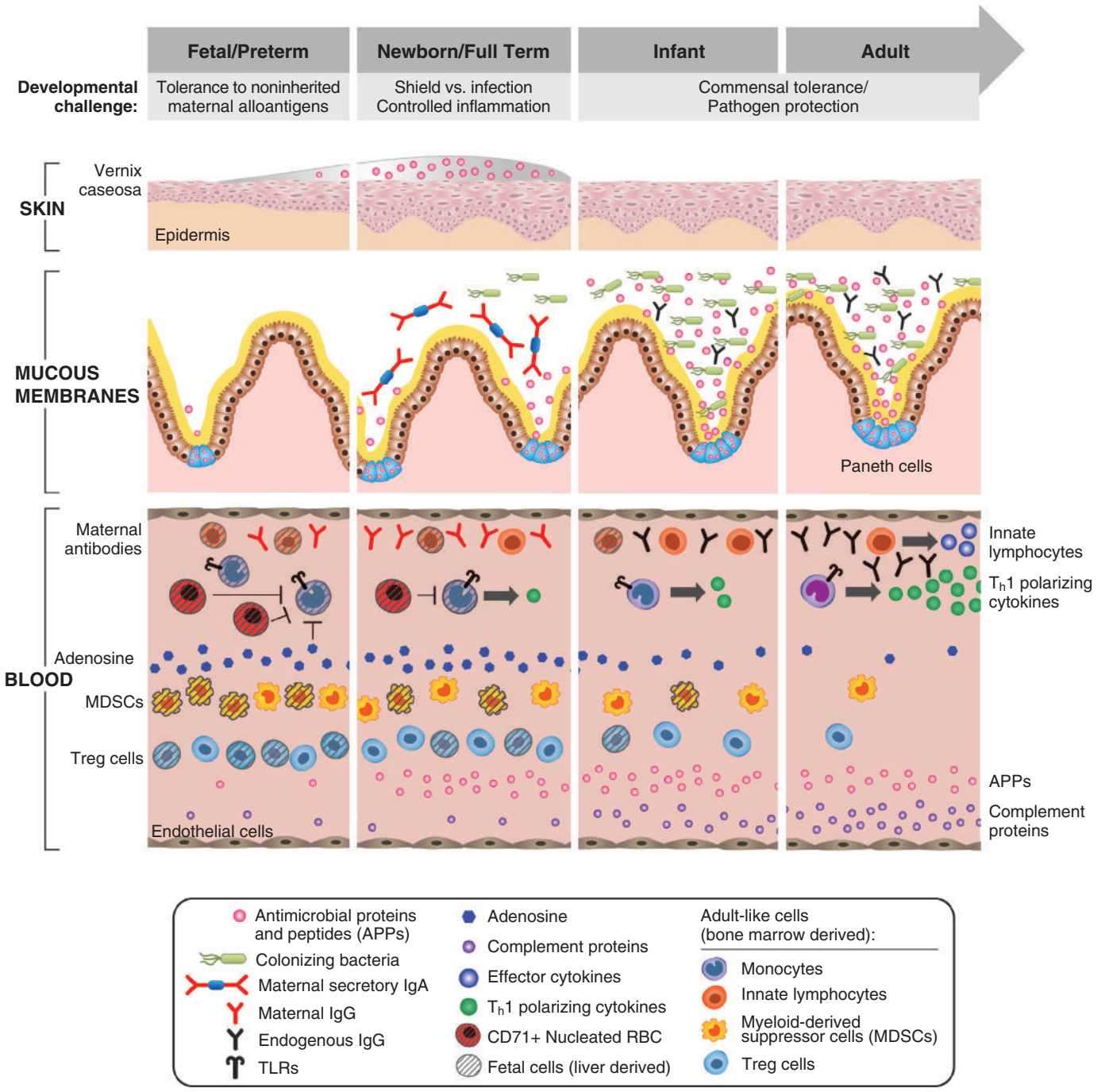
Complement

Central to the innate immune response is the complement system that consists of >40 plasma, cell surface, and regulatory proteins that interact to regulate multiple physiologic functions, including resistance to pyogenic infections, interaction between innate and adaptive immunity, and elimination of immune complexes, products of inflammatory injury, and apoptotic self cells.[95] Components of the complement system recognize and lyse bacteria, opsonize microorganisms, release anaphylatoxins, solubilize immune complexes, and induce B-cell proliferation and differentiation.

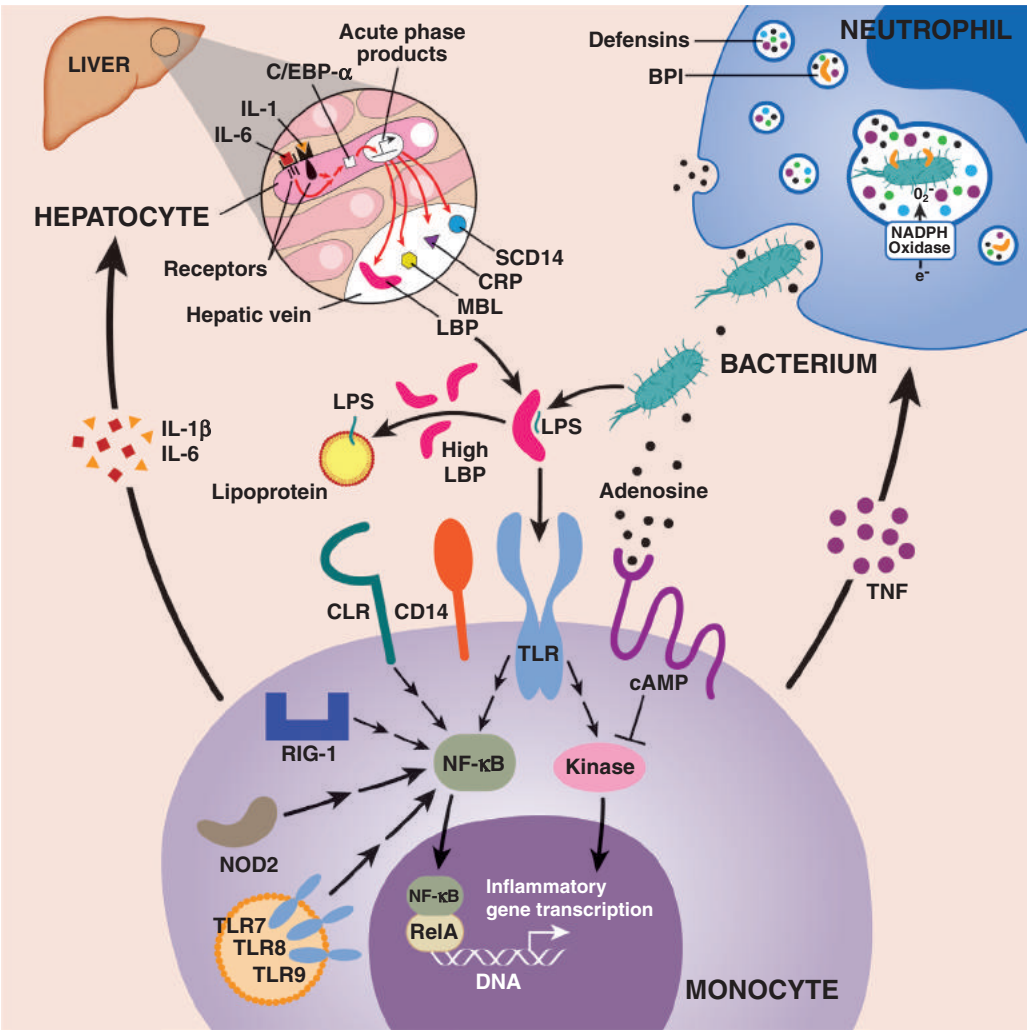
Activation of the complement cascade occurs via three pathways—classical, lectin, or alternative.[95,96] Several characteristics of the complement cascade are important for fetal–neonatal immunity. First, the complement system features both antibody-dependent specificity via the classical pathway activation triggered by interaction of antigens with antibodies, and antibody-independent activation of the alternative and lectin pathways initiated by pathogen-associated structures such as endotoxin and polysaccharides. Thus for the fetus or infant who has not received from the mother or has not yet produced antigen-specific immunoglobulin (Ig) G for immunologic recognition, the alternative and lectin pathways may be critical for triggering the effector functions of the complement cascade.[97,98] Second, the enzymatic activation of the complement cascade enables rapid functional amplification: deposition of a single Ig molecule or C3b fragment can generate enzymatic cleavage of thousands of later-acting components and thus multiple complement activities.[95,99] In addition, the alternative pathway can be amplified via a positive feedback activation mechanism, because C3b, an activation product of the alternative pathway C3 convertase, is a component of this convertase.[100] As the fetus and newborn are particularly dependent on antibody-independent pathogen recognition for immunologic responsiveness, the positive amplification loop of the alternative pathway may be particularly critical for rapid generation of complement effector functions in early life in the absence of antibody-based recognition. Third, the continuous activation of the alternative pathway requires rigorous regulation in the fetus to avoid tissue damage during organ remodeling.[101] Finally, the contributions of the lectin pathway to fetal–neonatal complement activation and fetal well-being are still under investigation.

Studies of fetal and neonatal complement have focused on quantification of serum concentrations of individual components, examining maternal–fetal transport of these proteins, assessing specific effector functions of the classical and alternative pathways, and investigating contributions of complement activation to common neonatal diseases. Detectable concentrations of C3 (1% of adult levels) and C1 inhibitor (20% of adult levels) can be measured as early as 5 to 6 weeks' gestation.[102] By 26 to 28 weeks' gestation, both C3 and C1 inhibitor concentrations increased to 66% of adult levels. Functionally and immunochemically measured classical and alternative pathway protein concentrations in UCB increase with advancing gestational age, such that impairment in CH50 is particularly evident in the preterm,[103] and at full-term gestation the concentrations are only approximately 50% to 75% of adult concentrations.[104,105] Although neonatal UCB lectin pathway component concentrations are lower than those in older children and adults, the correlation between mannose-binding lectin (MBL) and gestational age has not been consistently observed.[106,107] Of note, on the basis of studies of genetically determined, structurally distinct complement variants in maternal and umbilical cord serum, no transplacental passage from the mother to the fetus of C3, C4, factor B, or C6 has been observed.[108]

Much remains to be learned regarding regulation of complement effector functions in the fetus and newborn. Activation of the alternative pathway or the lectin pathway enables opsonization of invading microorganisms without specific Ig recognition. Accordingly, for preterm infants or those without organism-specific maternal IgG, alternative or lectin pathway activation provides a critical mechanism for engaging complement effector functions.[107,109,110] The functional contribution of the classical pathway to effector functions has been assessed through the use of blood-mediated opsonophagocytosis by polymorphonuclear leukocytes of group B streptococci (GBS) type Ia.[111] This GBS serotype may be opsonized by classical pathway components in the absence of specific antibodies and thus enables characterization of classical pathway function. In 8 of 20 neonatal



• **Fig. 32.3** Ontogeny of Skin, Soluble, and Cellular Innate Defense Systems. Host-protective barrier functions include physical, chemical, and functional components of the skin and mucous membrane epithelia of the fetus, neonate (birth to 28 days of age), and infant (1 month to 1 year of age). Skin: while physical and chemical barriers are impaired in early life, especially in the preterm newborn, the vernix caseosa and skin epithelia of full-term newborns robustly express antimicrobial proteins and peptides (*APPs*). Mucous membranes: in parallel with and induced by an increasingly complex microbiota, the newborn intestinal mucosal epithelium rapidly changes structurally, with an increase in the population of crypts and crypt-based Paneth cells, as well as functionally with increasing APP expression. Blood: the composition of neonatal blood is distinct, with relatively low concentrations of complement components and APPs and high concentrations of the immunosuppressive purine metabolite adenosine. Plasma also contains maternal antibodies that are transferred beginning midgestation and supplemented by postnatal factors derived from breast milk. Innate immunity is detectable from the end of the first month of gestation, with changes driven largely by the increasing exposure to environmental microbes. Neonatal antigen-presenting cells such as blood monocytes express pattern recognition receptors (e.g., Toll-like receptors, *TLRs*) with distinct functional responses, including limited T_h1-polarizing cytokine production, to most stimuli. Adaptive immunity develops from 4 weeks of gestation onward, with changes driven by an evolving chimerism reflecting fetal (liver-derived, *shaded cells*) regulatory T (*Treg*)-cell–rich lymphocytes, and more adultlike (bone marrow derived, *unshaded cells*) lymphocytes with distinct epigenetically encoded functional programs. *Ig*, Immunoglobulin; *RBC*, red blood cell. (Modified from Kollmann TR, Kampmann B, Mazmanian SK, et al. *Protecting the newborn and young infant from infectious diseases: lessons from immune ontogeny*. Immunity. 2007;46:350–363.)



• **Fig. 32.4** Innate Detection, Signaling, and Effector Functions of Blood Phagocytic Leukocytes and Hepatocytes. Innate immune signals are detected via signaling loops. Monocytes express pattern recognition receptors, including C-type lectin receptors (*CLR*s), CD14, and Toll-like receptors (*TLR*s). TLR-mediated monocyte activation engages signaling pathways resulting in kinase and nuclear factor κB (*NF-κB*) pathway activation, culminating in inflammatory gene transcription, including generation of cytokines that amplify an antiinfective response. For example, interleukin (*IL*)-1β and IL-6 engage cognate cytokine receptors on hepatocytes, inducing acute-phase response, including production and secretion of lipopolysaccharide (*LPS*)-binding protein (*LBP*), mannose-binding lectin (*MBL*), the pentraxin C–reactive protein (*CRP*), and soluble CD14 (*SCD14*). These molecules recognize and modulate inflammatory activity of microbial products. For example, LBP at low concentrations delivers LPS to TLR4, thereby enhancing signaling but at higher LBP concentrations detoxifies LPS by delivering it to plasma lipoproteins. Monocyte production of tumor necrosis factor (*TNF*) activates neutrophils that deploy antimicrobial systems that are oxygen dependent, such as nicotinamide adenine dinucleotide phosphate (*NADPH*) oxidase, as well as those that are oxygen independent, such as membrane-active antimicrobial proteins and peptides, including defensins and bactericidal/permeability-increasing protein (*BPI*), an LBP homologue that binds to and neutralizes endotoxin. Soluble mediators, such as the antiinflammatory purine metabolite adenosine, can limit proinflammatory responses. *cAMP*, Cyclic adenosine monophosphate; *C/EBP-α*, CCAAT/enhancer binding protein; *NOD2*, nucleotide-binding oligomerization domain-containing protein 2; *RelA*, NF-κB subfamily protein; *RIG-1*, retinoic acid-inducible gene 1.

serum samples examined, decreased bactericidal activity was detected and correlated with significantly lower functional activity of C1q and C4. These studies did not determine whether this decrease was mediated by an inhibitor of function or by an intrinsic change in functional activity of these components in neonatal sera. Studies of MBL concentrations and pathway activity suggest a contribution of the lectin pathway to neonatal susceptibility to infection.[97,107,112–114] Complement regulatory proteins (e.g., C4b-binding protein and factor H) also contribute to neonatal susceptibility as suggested by the failure of neonatal serum to reduce invasion by GBS and *Escherichia coli* into human brain microvascular endothelial cells.[109] In vitro experiments in which killing of *E. coli* by neonatal serum samples was limited by C9, but not by other classical pathway components, suggest that this terminal complement component is apparently important for cytolysis of this pathogen.[115,116] Although relatively

lower concentrations of complement components likely contribute to poor control of bacterial replication, these complement concentrations are nevertheless sufficient, via C3- and factor B-dependent activity in the alternative pathway, to enhance GBS-induced production of TNF-α by monocytes in human newborn UCB tested in vitro.[117]

In addition to relatively low serum concentrations of classical, alternative, and lectin pathway complement proteins, additional complement functions important for fetal and neonatal well-being can contribute to reduced capacity to activate the classical and alternative pathways. For example, fetal and neonatal serum demonstrates reduced concentration of C4b-binding protein, a critical regulator of classical pathway C3 convertase activity.[118–121] Lower C4b-binding protein concentration increases the functional anticoagulant activity of protein S, with which it complexes and thereby contributes to decreased coagulation function of the fetus and newborn. Consideration of complement components that also express nonimmunologic functions will likely be important in characterizing developmental regulation of complement component production.

Complement activation contributes to tissue injury in several common neonatal diseases, including neonatal hypoxic–ischemic encephalopathy, NEC, meconium aspiration syndrome, and intrauterine growth restriction and fetal loss.[122–127] Unregulated complement activation may occur in selected infants undergoing extracorporeal membrane oxygenation therapy, raising concern for inflammatory injury on that basis.[128,129] C5a is present in the cerebrospinal fluid of human newborns, at especially high concentrations in those born preterm,[130] raising the possibility that complement activation in the neonatal brain may contribute to preterm brain injury.

Overall, study of the complement system in early life, including characterization of the developmental and genetic regulation of this important group of plasma and cell surface proteins, promises to shed further light on immune ontogeny in relation to health and disease.

Antimicrobial Proteins and Peptides

The human body expresses natural antibiotics, including APPs that act alone and in combination with endogenous (e.g., complement) and exogenous (e.g., conventional antibiotic) systems to prevent infection and/or eliminate invading microorganisms.[131] APPs are expressed by a range of cells, including epithelial cells and leukocytes, especially neutrophils (see Fig. 32.4), and are found associated both with cells and in plasma. Plasma levels of APPs vary with gestational age such that preterm plasma is relatively deficient in multiple APPs, likely contributing to reduced microbicidal capacity (see Fig. 32.3).[132] Examples of APPs include (1) lactoferrin, an 80-kDa protein with iron-binding and direct membrane perturbing properties found in tear fluid, saliva, and neutrophil secondary granules, (2) the 5-kDa bactericidal/permeability-increasing protein (BPI), expressed on certain mucosal epithelia as well as neutrophil primary granules, with high affinity for LPS that enables it to neutralize the inflammatory activity of endotoxin and targets its microbicidal activity toward Gram-negative bacteria, (3) 14-kDa phospholipase A_2, an acute-phase reactant expressed in liver with ability to enzymatically kill a range of gram-positive pathogens, and (4) 4-kDa disulfide-rich defensin peptides of neutrophil primary (azurophilic) granules with broad microbicidal activity.[131] Ongoing efforts are aimed at developing congeners of APPs as novel antiinfective agents for individuals who are relatively APP deficient, including preterm infants and those undergoing chemoradiotherapy.[133] For example, oral administration of lactoferrin to human preterm newborns has shown promise in reducing the incidence of sepsis and NEC.[134]

Innate Lymphoid Cells, Including Natural Killer Cells

Innate lymphoid cells (ILCs) are derived from a common lymphoid progenitor, are defined by the absence of antigen-specific B-cell receptors (BCRs) or TCRs, and do not express myeloid or DC markers.[135] ILCs are divided into subgroups based in part on the cytokine profile they produce: (1) group 1 ILCs produce IFN-γ and are functionally dependent on the transcription factor T-bet; (2) group 2 ILCs produce type 2 cytokines (e.g., IL-4, IL-5, IL-9, and IL-13) in response to helminth infection and are dependent on RORα and GATA3; and (3) group 3 ILCs produce IL-17A and/or IL-22 and are dependent on the transcription factor RORγt.

NK cells are the most studied of the ILCs. These group 1 ILCs constitute approximately 10% to 15% of all peripheral blood lymphocytes. They are present in the spleen, lungs, and liver and are also rarely found in lymph nodes and thoracic duct lymph.[136] NK cells represent up to 70% of all lymphocytes in the maternal decidual tissue.[137] They demonstrate distinct morphology, function, and surface molecule expression, including expression of CD16 (Fc gamma receptor FγRγIII) and CD56 (nerve cell adhesion molecule 1). Mature NK cells appear larger and more granular than T or B cells[138] and express both activating and inhibitory receptors that are used to selectively identify and kill virally infected cells and tumors.[139] The presence of MHC class I molecules on potential target cells induces signals that suppress NK-cell function. MHC class I–deficient target cells activate NK-cell function, triggering release of lysosomal granules containing serine proteases, perforin, and transforming growth factor beta (TGF-β), thereby disrupting the target cell membrane and inducing an inflammatory response. Fetuses and neonates demonstrate reduced NK-cell activity compared with adults.[140,141]

NK cells are derived from a common hematopoietic progenitor that retains T-cell and B-cell developmental potential.[142] NK cells first make their appearance in fetal liver as early as 6 weeks' gestation. Committed $CD34^+CD56^-$ NK cell progenitors have been identified in the fetal thymus, bone marrow, and liver. In the human neonate, the NK-cell population is immature: only half of all NK cells express CD56, and the NK-cell cytolytic activity is lower.[143] This functional reduction in NK-cell activity may contribute to the severity of neonatal herpes simplex virus (HSV) infections. Profound defects in NK-cell activity result in familial hemophagocytic lymphohistiocytosis (HLH), a disease characterized by fever, hepatosplenomegaly, cytopenia, hyperferritinemia, and hemophagocytosis. Familial HLH arises from mutations in genes that encode proteins involved in the granule-exocytosis pathway and can be fatal without bone marrow transplant.[144,145]

NK-cell receptors are fundamentally different from TCRs and BCRs. NK-cell receptor gene expression does not require gene segment rearrangement, and the receptors are not clonally distributed. Instead, NK cells use an array of stimulatory and inhibitory receptors to regulate their cytolytic functions.[146] A cluster of 10 or more genes encoding killer-cell Ig-like receptors (KIRs) is located on human chromosome band 19q13.4.[139] Each of these type I glycoproteins recognizes a different allelic group of HLA-A–, HLA-B–, HLA-C–, or HLA-G–encoded proteins, and each KIR is expressed by only a subset of NK cells. Another family of

Ig-like receptor genes termed *ILT* is present near the KIR locus at 19q13.3. These receptors are not as restricted as the KIRs and bind multiple HLA class I molecules. A third inhibitory receptor gene locus is on chromosome band 12p12-p13, encoding a C-type lectin inhibitory heterodimeric receptor called *CD94/NKG2* that binds HLA-E. Those KIRs, ILT receptors, and CD94/NGK2 molecules with long cytoplasmic tails and two immunoreceptor tyrosine-based inhibitory motifs (ITIMs) function as inhibitory receptors. On phosphorylation, the two ITIMs recruit and activate the Src homology domain 2 (SH2)-containing phosphatases, which turn off the kinase-driven activation cascade.[147] The KIR family member KIR2DL4 is distinct from other KIRs in structure and distribution. KIR2DL4 binds HLA-G and has a single ITIM in the cytoplasmic tail and a lysine in the transmembrane region, enabling association with adaptor proteins. This inhibitory receptor was found on all decidual NK cells in the placenta at term but not on circulating maternal NK cells, suggesting that expression of KIR2DL4 is induced during pregnancy.[148]

Other KIRs or members of the C-type lectin receptor superfamily are activating receptors.[149] These receptors lack the long cytoplasmic tail of the inhibitory receptors and therefore do not contain ITIMs. Instead, they have a charged amino acid in the transmembrane region that enables receptor association with the adaptor molecule DAP12.[150] This adaptor contains an immunoreceptor tyrosine-based activation motif (ITAM) that allows these receptors to activate NK cells. The physiologic role of these HLA class I–specific activating receptors remains unknown. NK cell–activating receptors also include *natural cytotoxicity receptors* (NKp46, NKp30, NKp44), proteins that are Ig superfamily members with little similarity to one another or to other NK-cell receptors.[149] These receptors are highly specific for NK cells and apparently interact with non-HLA molecules.

CD244 (2B4) is a member of the signaling lymphocyte activation molecule (SLAM) family of receptors expressed on all human NK cells.[151] On interaction with the ligand CD48 on target cells, NK-cell signaling proceeds via interactions between the immunoreceptor tyrosine-based switch motif (ITSM) (switch motif) in the cytoplasmic tail of CD244 and one of two SH2 domain-containing adaptor proteins, SLAM-associated protein (SAP) and Ewing sarcoma–associated transcript 2 (EAT-2). SAP interactions trigger activation, as evidenced in humans with X-linked lymphoproliferative disease, caused by loss-of-function mutations in the SAP linker. In the absence of SAP, interactions with EAT-2 may be inhibitory.[146]

Recent studies have explored the potential contribution of ILCs beyond NK cells. A role for lung group 2 ILCs in mediating respiratory syncytial virus (RSV)-induced IL-33–driven T_h2-biased immunopathology has been demonstrated in neonatal mice.[152] IL-23–responsive group 3 ILCs played a role in the pathogenesis of neonatal intestinal inflammation in a murine model.[153] Much remains to be learned regarding the ontogeny of ILC function with respect to both the quantity and the quality of these cells in the very young.

Polymorphonuclear Neutrophils

Neonatal polymorphonuclear neutrophils (PMNs) are present at early stages of gestation, but their functional capacities are different from those of adult PMNs. Progenitor cells that are committed to maturation along granulocyte or macrophage cell lineages (granulocyte–macrophage colony-forming units) are detectable in the human fetal liver between 6 and 12 weeks' gestation in similar proportions as in adult bone marrow.[154] Human fetal blood has detectable granulocyte–macrophage colony-forming units from 12 weeks' gestation to term.[154] Although these progenitor cells are detectable in the fetus and newborn, developmental differences between adult and mature neonatal PMNs have been demonstrated—in signal transduction, cell surface protein expression, cytoskeletal rigidity, rolling adhesion, microfilament contraction, transmigration oxygen metabolism, intracellular antioxidant mechanisms, and neutrophil extracellular trap formation.[90,155–158] The magnitude of PMN functional differences correlates with the maturity of the infant and begins to decrease within the first few weeks after birth.[157]

In addition to intrinsic age-dependent differences in PMN function, age-dependent cell extrinsic soluble factors may developmentally regulate induction of specific functions and maturation of these cells.[154,159] For example, low concentrations of the chemoattractant complement component C5a in neonatal sera might impair establishment of chemoattractant gradients at sites of inflammation. In addition, elevated concentrations in human neonatal blood plasma of adenosine,[160] an endogenous purine metabolite that acts via seven-transmembrane adenosine receptors to inhibit inflammatory leukocyte responses, could contribute to inhibition of newborn neutrophil function.

Systemic bacterial infection in newborns is frequently accompanied by profound neutropenia, prompting investigation of neutrophil kinetics in infected infants.[161,162] These studies have suggested diverse, developmentally specific regulatory mechanisms required for mobilization of the neutrophil response to infection. The absence of detectable neutrophil precursors in bone marrow aspirates of infected infants and systemic neutropenia motivated studies of neutrophil replacement therapy in neutropenic, infected infants.[163] Although this approach has been successful in some cases, the results have not been uniformly beneficial, and a Cochrane review suggests a need for adequately powered multicenter trials of granulocyte transfusions in neutropenic septic neonates.[164] Metaanalysis suggesting that granulocyte colony-stimulating factor or granulocyte–macrophage colony-stimulating factor may reduce mortality in newborns when systemic infection is accompanied by severe neutropenia requires confirmation in adequately powered trials.[165] Heterogeneity in the impact of neutrophil modulation may reflect the importance of individualizing immunologic interventions for the genetic background, developmental stage, and pathogenic microorganism being treated.

Monocytes, Macrophages, and Dendritic Cells

Cells committed to phagocyte maturation, including granulocyte or monocyte–macrophages, are detectable in the human fetal liver by 6 weeks' gestation and in peripheral fetal blood by 15 weeks' gestation. Unlike granulocytes, whose tissue half-life is hours to days, macrophages migrate into tissues and reside for weeks to months. In a tissue-specific fashion, these cells regulate availability of multiple factors, including proteases, antiproteases, prostaglandins, growth factors, reactive oxygen intermediates, and a range of cytokines and chemokines. Importantly, monocytes can migrate from the bloodstream to tissue sites, becoming tissue-based DCs. Monocytes, macrophages, and DCs share the ability to present antigens to T lymphocytes, thereby triggering the classic adaptive immune responses.

Compared with their adult counterparts, newborn monocytes, macrophages, and DCs demonstrate reduced chemotaxis and

phagocytosis as well as distinct TLR signaling that is polarized toward T_h2 and antiinflammatory cytokine production.[92] The distinct function of newborn APCs reflects both intrinsic characteristics, including reduced nucleosome remodeling for IL-12 p70 production[166] as well as the modulatory effects of age-specific extrinsic factors such as the antiinflammatory purine metabolite adenosine, the level of which is relatively elevated in human newborn UCB plasma.[159,160]

Stimulation of monocytes results in a change in innate "set-point" such that responses to subsequent stimuli are altered.[167] This phenomenon, reflecting adaptive features of the innate immune system that are mediated by epigenetic changes, has been termed *trained immunity*[168] and may contribute to the heterologous beneficial ("nonspecific") effects of live attenuated vaccines (Goodridge et al., 2016). Recent studies indicate that trained immunity varies by age, with human neonatal monocytes demonstrating distinct immunometabolic BCG-induced training resulting in tolerogenic cytokine responses to subsequent LPS stimulation in vitro.[169] Much remains to be learned regarding the scope, ontogeny, and mechanisms underlying innate training/innate memory.

Adaptive Immunity

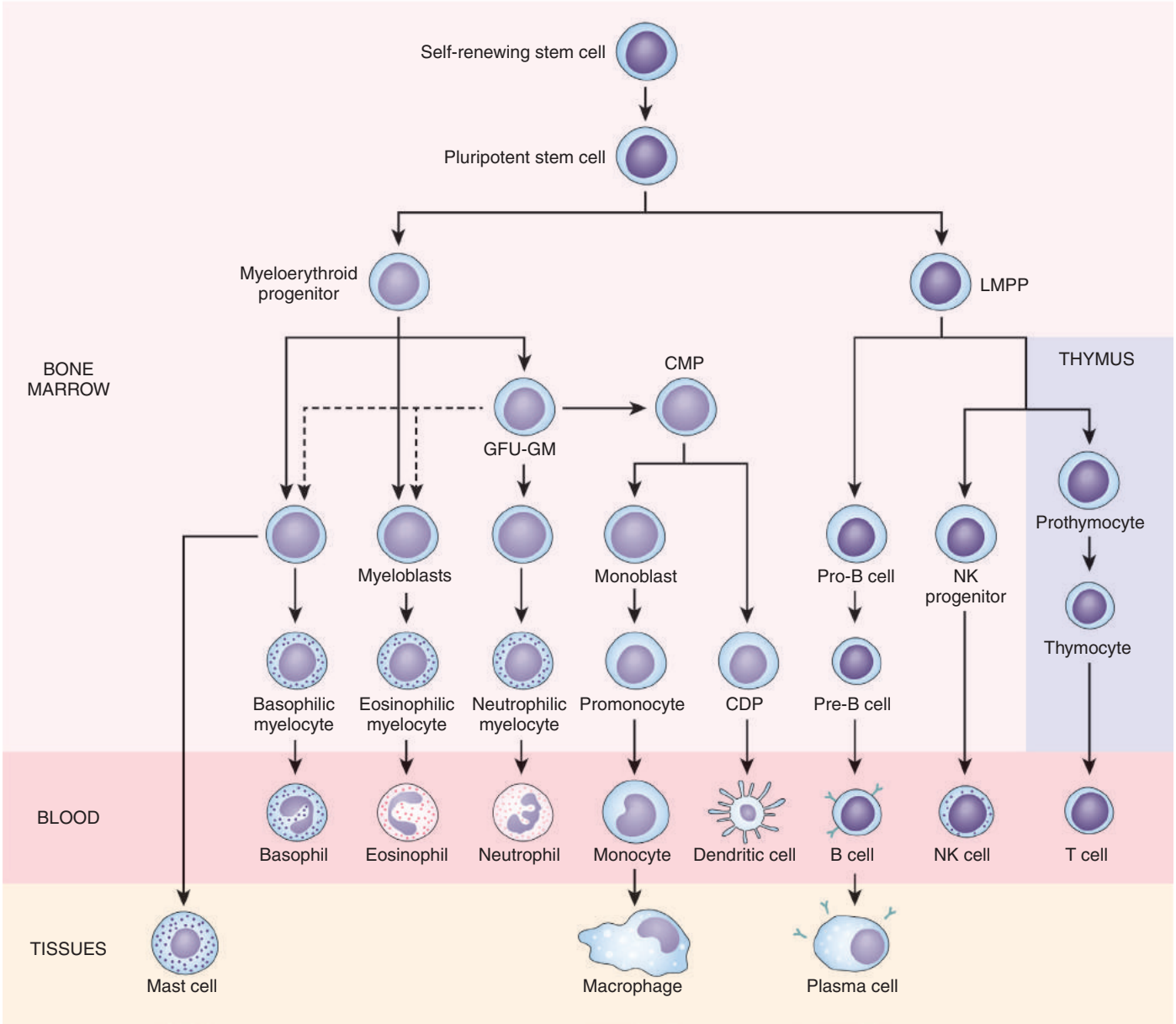
Antigen-specific T and B lymphocytes bearing TCRs and BCRs, respectively, play multiple critical roles in adaptive immunity. T cells responding to a specific antigen secrete cytokines and kill infected target cells and tumor cells by cell-mediated cytotoxicity. These functions of CD4 and CD8 T cells depend on their TCRs specifically recognizing antigenic peptides bound to MHC molecules. Several T-cell populations have a more restricted expression of TCRs that recognize ligands other than peptide/MHC ligands and have an innate-like role early in the immune response; these include NK T (NKT) cells, mucosal-associated invariant T (MAIT) cells, and T cells expressing γδ TCRs (γδ T cells). As discussed in the section entitled Role of Regulatory T cells in Pregnancy, $CD4^+$ Treg cells serve as negative regulators of effector responses. Analogously, antigen-specific B cells can be divided into populations that are involved in the conventional immune response and that depend on CD4 T-cell help for their differentiation into antibody-secreting cells; those that have an innate immunity-like function and act early in immune responses, such as marginal zone B cells; and those with regulatory-like suppressive function ("Breg cells").

All major lymphocyte lineages, which include T cells, B cells, and ILCs, which lack antigen-specific receptors and include NK cells,[170] develop from $CD34^+CD38^{dim}$ pluripotent hematopoietic stem cells (HSCs) found in the fetal liver and bone marrow in a perivascular niche (Fig. 32.5).[171] The process of lymphocyte differentiation and hematopoiesis has traditionally been viewed as a linear progressive narrowing of differentiation potential based on the sequential expression of specific transcriptional regulators. However, the pathways of development of the human myeloid, erythroid, and megakaryocyte lineages may undergo major shifts during ontogeny. For example, in fetal liver, the HSCs and their $CD34^+CD38^+$ progenitor cell derivatives have a similar ratio of cells with multipotent versus unilineage potential, whereas in bone marrow, which is the definitive site of hematopoiesis starting in the second trimester of gestation, $CD34^+CD38^+$ progenitor cells predominantly have unilineage potential.[172] Another example is that the HSCs of UCB have a greater potential to differentiate into the T lineage than HSCs of adult bone marrow.[173]

T Lymphocytes

Most T cells develop in the thymus, which includes cell types of nonhematopoietic origin, such as epithelial cells, as well as multiple cell types of hematopoietic origin, including the developing immature T cells or thymocytes, DCs, mononuclear phagocytes, and small numbers of B cells. The thymic epithelial cells are derived from the third branchial cleft and the third or fourth branchial pouch, a process that is perturbed in DiGeorge syndrome, resulting in thymic epithelial hypoplasia. Thymic lobes can be divided into four regions, which, proceeding from outward to inward, are the subcapsular region, cortex, corticomedullary junction, and medulla. Prothymocytes, which are bone marrow–derived $CD34^+CD38^+CD62L^+$ lymphoid cells, have the capacity to commit to the T-cell or other lymphocyte lineages depending on their receipt of instructive signals.[174] Circulating prothymocytes enter the thymus via vessels at the cortical–medullary junction. The prothymocyte becomes committed to the T-cell lineage by the engagement of its surface notch 1 receptor by ligands displayed on the thymic epithelium, such as delta-like ligand 4. This engagement results in an early T-cell progenitor that acquires expression of CD1a, CD2, CD7, and progressively loses its initial capacity for B-cell, myeloid, or NK-cell differentiation and becomes a fully T-lineage committed pro-T cell.[175] The pro-T cell migrates to the subcapsular region just below the outer capsule.

The subcapsular pro-T cell expresses all of the internal proteins required for V(D)J recombination, including the recombinase activating gene (RAG) 1 and RAG2 endonucleases that make double-stranded breaks in DNA; the proteins involved in nonhomologous end joining repair (e.g., Artemis, XLF [Cernunnos], and DNA ligase IV); and those that are essential for generating junctional diversity at complementarity determining region (CDR) 3 (e.g., terminal deoxynucleotidyl transferase [TdT]). CDR3, which is the most variable in amino acid sequence, is located at the center of the antigen-specific binding site of both TCRs and BCRs. The pro-T cell lacks most cell surface proteins characteristic of mature peripheral T cells, including CD3, CD4, and CD8, and is therefore also referred to as a *triple-negative thymocyte* (Fig. 32.6). The pro-T cell is the first stage in which there is VDJ rearrangement of TCR gene loci, with the TCRγ gene rearrangement occurring most frequently.[176] If this rearrangement is productive (i.e., capable of expressing a full-length TCRγ chain protein) and the thymocyte subsequently undergoes a productive TCRδ gene arrangement, a γδ TCR heterodimer is expressed on the cell surface, allowing the thymocyte to differentiate into a mature γδ T cell that emigrates from the thymus into the periphery. More frequently (>95% of the time) these TCRγ and/or TCRδ gene rearrangements are nonproductive, and the pro-T cell attempts TCRβ chain gene rearrangement. If this arrangement is productive, the TCRβ chain is expressed on the cell surface in association with an invariant pre-T alpha chain forming the pre-TCR complex, which defines the pre-T-cell stage of development. Like the mature TCR, the pre-TCR is associated with the CD3 complex of proteins, which includes CD3γ, CD3δ, CD3ε, and CD3ζ (also known as CD247) chains, all of which have cytoplasmic tails containing specific amino acid sequences called ITAMs. These ITAMs serve as molecular targets for tyrosine phosphorylation and binding by tyrosine kinases, such as Lck and zeta chain-associated protein of 70 kDa (ZAP-70), which generate intracellular signals leading to the induction of target genes.[177] These signals direct the pre-T cell

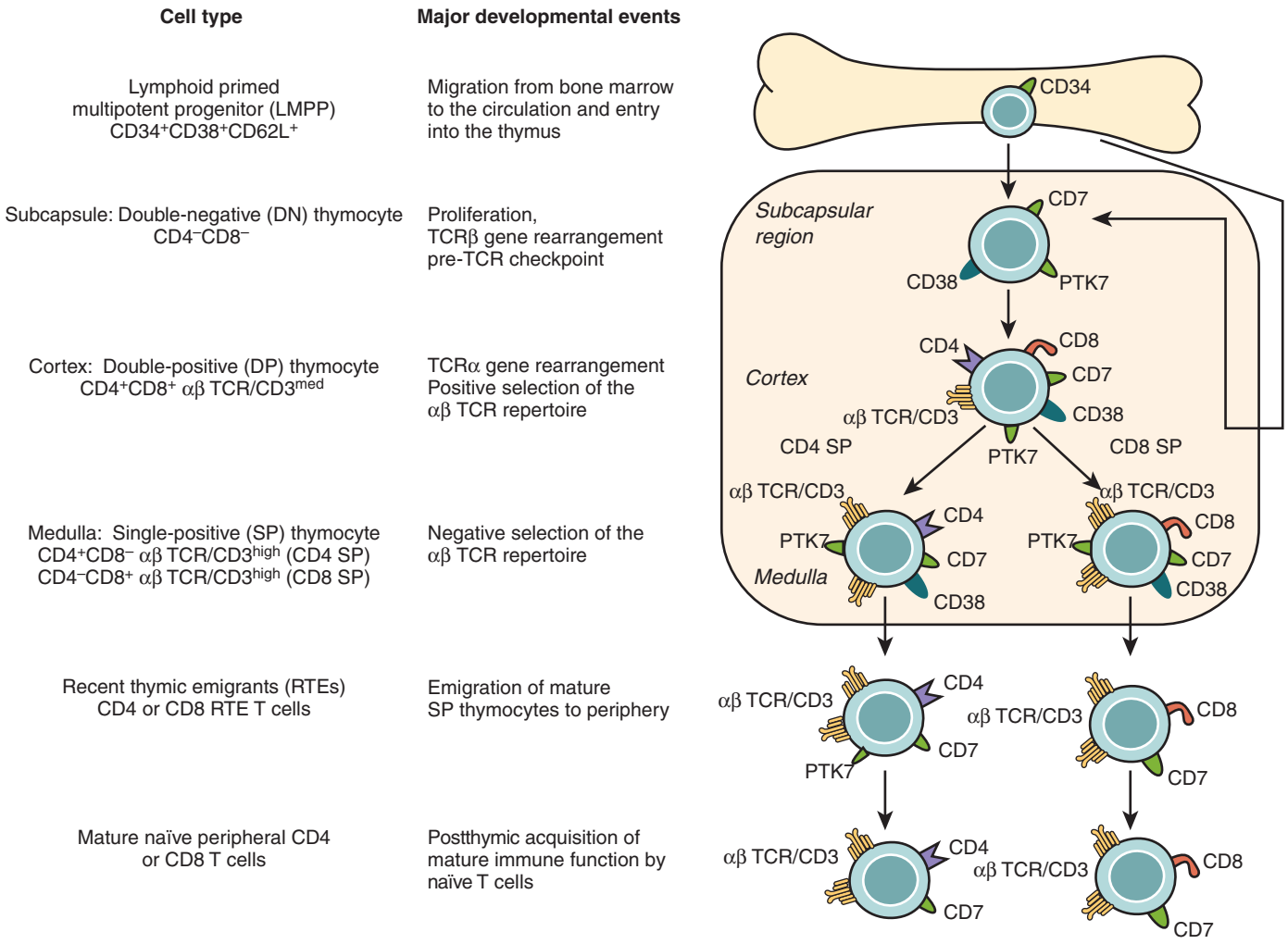


• **Fig. 32.5** Myeloid and Lymphoid Differentiation in the Bone Marrow, Blood, and Tissues. *CDP*, Committed dendritic cell progenitor; *CFU-GM*, colony-forming unit granulocyte–macrophage; *CMP*, common myeloid progenitor; *LMPP*, lymphoid primed multipotent progenitor; *NK*, natural killer.

to (1) proliferate, (2) upregulate expression of CD4 and CD8 and become a double-positive ($CD4^+CD8^+$) thymocyte, (3) migrate from the subcapsular area to the thymic cortex, and (4) start rearrangement of the TCRα chain gene locus (see Fig. 32.6).

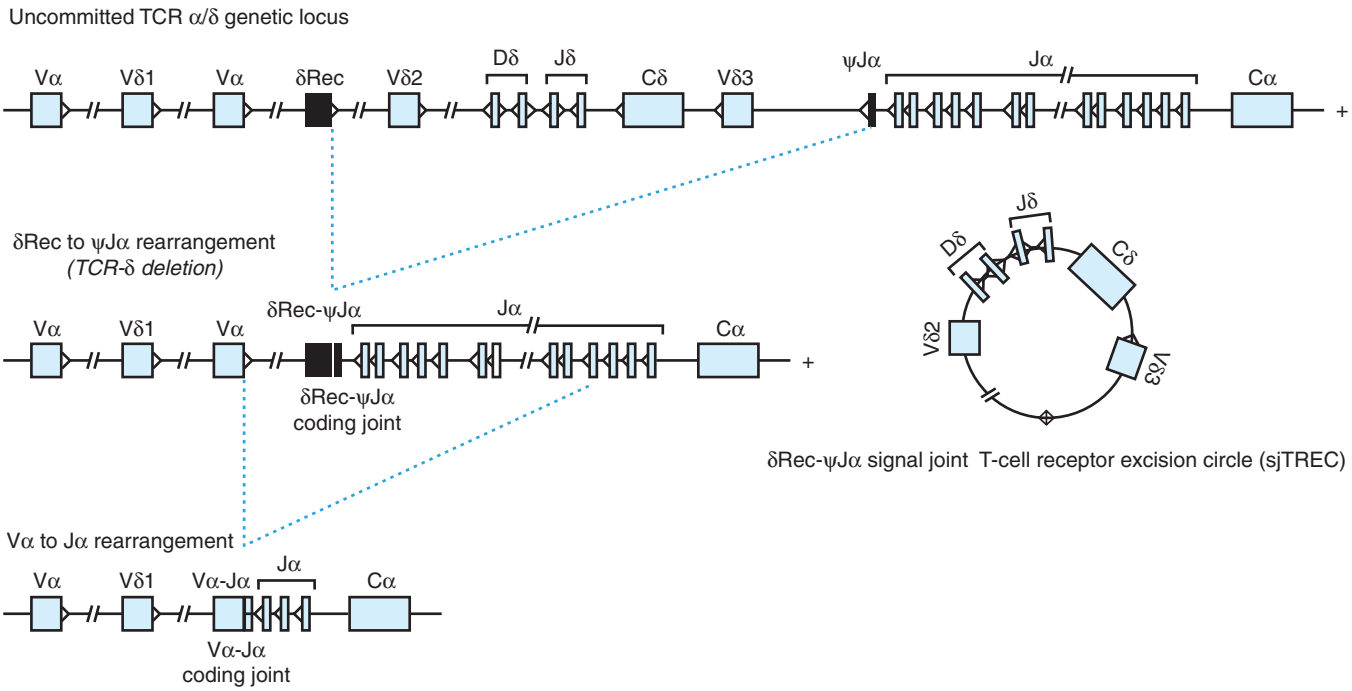
Rearrangement of the TCRα chain gene by $CD4^+CD8^+$ thymocytes is a two-step process in which there first is an internal deletion of a ψδ rec segment that brings the unrearranged Vα segments in close proximity with J_α segments and the C_α constant region.[178] The intervening DNA, which is excised as a circular product with fused signal joint sequences, referred to as a *signal joint TCR excision circle* (sjTREC), is highly stable within the cell (Fig. 32.7). The sjTREC content of the T-lineage cell subsequently decreases mainly as a result of cell proliferation, which under normal conditions is minimal until the mature T cell undergoes antigen activation-induced clonal proliferation. Thus, the measurement of the sjTREC content by peripheral blood T cells is an indirect but useful assessment of the adequacy of production of new T cells by the thymus. The measurement of sjTRECs in neonatal blood spots is routinely used for newborn screening in all 50 United States for identifying infants with impaired production of T cells by the thymus as occurs in most forms of severe combined immunodeficiency (SCID) (see Specific Immunologic Deficiencies of the Newborn and Their Diagnosis).

Double-positive thymocytes that have productive TCRα chain gene rearrangements replace their pre-TCR with a TCR consisting of an αβ TCR heterodimer in association with the CD3 proteins (see Fig. 32.6). The next major checkpoint of thymocyte development is positive selection, in which the αβ TCR of the $CD4^+CD8^+$ thymocyte is tested for whether it has significant affinity for either the MHC class I alleles (HLA-A, HLA-B, and HLA-C in humans) or the MHC class II alleles (HLA-DR, HLA-DP, and HLA-DQ in humans) expressed by thymic cortical epithelial (TCE) cells.



• **Fig. 32.6** Stages of Human αβ T-Cell Receptor–Positive Thymocyte Development. Prothymocytes from the bone marrow or, before the third trimester, fetal liver, which express CD34, CD38, and CD62 ligand (*CD62L*), but lack CD4, CD8, or CD3/T-cell receptor (*TCR*), enter the thymus via vessels at the junction between the thymic cortex and medulla. They differentiate to progressively more mature αβ TCR+ thymocytes, defined by their pattern of expression of the αβ TCR-CD3, CD4, CD8, and CD38. Protein tyrosine kinase 7 (*PTK7*) is expressed throughout thymocyte development. The TCRβ chain gene and then the TCRα chain gene are rearranged in the outer cortex. Positive selection occurs mainly in the central thymic cortex by interaction with thymic epithelial cells that express major histocompatibility complex (MHC) class I and class II proteins, and negative selection occurs mainly in the medulla by the interaction with thymic DCs that display MHC associated with self-peptides that are derived from medullary thymic epithelial cells. Following these selection processes, medullary thymocytes emigrate into the circulation and colonize the peripheral lymphoid organs as CD4 and CD8 T cells with high levels of αβ TCR/CD3. These recent thymic emigrants (RTEs) also have a high content of signal joint TCR excision circles (sjTRECs), which are a circular product of TCR gene rearrangement (see Fig. 32.7). In adults, most RTEs have modest levels of surface expression of PTK7 but probably lack CD38. In contrast, in neonates, most peripheral T cells have high levels of surface expression of PTK7 and CD38 and have relatively high amounts of sjTRECs compared with adult peripheral T cells.

All MHC class I and class II molecules during their biosynthesis have peptides loaded into their peptide-binding grooves. In the absence of infection or vaccination with foreign proteins, these peptides are derived from self-proteins, as is the case for the MHC of TCE cells. A specialized set of MHC class I-binding peptides may be generated by a special type of proteasome expressed by TCE cells (the thymoproteasome) and that these play an important role in increasing the antigen responsiveness of mature peripheral T cells.[179] In positive selection, thymocytes with TCRs that are unable to bind to MHC/peptide complexes on TCE cells with sufficient affinity to generate intracellular signals die by apoptosis, the default pathway. In cases where the TCR binding to MHC generates a relatively weak to moderate signal, the thymocyte is positively selected for survival. A large range of "analog" signals—from relatively weak to medium strength—are converted by the intracellular signaling protein Themis into a single "digital" outcome of thymocyte survival and maturation.[180] In cases where the TCRs of CD4+CD8= thymocytes receive very high levels of signaling, double-positive cortical thymocytes undergo apoptosis.[181] CD4+CD8+ thymocytes with TCRs that receive



• **Fig. 32.7** Sequential rearrangements in the T-cell receptor (*TCR*) α/δ genetic loci generate signal joint TCR excision circles (sjTRECs) and Vα–Jα rearrangements. Rearrangement of the δRec segment to the J_α segment commits the thymocyte to the αβ TCR lineage as this deletes the C and J segments that are necessary to encode a productive TCRδ chain. The δRec–ψJ_α rearrangement also generates an sjTREC, which is commonly used for monitoring peripheral T-cell populations for their recent thymic origin. The δRec–ψJ_α rearrangement and excision of an sjTREC are followed by TCRα (V_α–J_α) rearrangements, which if productive result in expression of an αβ TCR/CD3 complex on the thymocyte cell surface. Most thymocytes that express αβ TCRs have molecular evidence of nonproductive rearrangements of portions of the TCRδ gene locus (not shown).

MHC class II/peptide survival signals lose CD8 expression and upregulate CD3 expression, thereby becoming $CD4^+CD8^-$ thymocytes that are $CD3^{high}$. $CD4^+CD8^-$ thymocytes also begin to acquire a gene expression pattern that is characteristic of mature peripheral CD4 T cells that is required for their capacity to carry antigen-induced effector functions, such as IL-2 secretion and CD40-ligand (CD40L) expression. $CD4^+CD8^+$ thymocytes with TCRs that receive MHC class I/peptide survival signals lose CD4 expression and upregulate CD3 expression, thereby becoming $CD4^-CD8^-$ thymocytes; they also begin to express genes that are characteristic of peripheral CD8 T cells and that are required for their antigen-induced capacity to become cytotoxic cells. These $CD4^+CD8^-$ thymocyte-specific versus $CD4^-CD8^+$ thymocyte-specific outcomes of positive selection are directed by the master transcription factors ThPOK (encoded by the Zbtb7b gene) and Runx3, respectively.[182]

A small subset of thymocytes bearing TCRα chains containing the $V_\alpha24J_\alpha18$ segments in association with TCRβ chains containing $V_\beta11$ segments interact with relatively high affinity with CD1d, an MHC class I-like protein that is expressed on double-positive thymocytes.[183] This TCR interaction leads to the thymocyte-positive selection for NKT-lineage cells, which are distinct in function from conventional T cells in having the ability to secrete rapidly large amounts of cytokines, such as IFN-γ and IL-4, during the early phase of innate immune responses to pathogens.[184]

Another subset of thymocytes bearing TCRα chains with $V_\alpha7.2$-$J_\alpha33$ segments paired with TCRβ chains using either $V_\beta2$ or $V_\beta13$ interact with high affinity with an MHC class I-like protein, MR1, on non-hematopoietic cells, resulting in the positive selection of mucosal-associated invariant T (MAIT) cells.[185] MAIT cells, which are predominantly CD8 single positive, are found in greatest amounts in mucosal tissues, such as the intestine, lung, and liver.[186]

Positively selected single-positive $CD4^+CD8^-$ or $CD4^-CD8^+$ thymocytes move into the medulla, where they undergo a final selection process before emigrating from the thymus as CD4 and CD8 T cells called *negative selection*. This selection process, which is an important mechanism for maintaining tolerance of T cells to peptides derived from self-proteins, involves the exposure of the mature thymocytes to medullary APCs expressing a highly diverse repertoire of peptides derived from self-proteins. These self-proteins include those that are normally expressed in a tissue-restricted manner (e.g., peptides derived from insulin, which is produced by pancreatic beta islet cells or parathyroid hormone) or that are characteristic of only certain stages of early development.[187] This unusual pattern of protein expression by medullary DCs and B cells is the result, at in part, of a nuclear protein encoded by the autoimmune regulator (AIRE) gene.[188,189] AIRE stochastically relieves the transcriptional repression of these tissue-specific and developmentally regulated genes and the proteins they encode are transferred to thymic $CD11c^+$ dendritic cells, which play a major role in presenting these self-peptide/MHC complexes to medullary thymocytes (Breed et al., 2018). Thymocytes that have high levels of signaling for peptides derived from self-proteins are induced to undergo apoptosis, whereas intermediate levels of signaling by $CD4^+CD8^-$ thymocytes result in their differentiation

into Treg cells.[190] Thus, thymically derived Treg cells have TCRs that have substantial affinity for self-proteins expressed in a tissue-specific or developmental-specific manner.

Recent Thymic Emigrants and the Naïve T-Cell Compartment

Following negative selection, single-positive mature thymocytes undergo additional maturation before exiting the thymus as recent thymic emigrant (RTE) naïve T cells, including upregulation of CC-chemokine receptor 7 (CCR7), IL-7 receptor alpha chain (CD127), L-selectin (CD62L), and Smad interacting protein 1.[191] In humans, this maturation also includes downregulation of the CD45R0 isoform and upregulation of the CD45RA isoform of the CD45 protein tyrosine phosphatase. The fully mature thymocyte then enters the circulation as an RTE naïve T cell that is $CD45RA^+CD45R0^-CCR7^+CD62L^+$ and that retains surface expression of protein tyrosine kinase 7 (PTK7), a protein that is highly expressed during intrathymic development.[192] The RTE naïve T cell recirculates between the peripheral lymphoid tissue and the blood. The entry of the circulating naïve T cells involves the interaction of T-cell surface adhesion molecules, such as L-selectin (CD62L) with sialomucins expressed on high endothelial venules and the T-cell CCR7 chemokine receptor with its chemokine ligands, which are expressed within peripheral lymphoid tissues. RTE naïve T cells also undergo postthymic antigen-independent maturation over a period of several months with the loss of PTK7, a decrease in the capacity for activation-induced chemokine (C-X-C motif) ligand 1 (CXCL)8 (IL-8) production,[193] and an increase in the capacity for IFN-γ production.[192] $PTK7^+$ RTE naïve T cells also undergo one to two homeostatic cell divisions as part of this maturation.[192]

In the human embryo the first naïve, mature T cells appear in the circulation and lymphoid organs at approximately 11 to 12 weeks' embryonic development and have been found in the fetal intestine at 11 weeks' gestation.[194,195] Thymopoiesis normally continues at least through age 40 years as indicated by the presence of circulating $PTK7^+$ RTEs[192] and by the results of in vivo metabolic labeling studies with deuterium.[196] Thymectomy early in life (e.g., as part of open heart surgery for congenital heart disease or for the treatment of certain autoimmune diseases, such as myasthenia gravis) results in a substantial loss of naïve T cells, including $PTK7^+$ RTEs[192] and CXCL8-expressing RTEs.[193] Thymectomy also results in an oligoclonal memory T-cell compartment.[197,198] Some children who have undergone neonatal thymectomy appear to regenerate thymic tissue, which is associated with the reacquisition of naïve T cells with RTE features.[193] In individuals who do not recover thymic function, the decay process is accelerated by chronic cytomegalovirus (CMV) infection, resulting in an immunosenescent T-cell phenotype similar to that seen in elderly individuals and associated with increased morbidity and mortality.[199] Naïve T cells of the peripheral lymphoid compartment can undergo homeostatic expansion in response to cytokines, such as IL-7, and this proliferation may be particularly important in disease states that impair the production of RTEs and result in profound peripheral lymphopenia, such as treatment with chemotherapy or human immunodeficiency virus (HIV) infection.

Naïve CD4 T-Cell Activation into Effector T_h1, T_h2, T_h17, and Follicular Helper T cells

Naïve T-cell activation requires a complex molecular signaling cascade that involves the reorganization of signaling molecules of the T-cell membrane into an "immunologic synapse" with the APCs bearing MHC/peptide.[200] For naïve T-cell activation, $CD11c^+$ DCs are particularly effective as APCs. TCR engagement by a high-affinity MHC/peptide ligand results in phosphorylation of components of the CD3 complex associated with the αβ TCR. The CD3 proteins contain cytoplasmic tails with specific amino acid sequences called ITAMs, which serve as molecular targets for the tyrosine kinase Lck, which is associated with the cytoplasmic domains of CD4 and CD8. The CD3ζ chain is thought to be the most critical component and is found as a homodimer. The tyrosine phosphorylated CD3ζ chain binds the tyrosine kinase ZAP-70, which, in turn, phosphorylates linker for activation of T cells (LAT), a large protein that serves as a docking site for multiple signaling molecules. Full naïve T-cell activation also requires costimulation by engagement of CD28 on the T-cell surface by CD80 or CD86 on the APC. Together, signaling generated by the TCR and CD28 results in the activation of several parallel signaling pathways, including those of the increased free calcium/calcineurin/NFAT, Ras/ErkAP-1 (fos/jun), and protein kinase C-theta/nuclear factor κB (NF-κB) pathways. This results in entry of the transcription factors nuclear factor of activated T-cells (NFAT), activator protein-1 (AP-1), and NF-κB into the nucleus, where they bind to the *cis*-regulatory elements of hundreds of genes and alter their transcription.[200]

In response to signals generated through binding to the TCR/CD3 complex and CD28, cord blood naïve CD4 T cells have both enhanced calcium fluxes and activation of the Ras-Erk-AP-1 pathway.[201] However, both AP-1-dependent transcription[201] and nuclear translocation of NFAT[202] are impaired in comparison to adult naïve CD4 T cells. Later in infancy, calcium fluxes by activated naïve CD4 T cells are reduced compared to adult. Together, these results suggest that the outcome of neonatal and young infant naïve CD4 T-cell activation is suboptimal and may contribute to impaired development of effector function and memory formation. On the other hand, neonatal naïve CD4 T cells appear to be in cell cycle at baseline and undergo more robust expansion in response to IL-7 than their adult counterparts.[203]

After their activation, naïve CD4 T cells differentiate into effector T_h1, T_h2, T_h17, or follicular helper T (T_{fh}) cells.[204–206] Differentiation into peripheral Treg cells can also occur, and these cells are discussed in the section entitled Regulatory T cells. Each of these CD4 T cell types is defined by prototypical master transcription factors and by secretion of a characteristic profile of cytokines in response to antigenic stimulation and preferential expression of particular chemokine receptors. The cytokine milieu produced by non-T cells in the local environment during antigen presentation is a primary factor influencing the developmental fate of a naïve T cell following activation.[207] The outcome of differentiation may also be influenced by the strength of TCR-mediated signaling, at least in some contexts, such as during naïve CD4 T-cell interactions with CD11c+ dendritic cells.[208]

Activated T_h1 cells produce IFN-γ, which is the signature T_h1 cytokine, IL-2, lymphotoxin α, and TNF-α, and also express surface CD40L (CD154). IL-12 produced by DCs and IFN-γ produced by NK cells promote T_h1 cell development by a signal transducer and activator of transcription (STAT) 4-dependent mechanism, and this results in expression of the master transcription factor T-bet. T_h1 responses are generally proinflammatory, and IFN-γ secretion is particularly important in activating mononuclear phagocytes for the control of intracellular bacterial pathogens, such as *Mycobacteria*, *Salmonella*, and *Listeria*. IFN-γ also increases MHC expression, which may be particularly important

in counteracting the attempt by herpesviruses to avoid antigen detection by their production of a number of proteins that decrease MHC class I and class II antigen presentation. Human neonatal CD4 T cells are biased against T_h1 polarization relative to adult T cells,[209] and this bias may continue into infancy and contribute to the increased vulnerability of infants to severe and disseminated tuberculosis.[210] Both CD4 T cell-intrinsic mechanisms[211] and APC-intrinsic mechanisms, such as decreased expression of IL-12 p70 by neonatal and infant DCs,[166,212] appear to contribute to blunted T_h1 immunity. However, T_h1 immunity is not depressed in all immunologic contexts in the fetus and neonate: The fetal intestine includes an innate-like CD4 T-cell population that express CD161, a marker for T cells with innate-like functional properties and that include the capacity to produce IFN-γ, which may normally play a role in tissue development/homeostasis rather than in host defense against microbes[213]; however, this cell type may contribute to immunopathology post-natally in intestinal inflammatory contexts, such as gastroschisis. In addition, as previously noted, premature infants may have increased T_h1 responses following in utero exposure to chorioamnionitis.[87]

Activated T_h2 cells produce IL-4, IL-5, and IL-13 and are important in the response to infections with multicellular parasites, such as helminths, and classic allergic diseases in which the level of IgE is elevated. Their development is facilitated by a number of non–T-cell–derived cytokines, particularly from epithelial sources, including thymic stromal lymphopoietin (TSLP), IL-25, and IL-33, as well as IL-4 produced by basophils. This non-T-cell-derived IL-4 activates STAT6, which, in turn, induces GATA3, the master transcription factor for T_h2 differentiation from naïve CD4 T cells. The T_h2 cytokines IL-4 and IL-13 induce Ig heavy chain class switching to the IgE isotype (the T_h2 phenotype). IL-5 is an eosinophil growth factor and promotes eosinophil survival in inflamed tissues. IL-13 promotes goblet cell hyperplasia and mucous secretion. Thus, T_h2 cells coordinate many of the characteristic responses of the skin and mucosal tissues to parasitic infection and allergens. Murine neonatal CD4 T cells appear to be intrinsically biased toward T_h2 polarization,[214] but in vitro studies using human UCB CD4 T cells and adult allogeneic DCs as an APC source have not observed this bias.[211]

Activated T_h17 CD4 T cells secrete the closely related cytokines, IL-17A and IL-17F, which act upstream to induce increased epithelial barrier function and to promote local production of antimicrobial peptides, inflammatory cytokines, and chemokine production by neutrophils and mononuclear phagocytes.[215] These collective effects lead to increased tissue resistance of the mucosa and skin to fungal and bacterial infection. In addition to T_h17 cells, IL-17 is produced by the ILC3 innate lymphocytes and by subsets γδ T cells, NK T cells, and MAIT cells and all of these IL-17-producing cell types are CD161 positive.

In humans, IL-17 is important for the control of mucocutaneous fungal infection, particularly with *Candida* spp. (Okada et al.[216]) and in limiting nasopharyngeal carriage with *Streptococcus pneumoniae* (Basha et al., 2017). Memory/effector CD4 T cells producing IL-22, which is often co-expressed with IL-17, also provides substantial protection from *C. albicans* infection (Sheri et al., 2016). There is also growing evidence for the importance of IL-17 produced by either T_h17 cells or $T_h1/17$ cells that express both cytokines[217] and recognize MHC class II/peptide complexes in limiting pulmonary infection with *Mycobacterium tuberculosis*[218,219] and, in young children, nontuberculous mycobacterial lymphadenitis.[220]

The differentiation of activated naïve CD4 T cells into T_h17 cells is promoted by IL-1β and TGF-β and cytokines that activate STAT3, including IL-6 and IL-23. Activated STAT3 induces the expression of RORγt, which is the master transcription factor for T_h17 cell differentiation.[221] In addition to their role in host defense, T_h17 cells are also prominently involved in the pathogenesis of inflammatory bowel disease, psoriasis, multiple sclerosis, rheumatoid arthritis, and other autoimmune diseases. Umbilical cord blood naïve CD4 T cells also include a small but detectable percentage of CD161+IL-23 receptor+CCR6+ RORγt+ cells that are capable of rapidly differentiating into T_h17 cells using an appropriate cytokine milieu.[222,223] However, it remains unclear to what extent this naïve T_h17 precursor cell is involved in the post-natal acquisition of memory/effector T_h17 cells in response to infections. Circulating T_h17 memory cell numbers are at a similar or lower frequency than T_h1 memory cells in young infants (Sheri et al., 2016), and it is plausible that limitations in T_h17 immunity early after birth contribute to the increased susceptibility of the neonate and young infant to infections with Candida, Mycobacteria, and extracellular bacteria. Activated T_{fh} cells secrete IL-21 and express surface CD40L, both of which provide essential signals for the activation and differentiation of B cells to produce antibody against protein antigens.[206] Human naïve CD4 T-cell differentiation into T_{fh} cells appears to be promoted by the combination of IL-12, IL-21, IL-23, and TGF-β and engagement of inducible T-cell costimulator (ICOS) on the T cell by ICOS ligand. The master transcription factor for T_{fh} differentiation is Bcl6, which is induced by ThPOK.[224] Whether the differentiation of human neonatal naïve CD4 T cells into T_{fh} cells is as robust as that of older children and adults is unclear. However, the observation that young infants, compared with older children, have reduced primary antibody responses to protein antigens, such as the hepatitis B vaccine,[225] could be a reflection of less robust T_{fh} cell generation following immunization.

Circulating Cord Blood CD4 T Cells with T_h1, T_h2, and T_h17 Memory Phenotypes

CD4 T cells with a naïve (CD45RA+CD45R0-CCR7+) phenotype predominate in the fetal circulation. However, approximately 1% to 6% of CD4 T cells in cord blood of healthy term infants have a CD25lowCD127highCD45R0 surface phenotype, a polyclonal αβ-TCR repertoire, and a capacity to produce cytokines and/or express chemokine receptors that are characteristic of T_h1 cells, T_h2 cells, and T_h17 cells.[226] Whether these memory CD4 T cells are the result of activation by microbial products, a possibility supported by a recent report of low levels of bacteria in the second trimester fetus,[53] or are tissue resident memory T cells that have re-entered from a non-lymphoid tissue site (see section entitled Tissue Resident Memory CD4 and CD8 T Cells) remains unclear.

Naïve CD8 T-Cell Activation into Cytolytic Effector Cells

Naïve CD8 T cells have a similar $CD45RA^+CD45R0^-CD62L^+CCR7^+$ surface phenotype as those of the CD4 T-cell subset and recirculate between the blood and secondary lymphoid tissue by the same mechanisms. As for naïve CD4 T cells, antigen presentation by $CD11c^+$ DCs is particularly efficient for CD8 T-cell activation, which results in the acquisition of cytolytic effector function mediated by perforin and granzymes and the expression of FasL. Many activated CD8 T cells also secrete T_h1 cytokines, such as IFN-γ and TNF-α.

Human naïve neonatal (cord blood) CD8 T cells have a transcriptome and chromatin landscape distinct from those of adults that is functionally biased toward acting in innate immune responses.[227] Compared to adult cells, neonatal naïve CD8 T cells express lower basal levels of transcripts for proteins involved in cytotoxicity, for example, FasL, granzymes B and H, perforin, and had lower basal and activation-induced levels of effector cytokines, for example, IFN-γ and IL-2. Moreover, neonatal naïve CD8 T cells induced less apoptotic cell death of allogeneic targets than their adult counterparts. In contrast to adult cells, neonatal CD8 T cells had a markedly greater increased rate of spontaneous (homeostatic) proliferation and markedly greater expansion to polyclonal stimulation, and the transcription of multiple genes involved in innate immunity, such as antimicrobial peptides, and more typical of innate cells, such as neutrophils. These enhanced innate-like responses may be due both to alterations in transcription factors and chromatin configuration[227] as well as enhanced basal signaling for a number of pathways.[228] Interestingly, the exposure of neonatal naïve CD8 T cells to IL-12 results in the rapid downregulation of the neutrophil-like gene expression pattern,[227] suggesting the possibility that there could be rapid reprogramming of CD8 T cells for more effective adaptive immunity following "tuning" of the immune system by exposure to the microbiome.

Compared to infants born at term, cord blood CD8 T cells from preterm infants are reduced in number and appear to have undergone substantial homeostatic expansion in utero based on the loss of CD27 and CD31 surface expression.[229] This homeostatic expansion in a relatively lymphopenic environment appeared to result in antigen-independent T-cell maturation as indicated by an exaggerated production of pro-inflammatory cytokines in response to TCR/CD3 complex stimulation.[229] Based on murine studies, such maturation, coupled, in some cases, with exposure to inflammatory stimuli, for example, premature rupture of membranes, would compromise the adequacy of the antigen-specific response and CD8 T-cell memory formation.

Congenital CMV infection induces robust CMV-specific fetal CD8 T-cell responses,[230] suggesting that this pathway for differentiation is intact with a strong source of antigenic stimulation although a lag in the development of these responses may still occur. There is limited information on the neonatal and young infant CD8 T-cell responses to acute viral infection. Studies of older infants and young children indicate a relatively robust CMV-specific CD8 T-cell response to primary infection.[231]

Antigen-Specific Memory T-Cell Responses

Memory T cells, which are generated as part of the primary antigen-specific T-cell response, are defined by function (i.e., more rapid effector response) and by expression of a $CD45RA^{-}CD45R0^{+}$ surface phenotype. A recently identified T memory stem cell (Tmsc) subset, which differentiates from naïve T cells early in the immune response, appears to play an important role in the long-term generation of antigen-specific CD4 and CD8 memory T cells.[232] This generation does not appear to require continued exposure of the Tmsc to antigen, accounting for the persistence of T-cell memory for decades following a single acute infection with pathogens that are completely cleared from the body. Tmsc give rise to central memory T cells, which have a $CD45R0^{+}CD62L^{+}CCR7^{+}$ surface phenotype and survey the peripheral lymphoid tissues for specific antigen. A second population of cells, called *effector memory T cells*, have a $CD45R0^{+}$ $CD62L^{-}$ surface phenotype, express a variety of chemokine receptors other than CCR7, and survey the nonlymphoid tissues for specific antigen. The extent to which effector memory cells are derived from central memory cells or directly differentiate from Tmsc cells remains unclear. Both central and effector memory T cells recirculate, with substantial numbers of both cell subsets found in the blood. A fourth memory cell population, T resident memory (Trm) cells, is found in nonlymphoid tissues, such as the skin and lung, and does not recirculate in the blood (see section entitled T Resident Memory CD4 and CD8 T cells). Studies comparing the memory CD4 T-cell responses of infants and young children with those of adults following primary CMV infection suggest that the infant/child responses are skewed toward central memory rather than effector memory responses, with an overall reduced production of T_h1 cytokines.[233] Similarly, the pertussis antigen-specific CD4 T-cell response of infants following the receipt of either a diphtheria, tetanus, acellular pertussis (DTaP) or a tetanus toxoid, reduced diphtheria toxoid, acellular pertussis [TdaP] (DTaP) vaccination series appears to be skewed toward central memory cells and limited IFN-γ production compared with that of TdaP-vaccinated adults.[234] Relative blunting of T_h1 memory cell responses in older infants and children likely also applies to the young infant and may contribute to the vulnerability of human infants to severe and disseminated primary tuberculosis.[210]

Regulatory T Cells

Treg cells have a $CD25^{high}CD127^{low}$ surface phenotype and are a distinct subset of CD4 T helper cells critical for maintenance of immune self-tolerance and homeostasis by suppressing aberrant or excessive immune responses harmful to the host.[235] Treg cells are defined by having an immune-suppressive phenotype with high, sustained expression of the transcription factor FOXP3 and a number of other Treg cell signature genes.[236] Mutations in the *FOXP3* gene can result in an inherited multisystem autoimmune disease characterized clinically by diarrhea, insulin-dependent diabetes mellitus, thyroid disorders, and eczema, called *X-linked neonatal diabetes mellitus, enteropathy, and endocrinopathy syndrome*.[237]

Natural (thymic) Treg cells develop as a distinct lineage in the thymus, while induced (peripheral) Treg cells arise in the periphery from conventional CD4 T cells in the presence of TGF-β, retinoic acid, and microbial antigens and metabolites, such as short chain fatty acids.[238] Naïve CD4 T cells of the second-trimester fetus are skewed toward differentiation into peripheral Treg cells rather than effector cells compared with adult naïve CD4 T cells by a Helios-dependent transcriptional mechanism.[239] Murine SCID/human chimera experiments suggest that this skewing is a feature of naïve CD4 T cells that arise from fetal HSCs rather than adult HSCs.[240] It is not known when in ontogeny hematopoiesis switches from the fetal-type HSC to the adult type, raising the possibility that at least some neonatal and infant T cells could be fetal HSC derivatives and, as a result, have distinct immune function. Defects in Treg cell function and number have been described in a series of different autoimmune diseases, including type 1 diabetes, systemic lupus erythematosus, rheumatoid arthritis, multiple sclerosis, and inflammatory bowel disease. Relevant to the interpretation of animal models for neonatal immunology, ontogeny of FoxP3-expressing Treg cells is much more advanced in humans than in mice.[241] $FoxP3^{+}$ Treg cells are almost completely lacking in neonatal mice, while they are abundant in the thymus and mesenteric lymph nodes in the human fetus as early as 20 weeks' gestation.[242] It appears that maternal cells cross the

placenta to reside in fetal lymph nodes, inducing the development of fetal Treg cells suppressing immunity against maternal antigens and probably also playing a role in immune homeostasis after birth.[47] Therefore, it does not seem surprising that Treg cells can be detected in neonatal tissues as early as 23 weeks' gestation.[243] Consistent with the increased concentration of Treg cells in fetal tissues between 12 and 20 weeks' gestation, UCB from preterm infants born between 24 and 31 weeks' gestation contains a higher percentage of Treg cells compared with that of term infants.[244] This imbalance persists until later childhood and may play a role in the immune challenges former preterm infants face. In general, the critical maturation events in the developing immune system that underlie the high vulnerability to both infection and inflammation in the (preterm) neonate remain largely understudied.[245]

γδ T Cells

About 2% to 5% of T-lineage cells of the thymus and peripheral blood express γδ TCR. Despite the potential for a highly diverse γδ TCR repertoire, γδ TCR use is highly restricted in terms of variable (V) segment use, with $V_\gamma 9V_\delta 2$ T cells being the predominant γδ T-cell subset in adult peripheral blood. Gamma–delta T cells, which can be more innate-like or more adaptive-like in their immune function depending on the context,[246] can produce T_h1- and T_h17-type cytokines, kill virally infected or tumor target cells by cell-mediated cytotoxicity, and functionally interact with other cell types of the immune system, such as B cells and NK cells. $V_\gamma 9V_\delta 2^+$ T cells are innate-like in their immune function in that they are not activated by their TCR-recognizing MHC/peptides but rather utilize other receptors, such as the butyrophilin subfamily 3 member A1 (BTN3A1) and butyrophilin 2A1, to recognize microbe- and host-derived phosphorylated prenyl metabolites (collectively referred to as *phosphoantigens*).[246,247] Older work suggested that this Vγ9Vδ2 T-cell predominance was due to postnatal expansion in response to exposure to bacterial-derived phosphoantigens. However, studies of the human fetus suggest otherwise in that $V_\gamma 9V_\delta 2$ T cells are also the predominant γδ T-cell subset in fetal blood and, like their adult counterparts, are programmed to rapidly respond to phosphoantigens with IFN-γ and cytotoxin release.[248] These fetal $V_\gamma 9V_\delta 2$ T cells expand after birth and acquire potent cytotoxic function by 10 weeks of age, most likely in response to environmental phosphoantigen exposure.[249] An example of a γδ T-cell response that is dependent on TCR recognition of antigen is illustrated by $V_\gamma 8V_\delta 1^+$ T cells, which were first identified in fetuses with congenital CMV infection.[250] These $V_\gamma 8V_\delta 1^+$ T cells have the ability to lyse CMV-infected target cells in a TCR-dependent manner and have the potential to serve as non-MHC-restricted antiviral adoptive immunotherapy (e.g., in the post-HSC transplant setting).[251] These CMV-reactive $V_\gamma 8V_\delta 1^+$ T cells have innate T-cell like features including invariant TCRs and low levels of TdT so that there are minimal CDR3 insertions. Moreover, they acquire stereotypical effector function (IFN-γ and granzyme expression) during intrathymic development.[252]

Natural Killer T Cells

NKT cells are so named because they express αβ TCRs in conjunction with CD161 (NKR-P1A), the human orthologue of the mouse NK1.1 protein, and other NK-cell markers, including CD56, CD57, and NKG2D. Unlike conventional αβ T cells, NKT cells that emigrate from the thymus into the periphery have a uniformly $CD45R0^+$ surface phenotype and the ability to rapidly secrete high levels of T_h1 and T_h2 cytokines and to carry out cell-mediated cytotoxicity. Human NKT cells have a highly restricted repertoire of αβ TCR (TCRα chains containing the Vα24Jα18 segments in association with TCRβ chains containing Vβ11 segments) and are positively selected during thymocyte development by the nonclassical MHC molecule CD1d rather than by MHC class I or class II molecules. The invariant TCR of NKT cells can recognize lipids bound to CD1 that are either endogenously produced glycolipids for example, α-glycosylceramide and α-galactosylceramide secreted by thymocytes and CD11c+ dendritic cells,[253] or human gut microbiota derived glycolipids, for example, α-galactosylceramide produced by *Bacteroides*.[254] The role of the invariant αβ TCR in NKT-cell immunity remains controversial, and it has been proposed that activation of these cells by cytokines, such as IL-12, may be more important for their mediating an innate-like immune response.[184] NKG2D-dependent but TCR-independent activation of NKT cells (e.g., for cytotoxic activity) can also occur. NKT cells with invariant TCR can be divided into a $CD4^+(CD8^-)$ subset that expresses CD62L and may be involved in recirculation in secondary lymphoid tissues and a $CD4^-CD8^-$ cell subset that may mainly serve as effector cells at sites of extralymphoid tissue inflammation. In addition to their potential role in antimicrobial host defense, NKT cells have also been implicated as negative regulators of certain T-cell-mediated immunopathologic responses, as relative deficiency of their numbers has been associated with certain autoimmune diseases, graft-versus-host disease following hematopoietic cell transplant, and asthma.

NKT-lineage cells are present in the human fetal thymus by the beginning of the second trimester of gestation, and their relative frequencies compared with those of the other thymocyte subsets decline with increasing gestational age because of the rapid expansion of thymocytes differentiating by the conventional positive selection. Fetal and postnatal thymic NKT cells, which are largely $CD4^+CD45R0^+$, express high levels of IL-7 receptors, and their development appears to be largely IL-7 dependent. Extrathymic fetal NKT-cell populations are found in the small intestine, where they may constitute up to 5% of all T cells in this tissue, and in the lung, spleen, and mesenteric lymph nodes.[255] Those NKT cells found in the fetal small intestine are distinct from those of fetal peripheral lymphoid tissue in having greater capacity to produce IFN-γ and lower levels of expression of CD62L. Murine studies suggest that commensal bacteria may be an important source for NKT-cell expansion and maturation, but the finding of a human NKT-cell population with mature features in the fetal intestine before most bacterial colonization of the gut occurs suggests a different mechanism, such as the presentation of endogenous glycolipids by CD1d, which is abundantly expressed in the fetal intestine. Circulating NKT cells in the neonate, infant, and young child constitute approximately 0.06% of circulating lymphocytes, a frequency that is only modestly lower than the mean of 0.2% for adult peripheral blood.[256] At all ages there is striking individual variation in values, with frequencies ranging from less than 0.001% to greater than 0.5% of circulating lymphocytes.

Mucosal-Associated Invariant T Cells

MAIT cells are a population of $CD8^+$ or $CD8^-CD4^-$ αβ T cells that predominantly express an invariant $V_\alpha 7.2$ and $J_\alpha 33$ TCRα chain. They are positively selected during thymic development by MR1, an MHC class I-like protein that is widely expressed on

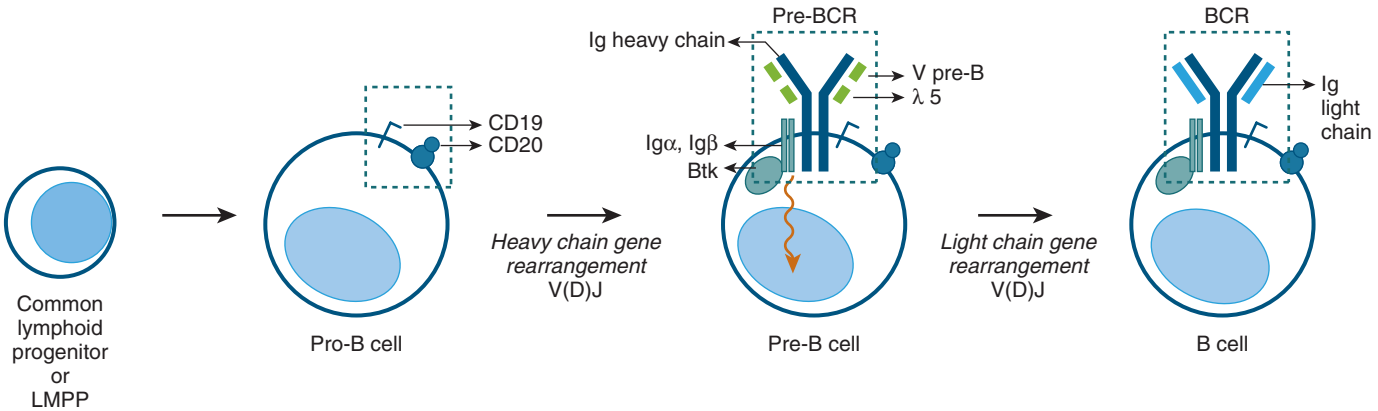
hematopoietic and epithelial cells.[257] There is growing evidence that MAIT cells serve a homeostatic role in tissue repair at epithelia where they are particularly abundant. Antigen for the MAIT cell αβ TCR consists of bacterial- or fungal-derived metabolites of the riboflavin biosynthetic pathway that bind to MR1 at a site analogous to the peptide-binding groove used by conventional MHC class I heavy chains.[258] These metabolites derived from the microbiota rapidly travel from mucosal surfaces to the thymus where they are captured by MR1 and serve in intrathymic MAIT cell selection.[259] MAIT cells produce T_h1 and T_h17 cytokines in response to polyclonal stimuli, including treatment with IL-12 or IL-18, and can kill bacterially infected epithelial cells by cell-mediated cytotoxicity. MAIT cells constitute up to 10% of circulating αβ T cells of healthy adults and uniformly have a memory ($CD45RA^-CD45R0^+$) cell surface phenotype. MAIT cells are the predominant αβ T-cell type in the liver and the lamina propria of the healthy intestine[260] and are also found in lung tissue. Murine studies have shown that MAIT cells can mediate protection against a variety of bacterial pathogens, including mycobacteria. Putative intrathymic MAIT cell precursors, which are $CD161^{high}V_\alpha7.2^+$ but $IL\text{-}18R^{low}$, are detectable at 18 weeks' gestation. MAIT cells with an activated and proliferative phenotype (i.e., $IL\text{-}18R^{high}CD45R0^+Ki67^+$) are easily detected in the small intestine, liver, and lung between 18 and 23 weeks' gestation.[261] These fetal intestinal $CD161^{high}V_\alpha7.2^+$ cells secrete IFN-γ and IL-22 in response to *E. coli* and anti-CD28 monoclonal antibody, but it is unclear if these cells are activated for these effector functions by MR1 antigen recognition. These cells may serve a TCR-dependent developmental/tissue repair homeostatic function as has been described for other MAIT cell populations associated with epithelia.[262] Studies using MR1 tetramers for analysis of circulating human T-cell populations suggest that the use of surrogate markers, such as expression of TCRs with $V_\alpha7.2$, may underestimate the phenotypic and functional diversity of MAIT cells.[263] MAIT cells in the UCB of neonates, which constitute approximately 0.7% of circulating αβ T cells, have a surface phenotype (e.g., $CD45RA^+$) that suggests that they may be RTEs that are in transit to the extralymphoid tissues, where they may mature and acquire a memory phenotype, as apparently occurs in the fetus.[261]

Tissue Resident Memory (TRM) CD4 and CD8 T Cells

Many non-lymphoid tissues of the body, including the lung, skin, and intestine, have substantial numbers of T cells that are mainly sessile and do not recirculate.[264] These resident T-cell populations include γδ T cells, NK T cells, MAIT cells, as well as αβ-TCR+ CD4 and CD8 T cells. In some tissues, such as the lung, substantial accumulation of CD4 and CD8 TRM cells occur only after birth in response to infection and the lack of these cells may contribute to the greater severity of viral infections in the neonate and young infant, for example, RSV infection of the lung.[265] CD4 TRM cells in the human lung may also be important for the control of pulmonary tuberculosis.[266] TRM cells are distinguished from other memory CD4 and CD8 T cell populations, such as effector memory cells, by their uniformly high surface levels of CD69 and CD103. At other sites, such as the intestine and skin, there may be relatively large populations of fetal CD4 and CD8 TRM cells with diverse TCR repertoires as early as the second trimester.[58,267,268] Fetal TRM populations mainly serve a homeostatic/tissue regulatory role and may have an activated phenotype in response to endogenous self-tissue-derived signals rather than due to microbial derived products.[269]

B Lymphocytes

B cells are lymphocytes that express BCRs, which consist of surface Ig in association with Igα and Igβ signaling molecules and the cytoplasmic Bruton tyrosine kinase (Btk) (Fig. 32.8). The engagement of the BCR by high-affinity antigen results in B-cell activation and clonal expansion and, in some instances, terminal B-cell differentiation into antibody-secreting plasma cells. Secreted antibodies form the humoral arm of the immune system and provide the main form of immune protection against many pathogens, particularly during the extracellular phases of their life cycle. Although the B-cell compartment is well formed before birth,



• **Fig. 32.8** Human B-Cell Development. A common lymphoid progenitor or lymphoid-primed multipotent progenitor (*LMPP*) cell gives rise to a pro-B cell that expresses surface CD19 and CD20 and that is committed to B-lineage–cell differentiation. The immunoglobulin (*Ig*) heavy chain gene locus undergoes V(D)J rearrangement, and, if productive, the B-lineage cell proceeds to the pre-B-cell stage, expressing a pre-B-cell receptor (*pre-BCR*). The pre-BCR consists of the Ig heavy chain in association with a surrogate light chain that consists of two polypeptides—V pre-B and λ5—and the proteins Igα and Igβ that are required for receptor complex surface expression and signaling. The Ig light chains undergo rearrangement in the pre-B cell, and, if productive, the pre-B cell differentiates into a B cell that expresses a B-cell receptor (*BCR*) that contains a mature surface Ig molecule. *Btk*, Bruton tyrosine kinase.

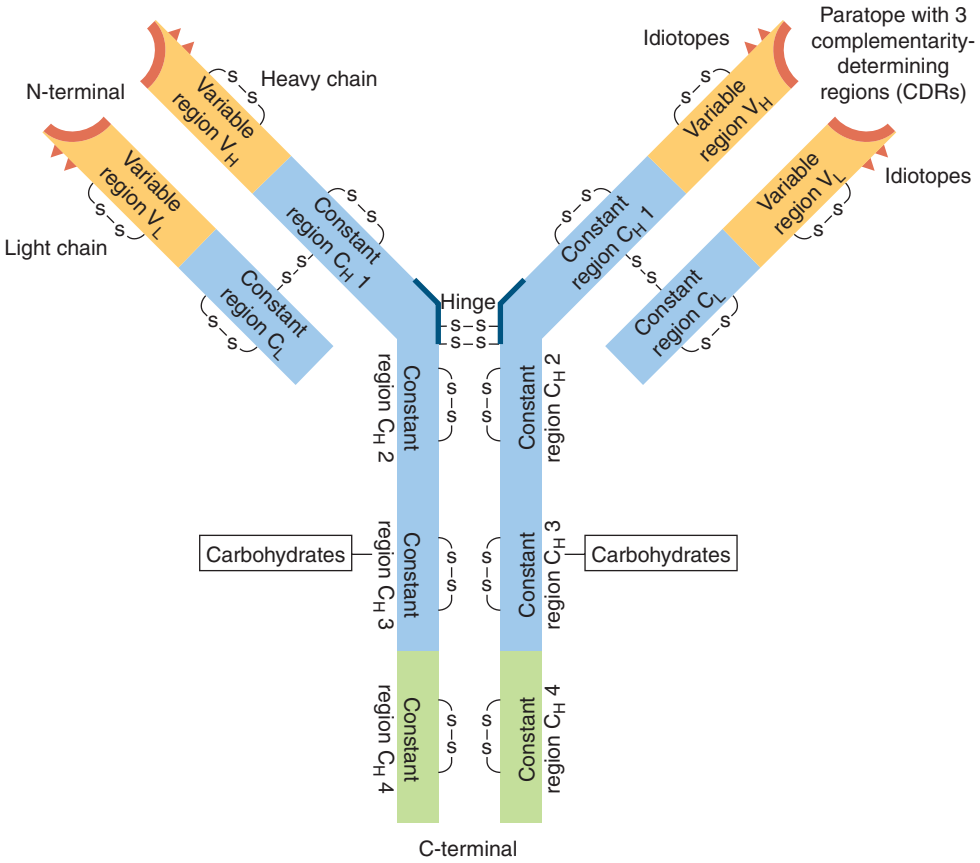
full diversification of the antibody repertoire and the efficiency of somatic hypermutation, which generates higher affinity antibodies, is not achieved until late in infancy.

B-Cell Development

The early phase of B-cell development, like T-cell development, is antigen-independent. In the human fetal bone marrow, a $CD10^-$ $CD19^+$ B-progenitor cell that is B-lineage committed undergoes dramatic expansion during the early second trimester.[270] This precursor gives rise to $CD19^+$ pro-B cells that have not undergone V(D)J recombination of the Ig genes and express CD34, TdT, RAG1, and RAG2. These cells undergo heavy chain D to J gene segment rearrangements followed by V to DJ rearrangement. As in TCR gene rearrangement, TdT inserts nucleotides between the segments to create additional junctional diversity at CDR3, which is encoded by the 3′ end of the V segment and the D and J segments. CDR3 is an important determinant of antibody specificity as it forms the central portion of the antigen-binding site. If the Ig heavy chain gene rearrangement is productive, it is expressed on the cell surface in association with a surrogate light chain consisting of the V-preB and λ5 polypeptides. Cells with a nonfunctional Ig heavy chain gene rearrangement or that express Ig heavy chain protein that assembles poorly with the surrogate light chain die by apoptosis. The pre-BCR signals use Btk (Fig. 32.9), which accounts genetic deficiency of Btk (X-linked agammaglobulinemia) resulting in a maturational arrest at the pre-B-cell stage. The next stage involves V_L to J_L light chain gene rearrangement, and, if productive, the newly expressed Ig light chain replaces the surrogate light chain. The light chain can be derived from either the kappa chain (60% of B cells) or the lambda chain (40% of B cells) gene clusters. The completed BCR is antigen specific and contains a surface IgM molecule. B-lineage cells also express surface IgD with the same antigen specificity as IgM, which is generated by alternative exon usage in the constant region of the Ig heavy chain gene. B-cell development initially occurs in the fetal liver, with a switch to the bone marrow beginning in the second trimester of gestation.

B-Cell Preimmune Selection and Maturation

Immature B cells of the bone marrow that have successfully produced productive heavy and light chains express these as IgM on the cell surface. A large proportion of these BCRs are autoreactive (i.e., they bind with relatively high affinity to molecules on other cell types within the bone marrow microenvironment or peripheral lymphoid organs). An important immune tolerance mechanism is the testing of immature B-lineage cells of the bone marrow for self-reactivity. At this stage of B-cell development, BCR signaling as part of self-reactivity maintains RAG activity, allowing the B cell to undergo a secondary V-to-J light chain rearrangement, a process known as *receptor editing*. If this eliminates BCR autoreactivity and cell signaling, RAG activity ceases, and the B cell enters the circulation as a peripheral transitional B cell to complete its maturation. Otherwise, additional light chain rearrangement can occur. Most initially autoreactive immature B cells can be converted to nonautoreactivity by receptor editing, allowing most B cells with productive Ig heavy chain rearrangements to contribute to the final repertoire.



• **Fig. 32.9** Structure of Monomeric Immunoglobulin. (Modified from Mix E, Goertsches R, Zett UKL. Immunoglobulins: basic considerations. *J Neurol*. 2006;253:V9–V17.)

Persistently autoreactive B cells are probably eliminated either by a process of clonal deletion, which involves apoptosis induced by strong BCR signaling,[271] or by anergy, in which the B cells are functionally inactivated. Anergic B cells have been identified as having an $IgD^{+}IgM^{-}CD27^{-}$ surface phenotype and constitute ~3% of peripheral blood B cells in adults. These anergic B cells have BCRs with autoreactive antigen specificity that mediate a decreased calcium flux and tyrosine phosphorylation after their engagement.[272]

These tolerance mechanisms appear to be incomplete in the human fetus such that antigenically naïve B cells that mature in the fetal liver and bone marrow have the capacity to produce antibodies that are polyreactive and that bind both to apoptotic cells and to commensal bacteria from healthy adults.[273] This restricted polyreactive antibody repertoire may be beneficial for the removal of apoptotic cells during development and for influencing the assembly of the gut microbiota after birth.

$IgM^{high}IgD^{high}$ transitional B cells can be identified in the bone marrow and circulation by their high levels of expression of CD5, CD10, CD24, and CD38 and low levels of adenosine triphosphate-binding cassette subfamily B member 1 (ABCB1).[274] Analogous to RTEs, these circulating transitional B cells undergo a post-bone marrow phase of peripheral maturation into fully mature naïve B cells that involves an increase in CD21 and ABCB1 expression and a decrease in CD5, CD10, CD24, and CD38 expression. The maturation of transitional B cells into fully mature antigenically naïve B cells includes negative selection of B cells with autoreactive BCRs that have entered the periphery and that have somehow escaped central tolerance mechanisms in the bone marrow.[275,276] Although the mechanisms involved in this secondary checkpoint for B-cell tolerance remain poorly understood, they may involve, at least in part, the death of autoreactive B cells because of their greater dependence for survival on B-cell activating factor (BAFF), a member of the TNF ligand superfamily, than nonautoreactive B cells.

The transitional B-cell subset is also enriched in regulatory B cells (Bregs), which have the capacity to express IL-10 after strong stimulation and, like Treg cells, may negatively regulate adaptive immune responses. Neonates and infants also have a circulating Breg population with a $CD5^{hi}CD10^{-}CD24^{lo}CD38^{lo}IgM^{lo}IgD^{lo}$ surface phenotype that is distinct from most transitional B cells and adult Bregs. These neonatal Bregs have a high capacity to produce IL-10 after their infection by RSV via viral interactions with the BCR and the CX3CR1 chemokine receptor, with the secreted IL-10 inhibiting the in vitro generation of T_h1 immunity.[277] Studies of young infants with RSV bronchiolitis found an inverse correlation with the frequency of neonatal Bregs in the circulation and nasopharyngeal aspirates with the circulating levels of CXCR3+ T_h1 cells and a direct correlation with disease severity.[277] Together, these findings indicate that Bregs, like Tregs, may inhibit effector T-cell function and immune control of infection and that this effect may be particularly prominent in RSV infection of neonates and young infants. Most transitional B cells that escape negative selection become fully mature naïve $IgM^{high}IgD^{high}CD27^{-}$ $ABCB1^{+}$ follicular B cells and enter follicle areas of the secondary lymphoid tissue by the interaction of their CXCR5 chemokine receptors with chemokines in the follicle (CXCL13). These cells can recirculate between the follicles of the peripheral lymphoid organs, including the spleen, lymph nodes, and Peyer patches, and the blood and lymph.[278] Follicular B cells include most of those that are involved in adaptive immune responses to T-dependent antigens, such as proteins and protein–carbohydrate conjugates.

Fetal and Neonatal B-Cell Development and Surface Phenotype

B cells expressing surface IgM are present by 10 weeks' gestation. The frequency of B cells in the tissues rapidly increases, such that by 22 weeks' gestation the proportion of B cells in the spleen, blood, and bone marrow is similar to that of adults. The concentration of B cells in the circulation is higher during the second and third trimesters than at birth.[279] After birth, the concentration of B cells increases to peak levels between 6 and 12 months of age, which is followed by a gradual decrease until stable adult values are reached.

Approximately 70% to 75% of UCB B cells are of the transitional subset, whereas transitional B cells usually constitute 10% or less of adult peripheral blood B cells, and 25% to 30% are fully naïve B cells.[274,280] $CD27^{+}$ B cells, which include most memory B cells in adults, are at low levels or undetectable in UCB, consistent with the antigenic naïveté of the healthy newborn. Neonatal naïve B cells are functionally distinct from their adult counterparts, having significantly lower expression of the IL-4 receptor alpha chain and responsiveness to IL-4 as assessed by tyrosine phosphorylation of STAT6.[281] Circulating newborn B cells are deficient in the purine ectoenzyme CD73, impairing their capacity for extracellular purine salvage.[282] Newborn naïve B cells demonstrate adult-level expression of TLRs and CD40, but responses to stimulation of these receptors are distinct, including (1) impaired neonatal TLR2- and TLR7-mediated as well as (CD40+ TLR)-mediated but enhanced TLR9-mediated cytokine production and (2) impaired CD40-mediated Ig secretion.[283] With respect to the quantity of B cells, absolute B-cell numbers double in the first 6 months of life from those at birth, mainly due to expansion of fully mature naïve B cells and, to a lesser extent, transitional B cells.

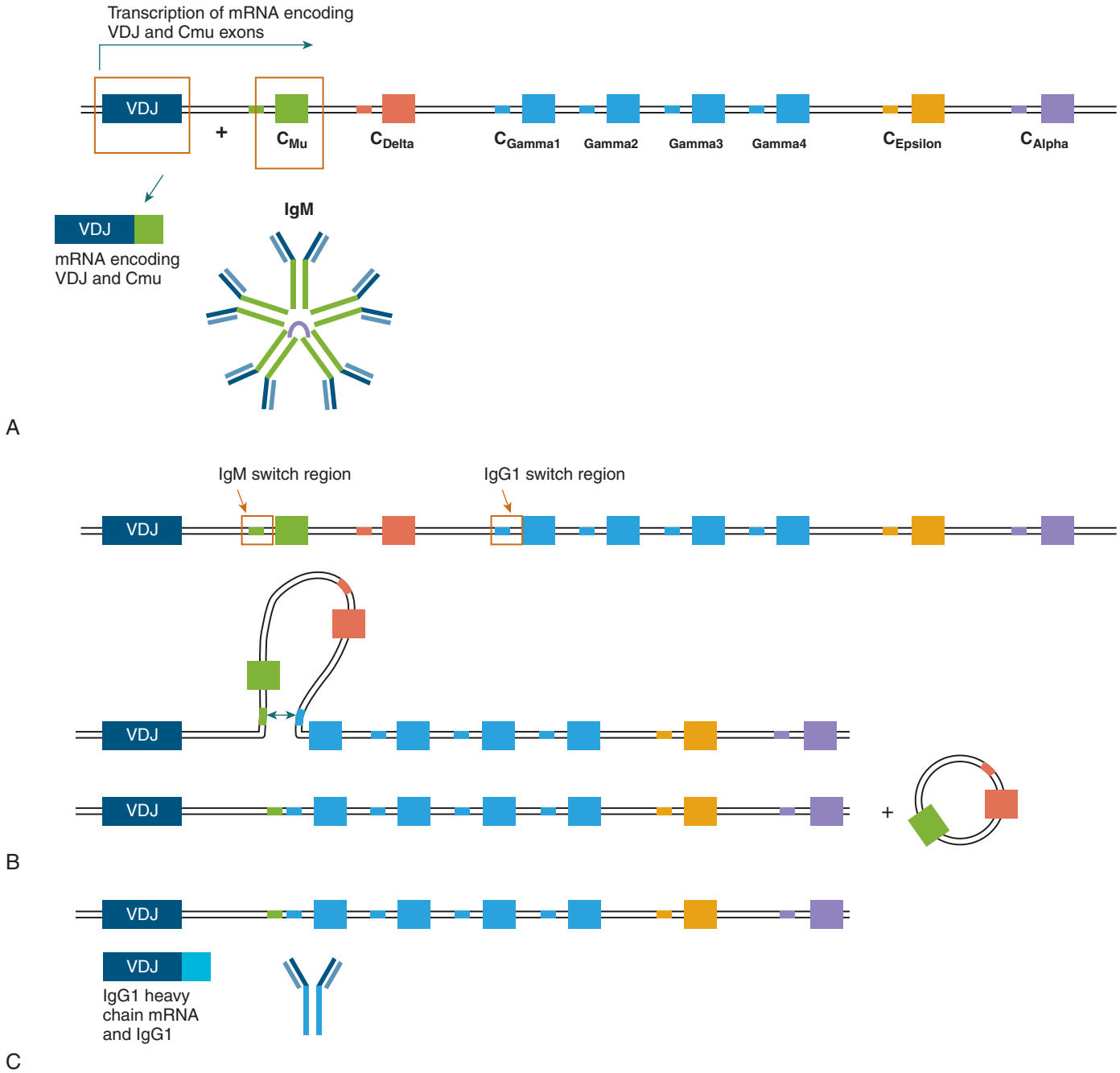
B-Cell Activation, Somatic Hypermutation, and Isotype Switching

When mature naïve B cells contact antigen through the BCR, a signal is transduced that promotes further growth and differentiation into memory B cells and plasma cells. BCR signaling involves activation of the tyrosine kinases spleen tyrosine kinase (Syk) and Btk, which are also involved in pre-BCR signal transduction, which results in transcriptional regulation by Elk-1, c-Myc, NF-κB, and NFAT.[284] The T-dependent B-cell response to protein antigens involves the internalization of protein bound to the BCR and its processing into peptides that are loaded onto MHC class II molecules. These peptide/MHC class II complexes that are expressed on the B-cell surface activate CD4 T cells via their TCR, resulting in a series of CD4 T cell/B-cell interactions, including between CD40L and CD40 receptor, between IL-21 and IL-21 receptor, between ICOS and ICOS ligand.

Both the B cells activated by protein antigen and the peptide antigen-specific CD4 T cells they activate and interact with enter the follicles of secondary lymphoid tissue, where these interactions continue. The activated B cells undergo massive clonal expansion and differentiation giving rise to histologically evident germinal centers.[285] Most B cells in the light zone of the germinal center undergo somatic hypermutation of the antigen combining site regions encoded by productively rearranged V, D, and J segments of the Ig heavy chain gene and the V and J segments of the light chain gene. Somatic hypermutation, which requires activation-induced cytidine deaminase and error-prone DNA polymerases expression by germinal center B cells, results in random point mutations that may lead to amino acid substitutions, premature

stop codons, or, less commonly insertions or deletions that result in frameshift mutations. B cells that have damaged immunoglobulin receptors as a result of somatic hypermutation are eliminated by apoptosis in the light zone.[286] B cells then enter the dark zone of the germinal center where B cells with BCRs with amino acid substitutions that have the highest affinity for antigen survive and those with lower affinity receptors die by apoptosis.[286] This affinity maturation process dramatically increases the ability of antibody to perform effector function, such as neutralization or opsonization. The peak of somatic mutation is approximately 10 to 12 days after immunization with a protein antigen.

Human B cells produce five isotypes of antibody (i.e., IgM, IgD, IgG, IgA, and IgE). The IgG and IgA isotypes can be, respectively, divided into the IgA1 and IgA2 subclasses and the IgG1, IgG2, IgG3, and IgG4 subclasses. During their process of differentiation from naïve B cells into memory B cells or plasma cells, B cells are able to change from expressing IgM to expressing other antibody isotypes without changing antigen specificity. This switching involves isotype recombination, the genetic replacement of the IgM-specific portion of the constant region (C_μ) of the heavy chain with a new isotype-specific gene segment (Fig. 32.10). As in V(D)J recombination, the intervening DNA



• **Fig. 32.10** Variable (V), Diversity (D), and Joining (J) Gene Segment Recombination. (A) Transcription of messenger RNA (mRNA) encoding VDJ and constant C_μ exons. (B) Recombination highlighting the immunoglobulin M (*IgM*) and the immunoglobulin G1 (*IgG1*) switch regions. (C) IgG1 heavy chain mRNA and protein.

is excised as a circle. Isotype recombination is mediated by switch regions that are positioned immediately upstream of each of the isotype-specific C regions. Successive multiple isotype switching by a single B cell can also occur—for example, IgM to IgG and then, on reactivation by antigen, IgG to IgE. Cytokines secreted by T cells or other cell types play an important role in promoting or inhibiting switching to a specific isotype. For example, IL-4 or IL-13 is required for isotype switching to IgE, a process that can be inhibited by the presence of IFN-γ.

IgA, which is the predominant Ig isotype secreted into mucosal secretions, has a distinct CD4 T-cell-independent and CD40L-independent pathway for its generation by isotype switching that utilizes the TNF ligand superfamily—a proliferation-inducing ligand (APRIL) and BAFF. These cell surface proteins, which are expressed by a variety of cell types, including intestinal epithelium, may play an important role in the development of IgA-secreting plasma cells in the gut in response to commensal bacteria.

Immunoglobulins

Igs, or antibodies, are a heterogeneous group of proteins that are detectable in plasma and body fluids and on the surfaces of mucosal barriers and B cells that bind specifically to antigens. The functions of Igs relevant to fetal and neonatal immunity are summarized in Table 32.2. There are five known classes of Igs: IgG, IgM, IgA, IgE, and IgD. Human IgM circulates as a pentamer or hexamer, and IgA circulates as a dimer. Multimers are formed in association with an additional J chain. The functions of individual Ig classes are different but overlapping (see Table 32.2). The prototype Ig molecule consists of a pair of identical heavy chains that determine the Ig class in combination with a pair of identical light chains, which are linked by disulfide bonds (see Fig. 32.9). Each Ig molecule contains two N-terminal, identical domains with antigen-binding activity that are formed by the variable regions of a heavy chain and a light chain. The antigen-binding site is formed by three complementarity-determining regions: CDR1, CDR2, and CDR3, which are hypervariable in their amino acid sequences as a result of V(D)J recombination of the heavy and light chain genes and, in some instances, somatic hypermutation. The C-terminal region of the two heavy chains forms the Fc (fraction crystallizable) region, which is not involved in antigen binding but mediates Ig effector functions. The principal functions of the Fc region include receptor-mediated phagocytosis (IgG), antibody-dependent cellular cytotoxicity (IgG), release of inflammatory mediators from mast cells and basophils (IgE), receptor-mediated transport through mucosa (IgA and IgM) and placenta (IgG), and complement activation (IgG1, IgG2, IgG3, and IgM). The five different isotype classes of human Igs (IgG, IgM, IgA, IgD, and IgE) are defined structurally by differences in the heavy chain constant regions (CH)1 and CH2 domains of the Fc fragments. Within isotypes, there are four IgG subclasses (IgG1, IgG2, IgG3, and IgG4) and two IgA subclasses (IgA1 and IgA2).

Immunoglobulin G

IgG is the predominant Ig isotype at all ages.[287] In adults, IgG1 is the predominant subclass, accounting for approximately 70% of total IgG, and IgG2, IgG3, and IgG4 account for ~20%, 7%, and 3% of the total, respectively. Passively derived maternal IgG is the source of virtually all of the IgG subclasses detected in the fetus and neonate. Because the IgG plasma half-life is about 21 days, these maternally derived IgG levels fall rapidly after birth. The levels of IgG synthesized by the neonate and that are derived from the mother are approximately equal at 2 months of age, and by 10 to 12 months of age nearly all of the IgG is infant derived. IgG values typically reach a nadir of approximately 400 mg/dL in term infants at 3 to 4 months of age and rise thereafter. Premature infants have lower IgG concentrations at birth that typically reach a nadir at 3 months of age; mean IgG values of 82 and 104 mg/dL are observed in infants born at 25 to 28 weeks' gestation and 29 to 32 weeks' gestation, respectively.

Murine studies suggest that commensal microbiota-specific serum IgG responses are frequent in adults and appear to arise by T-independent type 1 response involving engagement of B-cell TLRs. In pregnant mothers, these IgG antibodies can be transferred to the fetus via the FcRN and after birth appear to play an important role in limiting commensal bacterial translocation and potentially harmful anti-commensal T-cell responses.[288] This role for maternally-derived IgG may be particularly important early after birth prior to the full establishment of IgA-specific responses after 4 weeks of age.[289] Maternally derived anti-commensal IgG and IgA antibodies in breast milk may also play an important role in limiting undesirable anti-commensal immune responses.[290]

Other murine studies suggest that maternal IgG antibodies can inhibit neonatal and infant responses to vaccination in a titer-dependent manner by acting on germinal centers to reduce the output of memory B cells and plasma cells and altering the BCRs that emerge from somatic hypermutation.[291] These mechanisms may be relevant to vaccination in humans, as the titer of maternal antibodies in young infants is associated with impaired antibody responses for a variety of different childhood vaccines, including those that are inactivated or live-attenuated.[292]

Immunoglobulin M

IgM is the only isotype besides IgG that binds and activates complement. IgM has a half-life in the blood of 5 days and exists in either a pentameric or hexameric form, which confers a high avidity for antigen and highly efficient complement activation. The concentration of IgM in the blood increases from a mean of 6 mg/dL in infants born at less than 28 weeks' gestation to 11 mg/dL for those born at term.[293] This IgM is likely to be preimmune and not the result of a B-cell response to foreign antigens. Rather, it is likely enriched for polyreactive natural antibodies that may play an important role in innate defense against infection. Postnatal IgM concentrations rise rapidly for the first month and then more gradually, presumably in response to antigenic stimulation in both premature infants and term infants. By 1 year of age, the values are ~60% of those in adults. Because maternal–fetal transport of IgM does not occur, elevated (>20 mg/dL) IgM concentrations in UCB suggest possible intrauterine infections, although many infants with congenital infections have normal values.

Immunoglobulin A

IgA does not cross the placenta, and its concentration in UCB is usually 0.1 to 5.0 mg/dL, approximately 0.5% of the levels in maternal sera.[293] The concentrations are similar in term and premature neonates,[294] and both IgA1 and IgA2 are present. IgA has a half-life of 6 days in blood. Secretory IgA can be detected in the saliva of neonates as early as 3 days after birth.

The concentrations of IgA in serum increase to 20% of adult levels by 1 year of age and rise progressively through adolescence. Increased UCB IgA concentrations are observed in some infants with congenital infection, such as toxoplasmosis, and are common in those infected with HIV by vertical transmission. The amount of IgA produced daily is estimated to exceed that of all Ig isotypes combined. The overall importance of IgA in host defenses is only

TABLE 32.2 Characteristics and Functions of Immunoglobulins

	Molecular Mass (kDa)	Serum Concentration (g/L)	Serum Half-Life (Days)	Neutralization	Opsonization	Complement Activation	Epithelial Transport	Placental Transport	Sensitization for Killing by Natural Killer Cells	Sensitization of Mast Cells and Basophils
IgG1	150	10	21	++	+++	Strong classic, alternative	–	+++	++	+
IgG2	150	5	21	++	(+)	Classical, alternative	–	+	–	–
IgG3	170	1	7	++	++	Strong classical, alternative	–	++	++	+
IgG4	150	0.5	21	++	+	Alternative	–	(+)	–	–
IgA1	160	3	7	++	+	Alternative	+++	–	–	–
IgA2	160	0.5	7	++	+	Alternative	+++	–	–	–
IgM	900	2	5	+	+	Strong classical	+	–	–	–
IgD	180	0.03	3	–	–	Alternative	–	–	–	–
IgE	190	0.00003	3	–	–	–	–	–	–	+++

Ig, Immunoglobulin.
Modified from Mix E, Goertsches R, Zett UKL. Immunoglobulins: basic considerations. *J Neurol.* 2006;253:V9–V17.

partially understood, as individuals with complete IgA deficiency are typically asymptomatic. Recent studies suggest that mucosally secreted IgA may play an important role in regulating the composition of the human gut microbiome by a number of potential mechanisms, including suppressing or enhancing bacterial growth.[289,295] In cases of IgA deficiency, this role in regulating gut microbiota may be compensated for by mucosally secreted IgM.

Immunoglobulin E

Although IgE synthesis by the fetus is detectable as early as 11 weeks, the concentrations of IgE in UCB are typically low, with a mean of ~0.5% of maternal levels.[293] IgE concentrations are higher in infants born at 40 to 42 weeks' gestation than in those born at 37 to 39 weeks. The rate of postnatal increase differs and is greater in infants predisposed to allergic disease or greater environmental exposure to allergens.[296] The concentration of IgE at birth appears to have limited predictive value for later development of atopic disease for most individuals. Maternofetal transfer of IgE appears to be a common cause of elevated IgE concentration at birth.[297] Based on murine experiments, one potential mechanism for such transfer may be IgG anti-IgE complexes being transported via FcRN, which has substantial affinity only for IgG.

Immunoglobulin D

IgD is mainly produced by plasma cell derivatives of B cells from the aerodigestive mucosa that have undergone isotype switching from the Cμ to Cδ constant switch region and have an IgM–IgD+ surface phenotype.[273] IgD is detectable in serum from the UCB of term and premature infants, with mean levels of approximately 0.05 mg/dL.[293] These levels increase during the first year of life. Along with the IgA, IgM, and IgG isotypes, secreted IgD is enriched at mucosal sites of chronic infection and inflammation, such as the lung and tonsils, and can play a role in host defense by binding to pathogens as well as in microbial homeostasis by binding to some commensal microbes.[298] IgD binds to basophils via CD44 and galectin 9, and its engagement by antigen results in basophil activation and tissue infiltration. Elevated levels of IgD are also associated with certain monogenic autoinflammatory disorders, such as mevalonate kinase deficiency and familial Mediterranean fever.

Specific Immunologic Deficiencies of the Newborn

The most common reason for increased immunologic susceptibility to infection in newborns, besides prematurity, is iatrogenic immunosuppression caused by administration of corticosteroids for treatment or prevention of bronchopulmonary dysplasia. Such therapy is particularly associated with an increased risk of fungal infection, such as candidemia, particularly in the setting of indwelling intravascular catheters used for total parenteral nutrition.

Although less common than therapeutically induced immunodeficiency, genetically inherited primary immunodeficiencies (PIDs) can present in the neonatal period and may require prompt intervention for optimal outcome. There are approximately 450 recognized PIDs,[299] most of which are monogenic disorders.[300] The physician should attempt to differentiate infants with specific genetically regulated immunologic deficiencies from those with developmentally regulated, environmentally induced, or infection-related susceptibility to microbial invasion.[301] Documenting a full family history during a prenatal visit, including whether there is consanguinity or the family is of an ethnicity that is known to have a high incidence of a particular PID (e.g., those of Athabascan Indian heritage and SCID caused by Artemis deficiency), can be helpful in identifying families who may have a PID. However, the lack of such history does not exclude PID, as a patient can have de novo mutations causing PID. These de novo mutations are not present in either the biological mother or father or siblings and are the result of a mutation in the egg of the mother or sperm of the father, or in rare instances in the fertilized egg itself. Such de novo mutations are increasingly recognized as result of whole-exome sequencing of the biological mother, biological father, and affected child trios.[302] What follows is a discussion of specific PIDs that may present in the newborn period and are important to recognize promptly.

Severe Combined Immunodeficiency Syndrome

Epidemiology

SCID, which occurs in ~1 in 58,000 live births in the United States, is mainly an inherited severe immunodeficiency with marked deficiency of the de novo production by the thymus of antigenically naïve (CD45RA+CD45RO−) T cells with profound T-cell lymphopenia as a result. For a few etiologies, naïve peripheral T cells may be present in normal or near normal numbers that are unable to carry out immune function. The frequency of SCID in live births may be substantially higher in regions with high consanguinity, for example, parts of North Africa, the Middle East, and Southern India.

Pathophysiology

In SCID with profound T-cell lymphopenia there is either a reduced intrathymic production (most etiologies) or, rarely, a block in their egress from the thymus to the periphery [coronin 1A (CORO1A) deficiency.[303] The "combined" term in SCID reflects the fact that severe T-cell deficiency invariably compromises B-cell function, even if B cells are present in normal numbers. This is because CD4 T-cell help provided by T follicular helper cells in the form of surface CD40 ligand and secreted cytokines, such as IL-21, is required for B cells to produce antibodies to proteins. In addition, some forms of SCID result in the development of peripheral B cells that are functionally defective. In SCID, there is variable loss of NK cell numbers and other ILCs, depending on the specific gene defect involved. Thus, phenotyping by flow cytometry for T-cell, B-cell, and NK-cell subsets is useful in assessing the genetic etiologies and disease mechanisms that are likely to be involved.

SCID can be due to single X-linked or biallelic autosomal recessive (AR) gene defects (hereafter the specific genes involved in disease are capitalized and italicized).[303–306] Much less commonly, SCID can result from heterozygosity for gain-of-function mutations that inhibit hematopoietic cell function (e.g., certain Rac2 deficiency cases).[307] Most types of SCID due to monogenic defects are hematopoietic cells autonomous with the disease potentially curable by hematopoietic stem cell (HSC) transplantation or HSC-based gene therapy. There are a few rare genetic etiologies of SCID gene in which thymic hypoplasia and peripheral T-cell lymphopenia are due to defective thymic epithelial cell development, and these cases are important to identify as their definitive treatment requires a thymus transplant (see DiGeorge Syndrome section, below).

X-linked SCID, which comprises ~40% to 50% of cases of SCID in most series, is a form of T-B+NK- SCID in which T cells and NK cells are very low/absent in the peripheral blood but B cells are present in normal to increased numbers. X-linked SCID is due to deficiency of the IL-2 receptor gamma chain gene (*IL2RG*), which encodes for CD132, the common gamma chain protein. CD132 is an obligatory component of receptors for IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21. The IL-7 receptor, which consists of the IL-7R alpha chain (CD127) and CD132, is critical for thymocytes to grow in response to IL-7 such that *IL2RG* deficiency results in arrested T-cell development.[303,306] Although B cells develop normally and are present in normal or increased numbers, their lack of functional receptors for IL-21 results in their inability to respond to IL-21-mediated help for immunoglobulin production.[308] Deficiency of CD132 also results in the loss of functional surface receptors for IL-15, which results in NK-cell developmental arrest in the bone marrow.[303,306] T-B+NK- SCID can also result from AR inheritance of biallelic defects of the JAK3 gene, which encodes Janus kinase-3 that is associated with the cytoplasmic domain of CD132.[303,306]

Patients with $T^-B^-NK^+$ SCID have biallelic mutations of genes that encode proteins that are required for TCR and Ig gene rearrangement by V(D)J recombination, including RAG1, RAG2, Artemis (*DCLRE1C*), DNA-dependent protein kinase catalytic subunit (*PRKDC*), Cernunnos (*NHEJ1*), and DNA ligase IV (*LIG4*).[309] These mutations prevent the formation of pre-TCRs on thymocytes and pre-BCRs on immature B cells, resulting in early T-cell and B-cell maturational arrest and an absence of peripheral T cells and B cells. Except for RAG1 and RAG2, these proteins are also involved in non-homologous end-joining (NHEJ) gene repair for all cell types and these patients are highly susceptible to toxicity from irradiation that is sometimes used for HSC transplantation conditioning.

$T^-B^+NK^+$ SCID with severe T-cell lymphopenia and an AR inheritance pattern can be due to defects in the ability of the thymocyte TCRs to transmit intracellular survival or activation signals (e.g., biallelic genetic deficiencies encoding CD3-δ, CD3-ε, TCR-zeta [CD147], and CD45) or thymic epithelium differentiation is impaired (e.g., biallelic *PAX1* or *FOXN1* gene defects). A SCID-like clinical presentation with severe T-cell lymphopenia may occur for some causes of AR T-cell immunodeficiencies that more often present with a less severe phenotype and retention of greater numbers of T cells, such as cartilage hair hypoplasia syndrome (*RMRP*), zeta-associated protein of 70-kilodaltons kinase (*ZAP70*) deficiency, purine nucleoside phosphorylase (*PNP*) deficiency, sarcoma homology 2 domain-containing leukocyte protein of 76 kilodaltons (*SLP76*) deficiency, Coloboma, Heart defects, choanal Atresia, growth Retardation, Genital abnormalities, and Ear abnormalities (CHARGE) syndrome (*CHD7*), or tetratricopeptide repeat domain 7 deficiency (*TTCA7*) with intestinal atresias.[305] This T-B+NK+ SCID phenotype also occurs in coronin 1A deficiency, in which there is a block in emigration of mature thymocytes into the periphery but otherwise normal intrathymic T-cell development.

The $T^-B^-NK^-$ SCID phenotype is due to biallelic mutations in genes encoding the enzymes adenosine deaminase (*ADA*) and adenylate kinase 2 (*AK2*). Patients who lack ADA, an enzyme in the purine salvage pathway, accumulate deoxyadenosine triphosphate in red blood cells and lymphocytes, and the concentration correlates with disease severity. The ADA substrates, adenosine and deoxyadenosine, are found at increased levels in the serum. Developing thymocytes are particularly sensitive to these metabolic effects. ADA deficiency may also result in neonatal hepatitis, renal dysfunction, bone abnormalities (including flaring of the costochondral junction as seen on the lateral chest radiograph [termed *rachitic rosary*] and pelvic dysplasia), sensorineural hearing impairment, and cognitive impairment. Patients with AK2 deficiency, which is also known as *reticular dysgenesis*, have severe neutropenia and also sensorineural hearing impairment.

An AR T^+ SCID-like phenotype with severe immunodeficiency and opportunistic infections can also result in cases in which the production of T cells by the thymus is normal or modestly impaired but the peripheral T cells have severe functional defects. These defects can either be T-cell intrinsic, such as biallelic mutations of the genes encoding caspase recruitment domain family member 11 (CARD11), CD3γ, or the calcium channel proteins Stim1 and Orai1 that are involved in T-cell signaling, or that are T-cell extrinsic and are due to a lack of MHC class II–restricted antigen presentation. MHC class II deficiency is due to biallelic mutations of the regulatory factor X family member genes *RFXANK*, *RFXAP*, and *RFX5*, or of *CIITA*, which all encode proteins that positively regulate the transcription of the MHC class II genetic loci.[310]

T^+ SCID in which B cells are markedly decreased and NK cell numbers are normal can also occur in Omenn syndrome, which often presents in the neonatal period with diarrhea, intense erythroderma, alopecia, hepatosplenomegaly, and lymphadenopathy. Omenn syndrome was first described in cases of biallelic hypomorphic missense mutations of the *RAG1* gene or the *RAG2* gene but has also been reported in cases of SCID caused by *IL2RG*, *LIG4*, *IL7R*, *ADA*, *DCLREIC*, *AK2*, *RMRP*, *ZAP70*, *CHD7*, or *CARD11* mutations. Regardless of the genetic cause, the hematologic and immune phenotype is characteristic, with markedly increased eosinophilia, highly elevated serum levels of IgE, increased numbers of CD4 T cells, and markedly reduced numbers of CD8 T cells. Unlike CD4 T cells in healthy newborns, the CD4 T cells of Omenn syndrome are uniformly $CD45R0^+$ and lack CD45RA expression.

Mutations of genes that cause SCID that are hypomorphic (i.e., preserve some degree of normal protein function) can result in atypical forms in which the T-cell numbers may range from being only moderately depressed to normal values (e.g., certain missense mutations of the *IL2RG* gene in X-linked SCID).

T^+ SCID can also be the result of the engraftment of maternal T cells into the fetus. The clinical consequences of maternal engraftment can range from asymptomatic to a mild erythematous rash to full-blown symptoms of graft-versus-host disease (e.g., severe dermatitis, gastroenteritis, hepatitis, and lung disease). Maternal T-cell engraftment can also result in hemophagocytic syndrome, which can lead to secondary changes in the numbers of other cell types, such as B cells, NK cells, and neutrophils, which can obscure the SCID diagnosis.

Clinical Presentation

Most infants with SCID appear healthy at birth. In the neonatal period, a morbilliform rash, probably the result of attenuated graft-versus-host disease from transplacental passage of maternal lymphocytes may be the only symptom of SCID. Maternal T-cell engraftment may also result in hemophagocytic lymphohistiocytosis syndrome, with secondary cytopenias, such as neutropenia, predisposing to bacterial sepsis. Cases of SCID complicated by Omenn syndrome typically present as with severe erythroderma

and marked lymphadenopathy and hepatosplenomegaly. During the first several months of life, as acquired maternal antibody levels drop, failure to thrive and undue susceptibility to infection become universal features. Intractable diarrhea, pneumonia, and persistent thrush, especially oral thrush, constitute the triad of findings most frequently seen in infants with this disease. A diffuse pneumonia with severe hypoxemia due to *Pneumocystis jirovecii* is a classic presentation of SCID and usually occurs between 3 and 6 months of age although it rarely may occur in the neonatal period. CMV either acquired by passage through the birth canal or by breast-feeding can result in a severe/fatal disease that includes severe pneumonia or CNS involvement. Severe adenovirus pneumonia and/or hepatitis may also be a SCID presentation. Patients with SCID who receive live rotavirus vaccine, which is routinely first administered at 2 months of age, often develop persistent shedding and disease,[311] and such symptoms should raise the suspicion of SCID or other severe T-cell immunodeficiency. Similarly, BCGosis (disseminated infection with BCG) following neonatal vaccination should raise the possibility of SCID or other T-cell immunodeficiency disorders.[312]

Other manifestations that are characteristic of specific SCID etiologies include boney abnormalities (rachitic rosary) and noncholestatic hepatitis found in some cases of *ADA* deficiency, sensorineural hearing loss in *ADA* and *AK2* deficiency, intestinal atresias in *TTC7A* deficiency, skeletal dysmorphic features in cartilage hair hypoplasia and PAX1 deficiency, the absence of hair in cartilage hair hypoplasia syndrome and *FOXN1* deficiency.

Evaluation

The diagnosis of SCID is often suggested by an opportunistic or unusually severe infection in the setting of profound lymphopenia (<1000 lymphocytes per microliter). Only 10% of patients with SCID have lymphocyte counts in the normal range. Flow-cytometric analysis of T-cell, B-cell, and NK-cell populations, including the enumeration of CD4 and CD8 T cells and their naïve (CD45RA) and memory (CD45R0) subsets, is an essential part of the early workup. Based on the results of flow cytometry, the patient can often be assigned to one of four characteristic groups (T-B+NK-; T-B-NK+, T-B+NK+, and T-B-NK-) that point toward potential genetic etiologies. However, patients with high levels of maternal T-cell engraftment, hypomorphic mutations, for example, for *IL2RG*, Omenn syndrome, for forms of T+B+NK+ SCID may present a challenge in such an assignment. Clues to maternal engraftment include the finding of uniform expression of CD45R0 by the circulating T cells, and unusual CD4 T cell to CD8 T cell ratios. Omenn syndrome is suggested by normal or increased numbers of CD4 T cells that are uniformly CD45R0, decreased or absent CD8 T cells and B cells, and marked elevation of total serum IgE. The involvement of a clinical immunologist with expertise in primary immunodeficiency is strongly recommended early on in the diagnostic and treatment process given the potential complexity of interpretation and the importance of decisions on early therapy.

Other useful tests include measurements of ADA and purine nucleoside phosphorylase activity in red blood cells. Quantitative Ig levels are not particularly helpful in the diagnosis of neonatal SCID, because most IgG is maternal in origin, and IgA and IgM levels are often low in the neonatal period. Omenn syndrome may be suggested by elevation of IgE in conjunction with characteristic flow cytometry phenotypic features. A determination of T-cell mitogen responses, including to PHA, is helpful as a baseline for patients who will undergo HSC transplantation, as the PHA response is frequently used clinically to measure post-transplant T-cell immune reconstitution.

Routine neonatal screening for SCID using Guthrie card blood spots is carried out in all states of the United States, Puerto Rico, and the Navajo Nation.[313,314] The test is based on the real-time PCR detection of sjTRECs, generated during V(D)J recombination of the TCR α/δ gene locus (see Fig. 32.7) in total DNA isolated from the blood spots. sjTREC screening is effective at identifying most causes of SCID in which levels are markedly low in most instances. However, there are a few disorders in which sjTREC levels are normal, such as MHC class II deficiency and CARD11 deficiency, whereas sjTREC levels may be low in some otherwise healthy premature born infants. The turnaround time of the test is usually about 1 to 3 weeks. Abnormally low values of sjTRECs should lead to prompt flow-cytometric evaluation of lymphocyte populations as described above.

The importance of making a specific genetic diagnosis of SCID cannot be overemphasized, as this may have an important influence on expediting appropriate definitive therapy, for example, HSC transplantation versus thymic transplantation or HSC transplantation versus lentiviral gene therapy. Next generation sequencing panels for monogenic inborn errors of immunity include almost all of the known genetic etiologies of SCID disorder and can be very useful in helping target later confirmative Sanger sequencing.

Early consultation with an expert clinical HSC transplantation team is also highly recommended. In preparation for potential HSC transplantation, HLA typing of the patient and their parents is recommended as is an evaluation for maternal chimerism. Maternal engraftment can be readily diagnosed by means of T-cell chimerism assays, such as the polymerase chain reaction (PCR)-based short tandem repeats/variable number tandem repeats (STR/VNTR) system.[315]

The thymus gland is not seen on chest radiographs except in some cases of T^+ SCID or T-B+NK+ SCID because of defects in thymocyte egress, such as coronin 1A deficiency. The lack of thymus tissue by chest radiograph or computed tomography is not specific for a SCID disorder, and may occur in patients who appear well and who have relatively subtle abnormalities based on flow cytometry, for example, ataxia telangiectasia. Most of these monogenic non-SCID disorders can be readily diagnosed as part of the commercially available next generation sequencing (NGS) panels.

The possibility of complete DiGeorge syndrome (see section below) should be considered in SCID patients who have a $T^-B^+NK^+$ phenotype and/or who have features such as facial dysmorphism, congenital heart disease, velocardial insufficiency, or hypocalcemia/hypoparathyroidism. Since patients also have been identified having both DiGeorge syndrome from a hemizygous 22q11.2 deletion and SCID from a genetic deficiency, we routinely send the 22q11.2 deletion fluorescent *in situ* hybridization (FISH) chromosomal analysis on all patients being evaluated for SCID.

Management

After SCID is diagnosed and appropriate blood samples for total and antigen-specific immunoglobulin levels have been obtained, intravenous immune globulin (IVIG) usually is administered pending a definitive treatment plan. Only irradiated CMV-negative blood products should be administered. All live vaccines, including oral poliovirus vaccine, rotavirus vaccine, measles, mumps rubella (MMR) vaccine, varicella vaccine, vaccinia, BCG, attenuated influenza vaccine, and oral *Salmonella*

Typhi vaccine, are contraindicated. IVIG therapy obviates the benefit of vaccination with "killed" vaccines (e.g., inactivated poliovirus vaccine) by providing passive antibody. Therefore, inactivated or protein/polysaccharide component vaccines, although safe in SCID and other immunodeficiencies, typically are not administered after IVIG therapy is begun. CMV infection can be transmitted to the infant by breast milk or during passage through the birth canal and cause severe complications. For all infants with suspected or diagnostically confirmed SCID, the mother's CMV serostatus should be determined and the infant's CMV infection status should be determined by blood PCR. If the mother is seropositive, any breast feeding should be discontinued. Confirmed or suspected SCID patients should be put into an appropriate level of high-grade protective isolation, Detailed approaches used by expert clinicians for prophylaxis of herpesviruses, RSV, pneumocystis, and fungal infections of infants with SCID are available.[316]

SCID treatment usually requires allogeneic HSC transplant, ideally from an HLA-matched sibling.[317] In one approach, conditioning regimens, such as busulfan, are not used and recipients usually become chimeric, with only T and NK cells of donor origin. B-cell function is frequently deficient, and many patients continue to require Ig replacement therapy. As there is not replacement of totipotent HSCs of the recipient with those of the donor, there is eventually a drop-off in T-cell numbers, requiring that the patients receive a "boost" transplant from the original donor.[318] An alternative approach is to use standard conditioning regimens that result in the replacement of all hematopoietic cell types of the recipient with those of the donor, including HSCs. This approach has an increased risk of complications in the early posttransplant period but avoids the need for later transplants and often leads to normal B-cell function, obviating the need for potentially lifetime Ig replacement therapy. ADA deficiency can be treated with enzyme replacement. This treatment involves weekly injections of ADA coupled to polyethylene glycol. Response, consisting of decreasing deoxyadenosine triphosphate levels and increasing T-cell numbers, is seen in most patients within weeks. Finally, retroviral lentiviral gene therapy based on vector-mediated transfer of a therapeutic gene into autologous HSCs is a therapeutic option for the treatment of ADA SCID and is in late-phase clinical trials for X-linked SCID.[319] The correction of the T-cell immunodeficiency treatment for $T^-B^+NK^+$ SCID that is due to thymic epithelial abnormalities, for example, PAX1 or FOXN1 deficiency, CHARGE syndrome, and that requires thymic transplantation is discussed in the DiGeorge Syndrome section.

Outcome

SCID is a pediatric emergency and is invariably fatal if untreated. Most untreated patients die in the first year of life. HSC transplantation, which is the definitive therapy for most types of SCID has had excellent results in cases where an early genetic diagnosis is made. Nevertheless, there is still an appreciable mortality of ~5% with the HSC transplantation approach and further refinements with safe conditioning regimens and treatments for graft-versus-host disease may further improve this already excellent outcome. Recently published results for eight infants with X-linked SCID treated with lentiviral gene therapy suggest that this approach is safe, with a low risk to cause dysregulation of endogenous genes near the lentiviral insertion sites that could lead to oncogenesis, and is effective (based on immune reconstitution and the clearing of opportunistic infections).[320] The treatment of ADA SCID by lentiviral gene therapy has not encountered any major safety issues and has had a long-term outcome that appears to be equivalent or superior to that obtained with HSC transplantation.[321]

DiGeorge Syndrome (22q11.2 Deletion Syndrome)

Epidemiology

DiGeorge Syndrome, 22q11.2 deletion syndrome (22q11.2DS), is the most common human chromosomal microdeletion syndrome, occurring in approximately 1 in every 1000 fetuses and 1 in every 3000 live births. The syndrome affects both sexes equally and occurs in all major racial and ethnic groups. About 85% of cases are due to hemizygosity for a de novo stereotypical deletion of 3.0 mB between the low copy repeat (LCR) regions LCR22A and LCR22D. This 3.0 mB segment contains 90 known or predicted genes, including 46 protein-encoding genes, 7 genes encoding microRNAs, and 10 non-coding RNAs.[322] A quantitative T-cell immunodeficiency due to thymic hypoplasia occurs in up to 75% of patients that ranges from mild to moderate in severity more than 99% of the time. However, for unclear reasons, approximately 0.1% of patients with the 22q11.2DS and 3.0 mB deletion have a severe T-cell immunodeficiency with circulating naïve ($CD45RA^+CD62L^+$) T-cell numbers less than 50 per microliter.

Pathophysiology

The embryologic anlage of the thymus, parathyroid, and a portion of the great vessels is the endodermal epithelium of the third and fourth pharyngeal pouches.[322] When normal development of these structures is disturbed, thymic and parathyroid hypoplasia and congenital heart disease involving the great vessels can occur resulting in DGS. The molecular mechanisms by which 22q11.2 region haploinsufficiency perturbs thymic and parathyroid, great vessel, and cardiac development as well as that of other organs remains poorly understood. Haploinsufficiency of the TBX1 gene, which encodes the T-box1 transcription factor, appears to make a major contribution to pathogenesis, particularly for the effects on the thymus, parathyroids, and cardiac system, but there are many unanswered questions for why there is such variable penetrance of the DGS phenotype. Although the 22q11.2Del syndrome is the most likely cause of DGS, other etiologies include maternal gestational diabetes, maternal prenatal retinoic acid exposure, single gene disorders that include mutations in PAX1, TBX1 (which encodes the T-box1 transcription factor), or CHD7 (which results in CHARGE syndrome), or other chromosomal deletions, such as those involving the 10p13-14 region.[322] Like the 22q11.2Del syndrome, most of these etiologies usually result in a mild to moderate immune deficiency but can result in complete DGS with severe T-cell immunodeficiency; biallelic disease with PAX1 usually has a severe T-cell phenotype and less commonly presents with hypocalcemia/hypoparathyroidism.

Clinical Presentation

Infants with 22q11.2DS can exhibit abnormalities of calcium homeostasis during the neonatal period (hypocalcemia and tetany) and variable T-cell deficits, which are usually subclinical and only severe in less than 1% of cases. Often, the syndrome is suspected because of congenital conotruncal cardiac defects (about 60% of cases), low-set ears, midline facial clefts, hypomandibular abnormalities, and hypertelorism. The cardiac anomalies

associated with 22q11.2DS are variable but usually involve the outflow tract and the derivatives of the branchial arch arteries. These defects include interrupted aortic arch type B, truncus arteriosus, and tetralogy of Fallot. Children with 22q11.2DSalso exhibit a higher incidence of receptive-expressive language difficulties, cognitive impairment, and behavioral problems, including psychotic illness.[323,324]

Evaluation

About 95% of neonates and young infants presenting with clinical DGS will have the 22q11.2DS due to the 3.0 mB deletion and will be readily diagnosed by fluorescence in situ hybridization using standard probes.[322] Cases of DiGeorge syndrome lacking the 22q11.2 deletion should be evaluated for *TBX1* loss-of-function mutations, which can result in most of the features of DiGeorge syndrome, including T-cell immunodeficiency, hypoparathyroidism, and/or conotruncal cardiac lesions.[325] Other assays such as comparative genomic hybridization may reveal nested deletions that are distal to the clinically used FISH probes between LCR22B to LCR22D or between LCR22C to LCR22D or other chromosomal deletions that have been associated with DGS, for example, involving 10p13-14 and 11q23-ter. Clinical features suggestive of DiGeorge syndrome can also be observed in some cases of PAX1 deficiency and with *CHD7* mutations that cause CHARGE syndrome. CHARGE syndrome usually has additional phenotypic abnormalities, such as colobomas and choanal atresia that point to this as the etiology of DGS.

In the nursery, identification of infants with congenital conotruncal abnormalities or unexplained persistent hypocalcemia should prompt consideration of CHARGE. In most cases, the T-cell deficiency is not severe, with decreases in circulating CD4 T-cell and CD8 T-cell numbers to levels that are 25% to 50% of those normal for age. However, in about 1 in 400,000 deliveries, infants with features of DiGeorge syndrome may have severe T-cell lymphopenia—that is, circulating naïve ($CD45RA^+CD62L^+$) T-cell numbers less than 50 per microliter. Severe T-cell lymphopenia in association with hypoparathyroidism and conotruncal congenital heart disease is called *complete DiGeorge syndrome* and is due to the 22q11.2 deletion in about 50% of cases, CHARGE syndrome in about 25% of cases, and gestational diabetes in about 15% of cases.[326]

Management

Like SCID, complete DiGeorge syndrome is a medical emergency that prompts antibiotic prophylaxis for *P. jirovecii* and nontuberculous mycobacteria (azithromycin), Ig replacement therapy, and protective isolation. All patients should also be evaluated for their CMV infection status, and, if positive, antiviral therapy should be started. Patients who are not infected with CMV should not be breastfed if their mother is CMV IgG antibody positive and in cases of potential exposure to CMV from breastmilk, prophylaxis may include valganciclovir. About 30% of complete DiGeorge syndrome patients develop an extensive erythematous rash sometimes accompanied by gastrointestinal symptoms and hepatitis, which is referred to as *atypical complete DGS*. These patients often have increased levels of circulating T cells in their blood that express memory markers, such as CD45R0, with similar T cells found in skin biopsy. These T cells, which are produced by the patient and are not the result of maternal engraftment, are oligoclonal in their TCR usage and do not provide useful immune function. Patients harboring these autoreactive T cells should be treated with immunosuppressive drugs, such as calcineurin inhibitors and glucocorticoids, particularly if the T-cell lymphocytosis is marked and the patient has moderate to severe skin disease and extra-cutaneous sites are involved with T-cell mediated inflammation.

The only definitive therapy for complete DGS due to the 22q11.2 is transplant of thymic epithelial tissue. The transplant is derived from thymus tissue removed from otherwise healthy children as part of surgery for congenital heart disease and that is cultured for deplete thymocytes and enriched for the thymic epithelial cells.[327] Small pieces of thymic epithelia are placed within the quadriceps muscle, and the de novo production of CD4 and CD8 T cells begins approximately 4 to 6 months after transplant.

Outcome

The thymic transplant is successful in ~80% of cases, so that antibiotic prophylaxis, Ig replacement therapy, and protective isolation can be discontinued. Patients that have good engraftment with normalization of CD4 and CD8 T cell numbers for age can receive MMR vaccination. There is a relatively high risk of complete DGS patients developing autoimmune diseases, such as thyroiditis, following transplant, suggesting that the intrathymic mechanisms of tolerance, such as negative selection and/or Treg production, may not be as effective as the native thymus. In the absence of thymic transplant most patients with complete DGS often die by 3 years of age.

Combined Immune Disorders Involving T Cells and B Cells

Many monogenic immune disorders have complex phenotypes that involve both T and B cells in which the residual T-cell function is sufficient to result in a delayed presentation after the neonatal period. For example, Wiskott–Aldrich syndrome (WAS) is a combined immune disorder involving T cells, B cells, and APCs.[328] WAS affects males and is due to mutations of a gene located on the X chromosome that encodes WAS protein (WASp), an intracellular protein. WASp is expressed in all hematopoietic cells that interact with actin cytoskeleton and can also participate in transcriptional regulation. WAS is characterized by eczema (usually severe), thrombocytopenia, increased risk of malignancy, and susceptibility to recurrent sinopulmonary infections, severe herpesvirus infection, and, less commonly, classic opportunistic infections, such as infection with *P. jirovecii*. Other manifestations that may be present in the newborn period include petechiae and bruises, bloody diarrhea, and hemorrhage after procedures. In infants with any of these clinical findings, thrombocytopenia in a complete blood count report is an important clue to possible WAS. In contrast to immune thrombocytopenia purpura, WAS platelets have a low mean platelet volume, and this finding is pathognomonic of the disorder. Characteristic immunologic findings include moderately decreased numbers of T cells, particularly those of the CD8 subset, with relatively subtle defects in T-cell proliferation, decreased IgM levels, and increased IgA and IgE levels. Many of these immunologic findings may not be evident in the neonatal period. Flow cytometry can be used to evaluate leukocyte WASp expression to make a provisional diagnosis of WAS. As for children with SCID, all blood products given to children with WAS should be CMV negative and irradiated before administration to avoid T-cell engraftment and graft-versus-host disease. WAS can be cured by HSC transplant, with the best results obtained with transplant before 3 years of age. Lentiviral gene therapy is a promising alternative for treatment.[319]

B-Cell Immunodeficiencies

Most genetic defects that target B-cell immunity but leave T-cell immunity intact, such as X-linked agammaglobulinemia (XLA), are not detected in the neonatal period without special screening tests, such as flow cytometry to evaluate circulating B-cell numbers, intracellular expression of Btk by monocytes.[329] Because of maternal transfer of IgG during the last trimester of pregnancy, infants with selective immunodeficiencies of B cells typically do not develop clinically significant hypogammaglobulinemia and recurrent or severe sinopulmonary infections with encapsulated organisms until after 3 to 6 months of age. Moreover, the absence of B cells from the circulation does not usually result in clinically detectable lymphopenia on the complete blood count. Analogous to screening for severe T-cell deficiency, neonatal screening for quantitative B-cell immunodeficiency is possible by assaying by PCR for kappa receptor excision circles (KRECs), which are stable circular DNA byproducts of V(D)J rearrangement of the kappa light chain Ig gene in B-lineage cells.[330] Currently, KREC evaluation is not part of routine newborn screening in the United States.

Innate Immune Deficiency Disorders

Immune deficiencies that include neutropenia as part of a syndrome, such as SCID due to AK2 deficiency (reticular dysgenesis), or that result in isolated quantitative or qualitative defects of neutrophils often present in the neonatal period. This reflects the essential role of neutrophils in phagocytosing pathogenic bacteria and fungi and eliminating them by oxidative and nonoxidative mechanisms within the phagosome.[331] Bacterial infections, such as bacteremia with gram-negative organisms such as *Escherichia coli* or *Pseudomonas aeruginosa* or gram-positive organisms such as *Staphylococcus aureus*, are frequent with neutrophil disorders. Delayed umbilical cord separation and omphalitis are important clinical signs that should lead to evaluation for neutrophil deficiency, such as leukocyte adhesion defect, which is most commonly due to biallelic mutations of the *CD18* gene. An important clue to leukocyte adhesion defect is that the neutrophil count is always elevated above normal values, even in the absence of infection or other inflammatory stimuli. Chronic granulomatous disease (CGD) is due to mutations of genes encoding the phagocyte oxidase system, which is required for oxidative killing within the phagosome. The clinical infections characteristic of CGD, such as deep tissue infections with bacteria or fungi, can present during the neonatal period, although in most cases this occurs later in infancy.

Although relatively rare, monogenic immune deficiencies of innate immune cell signaling may present in the neonatal period with severe gram-positive or gram-negative bacterial infections or fungal infections. Among the best characterized are biallelic mutations of the genes encoding MyD88 or IL-1 receptor–associated kinase 4 (IRAK-4), which are cytoplasmic proteins that are required for both TLR and IL-1 receptor family (IL-1R) signaling.[332] The lack of IL-1R signaling results in an impaired febrile response, and the absence of any fever in a neonatal patient with a severe invasive bacterial or fungal infection should lead to an evaluation for these disorders. A useful screening test for MyD88 or IRAK-4 deficiency is to evaluate peripheral blood mononuclear cells for their production of TNF-α in response to incubation with TLR ligands, such as endotoxin, flagellin, or oligodeoxynucleotides containing unmethylated CpG residues. Such production is markedly impaired or absent in these patients.

Implications of Studies of Immune Ontogeny for Enhancing Neonatal Immunization

Hepatitis B vaccine, polio vaccines, and bacille Calmette–Guérin (BCG) vaccine are the only childhood vaccines routinely given at birth. Immunization shortly after birth offers important logistic advantages for providing protection against infectious diseases, as birth is the most reliable point of healthcare contact worldwide, especially in less developed countries.[333] However, the response of the neonate to vaccination with T-independent polysaccharide vaccines, such as unconjugated pneumococcal vaccine, is negligible and the humoral response to T-dependent antigens, such as proteins and protein–polysaccharide conjugate vaccines, is less robust and durable than that of the adult.[334] Thus, extending neonatal immunization to involve other routine childhood vaccines may require the development of formulations that are more immunogenic when administered in the first few days after birth.

A growing appreciation of distinct immunity with age has prompted efforts to develop vaccines that may induce robust protection in the newborn and the young infant.[335] One approach for improving the response to neonatal vaccination has been to combine vaccine antigens with adjuvants to enhance immune responses, focusing on adjuvants for which human neonatal APCs have robust adult-like responsiveness in vitro.[89,335,336] A promising example of this approach is to combine TLR agonists that are particularly active in early life such as imidazoquinoline TLR7/8 agonists with the 13-valent pneumococcal polysaccharide protein conjugate vaccine (Prevnar 13). Immunization of 1-day-old nonhuman primates with the combination of TLR7/8 agonist and Prevnar 13 resulted in a 10-fold to 100-fold increase in specific antibody titer that was associated with robust increases in the CD4 T-cell responses to the vaccine protein conjugate.[337] A similar approach could be potentially used for other inactivated vaccines that currently are routinely administered at 2 months of age. Together, these observations indicate compelling rationale to characterize early-life immune ontogeny to provide fresh perspectives on neonatal health and disease and inform novel approaches to protect the fetus and young infant.[1]

Acknowledgments

The authors acknowledge the writing by Calvin B. Williams, Eli M. Eisenstein, and F. Sessions Cole, the authors of a previous version of this chapter, on which this version is based in part.

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Neonatal Bacterial Sepsis and Meningitis

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KEY POINTS

- Group B streptococcus and *Escherichia coli* account for most of the cases of neonatal early-onset bacterial sepsis.
- Prevention of infection by maternal treatment is the main factor accounting for the decreased incidence of early-onset group B streptococcus sepsis but does not affect rates of late-onset group B streptococcus sepsis.
- Microbiologic blood cultures of adequate volume (at least 1.0 mL) represent the mainstay for the diagnosis of infection.
- Management of the asymptomatic newborn at risk of infection due to maternal risk factors is evolving, with evidence supporting evaluation and treatment of only a small proportion of asymptomatic infants.
- Ampicillin and gentamicin are recommended as initial therapy in neonates with suspected bacterial sepsis. Treatment can then be narrowed appropriately after the results of antibiotic susceptibility studies.

Neonatal sepsis is one of the leading causes of morbidity and mortality of newborns worldwide.[1] It is estimated that nearly 430,000 neonatal deaths occurred in 2013 due to neonatal sepsis, approximately 15% of all neonatal deaths. Thus, neonatal sepsis is a common and deadly disease process that requires an understanding of the pathogenesis and timely treatment to prevent severe morbidity or mortality.

Bacterial sepsis in newborns is commonly divided into two categories: early-onset sepsis (EOS) and late-onset sepsis (LOS) based upon time since birth. Traditionally, EOS is classified as either occurring in the first 72 hours or, in some cases, within the first 7 days after birth. In this chapter, we will define EOS as occurring within the first 72 hours after birth and LOS as occurring after 72 hours. Here, we will discuss early-onset neonatal bacterial infections, including the pathogenesis, epidemiology, clinical signs and symptoms, common bacterial pathogens, evaluation methodology, and prevention strategies. We will then explore late-onset neonatal bacterial infections and conclude with an evaluation of the medical literature surrounding neonatal bacterial meningitis.

Early-Onset Neonatal Bacterial Infections

Early-onset bacterial sepsis remains a major cause of neonatal morbidity and death, although the sepsis-associated death rates per 100,000 live births has declined significantly from 2000 to 2015. The introduction of intrapartum antibiotic prophylaxis (IAP) in pregnant women during labor and delivery contributed to much of this decline in mortality.[2–5] Mortality rates in infected premature and very immature neonates are significantly higher than those in term neonates. Significant improvements in neonatal intensive care and early identification of infected neonates have contributed to reduced mortality rates in the newborn period.

Pathogenesis of Early-Onset Neonatal Bacterial Infections

Vertical transmission is the main route through which EOS occurs and involves the passage of pathogenic bacteria from the mother to the neonate before or during delivery. Maternal chorioamnionitis and acute inflammation from presumed infection of the membranes surrounding the fetus in utero is a well-known risk factor for EOS.[6–9] Other potential routes for group B streptococcus (GBS) acquisition and EOS include ascending spread from the vaginal environment to the uterus following membrane rupture or fetal contact with pathogenic bacteria during passage through the birth canal at delivery. Less commonly, bacteria reach the fetus through the in utero transplacental passage, as suggested by the presence of high-grade bacteremia and severe sepsis that are clinically apparent at the time of birth in the presence of an intact membrane in neonates born via cesarean delivery.[10–12] Although organisms recovered from the amniotic sac in the mother are usually polymicrobial and include organisms such as GBS, group D enterococcus, aerobic gram-negative bacteria, and anaerobes such as *Bacteroides* spp., a single organism causing bacterial sepsis is the rule in sepsis of the newborn.[1,6,13]

Pathogenesis of neonatal sepsis is due to several key factors, including but not limited to timing and duration of exposure, inoculum size, the virulence of the pathogenic bacteria, and the immune status of the neonate. Although many microorganisms recovered from the amniotic cavity are thought to induce spontaneous preterm labor and possibly premature rupture of membranes, the exact mechanisms by which this may occur are debatable. Clinical or subclinical chorioamnionitis can incite a marked inflammatory response with the release of cytokines that can contribute to the onset of preterm labor and premature rupture of membranes. Other risk factors for clinical intra-amniotic infection include young maternal age, prolonged labor, prolonged rupture of membranes (≥18 hours), internal scalp fetal monitoring, the presence of urinary tract infections (UTIs), and a history of bacterial vaginosis.[14,15]

Epidemiology of Early-Onset Bacterial Infections

The incidence of early-onset bacterial infection is variable and ranges from 1 to 5 per 1000 live births.[16] Rates of neonatal sepsis are inversely related to gestational age. Notably, there has been a reduction in the rate of EOS in the United States since the implementation of IAP against GBS. Early-onset GBS infection rates in the United States have declined from 0.37 per 1000 live births in 2006 to 0.23 per 1000 live births in 2015.[17] However, despite IAP, late-onset rates have remained relatively stable at approximately 0.31 per 1000 live births.[17]

Infants with EOS frequently have one or more identifiable risk factors.[18,19] Prematurity is the single greatest risk factor for EOS. Because extremely low birth weight infants have impaired host defenses and since preterm birth may be associated with low-grade chorioamnionitis, it is not surprising that premature neonates have nearly 30 times higher rates of mortality from EOS compared to term neonates (1.6% in term neonates vs. 30% mortality in those at 25 to 28 weeks of gestation).[20–22] Neonatal susceptibility to GBS infection is increased with deficiencies in circulating levels of GBS type-specific antibody and complement, and is further heightened by neutrophil dysfunction seen in infants who are more premature.[23–26] Other risk factors for EOS are maternal age, health and nutrition, colonization with well-known pathogens (e.g., GBS), maternal fever, and longer duration of rupture of membranes.[19,27]

Bacterial Pathogens in Early-Onset Infections

The most common bacterial pathogens associated with EOS are GBS and *Escherichia coli*. Here we review these two primary pathogens in depth before briefly exploring other less common bacterial etiologies of EOS, such as *Listeria monocytogenes* and other gram-negative enteric bacilli.

Group B Streptococcal Infections

Transmission of GBS to Infants and the Role of Intrapartum Antibiotic Prophylaxis

GBS accounts for nearly 45% of culture-confirmed cases of EOS in term neonates and 25% among very low birth weight (VLBW) infants (weighing <1500 g at birth).[20,28] Approximately 20% to 30% of pregnant women in the United States are colonized with GBS.[29–33] Treatment of GBS-colonized women during pregnancy only temporarily eradicates the organism. Most women are recolonized within several weeks. Before the introduction of IAP, approximately 50% of neonates born to mothers known to be GBS carriers became colonized, and approximately 1% to 2% of colonized neonates developed GBS infection.[34–37] Since the widespread use of IAP, approximately 60% to 80% of GBS cases occur in infants born to mothers who screened negative for GBS.[38,39]

Detection of maternal GBS colonization has been emphasized since 1996, approximately. Early studies determined that the optimal sampling sites for GBS were the genitourinary and gastrointestinal tracts. The optimal time for performing prenatal cultures is between 36 0/7 and 37 6/7 weeks' gestation, and the highest culture yield is obtained when both the lower vaginal area and anal or rectal sites are sampled.[40,41] Furthermore, GBS testing should be performed on pregnant women who present in preterm labor or with the rupture of membranes (PROM) before 37 0/7 weeks' gestation. If GBS has previously been detected through urine culture during pregnancy, no further confirmation by vaginal-rectal culture is necessary.[40,41] By identifying the pregnant woman who is colonized with GBS, IAP can be provided in a timely manner. Since the introduction of IAP recommendations, the national incidence of GBS EOS has decreased from 1.8 cases (1990) to 0.23 cases (2015) per 1000 live births.[17] However, late-onset GBS incidence has not changed.

To prevent GBS EOS, IAP is recommended for all women with GBS colonization identified by antenatal recto-vaginal culture, with a history of GBS bacteriuria during the same pregnancy, with a history of a former infant with GBS disease, or for women who present in preterm labor or have PROM <37 0/7 weeks' gestation.[40,41] For women who present in labor ≥37 0/7 weeks' gestation with unknown GBS status should have IAP if maternal temperature ≥38°C or duration of rupture of membranes ≥18 hours.[40,41]

GBS is sensitive to penicillin, which is the drug of choice because of its narrow spectrum; the alternative is ampicillin. If a mother is allergic to penicillin but considered low-risk for anaphylaxis, the use of cefazolin is recommended. When patients are at a high risk of anaphylaxis, tests for antimicrobial susceptibility of prenatal GBS to clindamycin should be performed. In 2016, 42% of GBS isolates were found to be resistant to clindamycin.[40,41] Therefore if the prenatal GBS is sensitive to clindamycin, patients can receive clindamycin intravenously (IV) until delivery. Of note, erythromycin is no longer recommended as an IAP due to high resistance patterns.[40,41] When GBS is resistant to clindamycin, IV vancomycin every 12 hours until delivery is recommended. The high levels of resistance highlight the importance of antibiotic susceptibility testing on the group B streptococcal isolates from pregnant women. Only penicillin G, ampicillin, or cefazolin are considered adequate IAP.[40,41] If clindamycin or vancomycin are used, while likely capable of providing some protection against invasive early-onset GBS disease in the newborn, it is considered inadequate IAP coverage due to limited data on their clinical efficacy.

Group B Streptococcal Virulence Factors

There are 10 known serotypes of GBS (I, Ia, II-IX) that are distinguished by a specific polysaccharide capsule. The polysaccharide capsule helps the GBS evade complement deposition, opsonization, and phagocytosis by the infant's immune system and is considered the most important virulence factor.[42–45] Other virulence factors include proteases (such as C5a peptidase), which cleave complement and surface proteins (such as alpha and beta C-proteins) and promote evasion of human host defenses.[46] Serotype III is associated with an estimated 62% of invasive infant strains and 25% of colonizing strains and is also a common cause of GBS meningitis.[17,47] Globally, serotypes I to V are responsible for the vast majority, 98% of colonization and 97% of invasive infant strains.[31,48] Multivalent vaccines against specific capsular polysaccharides have been investigated through phase I and II clinical trials and demonstrated their safety and immunogenicity.[49–51] Further clinical trials are planned.

GBS Sepsis

While most EOS is classified as sepsis in the first 72 hours after birth, for GBS infection, early-onset disease (EOD) is classified in the first 7 days after birth. Subsequently, late-onset disease occurs from 1 week to 3 months of age, and very-late-onset disease occurs more than 3 months after birth (Table 33.1).[1] Although IAP has led to a significant decrease in the incidence of GBS EOD, there is no evidence that chemoprophylaxis prevents late-onset or very-late-onset disease.[52–54] GBS EOD is generally believed to be caused

TABLE 33.1 Manifestations of Early-Onset and Late-Onset Group B Streptococcal (GBS) Disease

Characteristic	Early Onset	Late Onset
Age at onset	Birth to 7 days of life	7 days to 3 months of life
Symptoms	Respiratory distress, apnea	Irritability, fever, poor feeding
Findings	Pneumonia, sepsis	Sepsis, meningitis, osteoarthritis
Maternal obstetric complications	Frequent	Uncommon
Mode of transmission	Vertical, in utero, or intrapartum	Nosocomial, horizontal
Predominant GBS serotypes	Ia, III, V*	III, Ia, V*
Effect of intrapartum antibiotic prophylaxis recommended by the Centers for Disease Control and Prevention	Reduces incidence by 85%–90%	No effect

*In decreasing order of frequency.

by ascending infection from the maternal birth canal to the uterine compartment, with subsequent fetal infection through colonization of the skin or aspiration of infected amniotic fluid.[41] The strongest risk factor for GBS EOD is maternal GBS colonization. However, even if a mother has screened negative for GBS, GBS EOD can still occur due to maternal colonization status during the time from screening to delivery. Evaluation of neonates at risk for GBS EOD is discussed separately below.

Escherichia Coli Infections

Historically, *E. coli* is the second most common pathogen causing sepsis and meningitis in newborns. The antigenic structure of *E. coli* is complex and is composed of approximately 150 somatic or cell wall O antigens, 50 flagellar H antigens, and approximately 80 capsular K antigens. However, a limited number of specific K antigen *E. coli* strains cause meningitis. Approximately 80% of the strains causing meningitis and 40% of the strains causing bacteremia or sepsis express the K1 antigen.[55] The capsular K1 polysaccharide antigen is highly homologous to the capsular antigen of group B *Neisseria meningitidis*. Because a high percentage of women have bacteriuria with strains of *E. coli* that express the K1 antigen or are colonized with it at the time of delivery, it is surprising that *E. coli* sepsis or meningitis is not more common.

Surveillance data from the National Institute of Child Health and Human Development Neonatal Research Network, a consortium of 16 US academic neonatal centers, revealed that in the era of widespread implementation of antibiotic prophylaxis, the rate of *E. coli* sepsis increased from 3.2 to 6.8 cases per 1000 live births.[56] This increase was observed in the 1998–2000 era and persisted from 2002 to 2003. Approximately 85% of *E. coli* infections in VLBW infants were ampicillin-resistant.[56] Yet, while most evidence suggests that IAP has not been associated with a concomitant increase in the incidence of *E. coli* or other non-GBS bacterial causes, other evidence demonstrates that the incidence of *E. coli* and ampicillin-resistant *E. coli* infections increased significantly among preterm infants.[57] Furthermore, neonates who developed *E. coli* infections with ampicillin-resistant strains are more likely to be born from mothers with IAP with ampicillin.[56,58,59] In VLBW neonates, the incidence of EOS with *E. coli* has increased, with nearly 85% of cases having resistance to ampicillin.[56,58] Thus, while the benefits of IAP on reducing EOS attributable to GBS are well-documented, the balance of preventing resistance by other bacterial pathogens is still under investigation.

Listeria Monocytogenes Infections

L. monocytogenes is a small, facultative anaerobic, gram-positive, motile bacillus that produces a narrow zone of beta hemolysis on blood agar plates. It can be confused with GBS unless a careful Gram stain, a catalase reaction, and other tests are performed. Most *L. monocytogenes* infections are due to three serotypes: 1a, 1b, and 4b. The last serotype has been described in most outbreaks of listeriosis.[60]

Most cases of listeriosis appear to be food-borne. Foods commonly contaminated by *L. monocytogenes* include raw vegetables such as cabbage, raw milk products, fish, poultry, processed chicken, beef, and hot dogs.[61] Transmission to the fetus occurs through either a hematogenous (transplacental) route or via an ascending infection through the birth canal. Frequently, infections with *Listeria* spp. early in gestation result in abortion; later in pregnancy, infection with *Listeria* spp. can result in the premature delivery of a stillborn or infected newborn. Approximately 70% of *Listeria*-infected women deliver before 35 weeks' gestation.

L. monocytogenes illness in the mother may be undetected as it can be a vague influenza-like illnesses that may not come to medical attention. In approximately half of the perinatal cases, illness in the mother has preceded delivery by 2 days to 2 weeks. An autopsy of stillborn neonates or those who die in the perinatal period from *L. monocytogenes*, granulomas are found throughout organs such as the liver and lungs, and infection is widely disseminated, including involvement of the meninges and even a skin rash due to microabscesses and granulomas called granulomatosis infantisepticum.[62] Treatment of *Listeria* spp. infection or bacteremia during pregnancy can prevent infection in the fetus.[63]

Like GBS infection, *Listeria* spp. infection may have either an early-onset or a late-onset presentation. Epidemics of neonatal *Listeria* spp. infection has been described after the ingestion of contaminated foods such as cheese or coleslaw. The first clearly documented food-borne (coleslaw) outbreak of listeriosis was in 1981 from the Maritimes in Canada; it was associated with a fatality rate of 27%.[61] There are reports of repeated abortions in women with colonization of *Listeria* spp. in the gastrointestinal tract, and cultures can be performed to detect fecal carriage in such women; selective media for *Listeria* spp. is recommended to isolate the organism from various foods or stool specimens. Rapid antigen tests based on nucleic acid amplification are available but not commonly used in clinical diagnostic laboratories. There is no vaccine for *Listeria* spp. infection, but preventive measures have included the surveillance programs from the US Department of Agriculture, prohibiting the sale of contaminated meats. Between 1996 and 2006, the incidence of *Listeria* spp. infections declined by 36%; however, an outbreak of the disease in 2002 related to contaminated turkey meat led to 54 illnesses, 8 deaths, and 3 fetal deaths in 9 states within the US.[60]

Miscellaneous Bacterial Pathogens

The bacteria responsible for early-onset neonatal sepsis have changed over time, and there are regional differences in the organisms commonly responsible for early-onset sepsis. In addition to the organisms mentioned previously, other bacterial pathogens associated with early-onset bacteremia or sepsis in newborns include *Enterococcus* spp., viridans group *Streptococcus* spp., *Klebsiella* spp., *Enterobacter* spp., *Haemophilus influenzae* (typeable and nontypeable), *Staphylococcus aureus, Streptococcus pneumoniae*, group A streptococcus and other beta-hemolytic streptococci, and coagulase-negative staphylococci (CONS).

Clinical Signs of Early-Onset Bacterial Infections

There is great variability in the clinical presentations of neonates with early-onset bacterial sepsis (Box 33.1). Most neonates exhibit respiratory distress in the first 12 hours of life, frequently immediately after birth. In these neonates, the progression may be rapid, with cardiovascular instability, shock, and death. Presentation within the first 12 hours of life suggests that the infection occurred at or near the time of birth or during the immediate postnatal period. Neonates with in utero hypoxia may gasp, inhaling contaminated amniotic fluid and setting the stage for early-onset pneumonia, bacteremia, and sepsis.

The signs of early-onset infection may be subtle, such as tachypnea, or may be more overt, with respiratory distress and hypotension. Because the signs of sepsis can be relatively nonspecific, such as poor feeding and increased sleepiness, they can be overlooked. The clinical signs of neonatal sepsis include, but are not limited to, temperature instability (hyperthermia or hypothermia); respiratory distress; cyanosis; jaundice; hepatomegaly; abdominal distention; feeding abnormalities; and neurologic abnormalities (including lethargy, apnea, and seizures).

Evaluation of Early-Onset Bacterial Infections

Laboratory Testing

Many laboratory tests have been evaluated for infants with possible sepsis, and the results must be interpreted with caution, assessing the sensitivity and specificity of a particular test as well as its positive and negative predictive accuracy.

BOX 33.1 Common Clinical Signs of Neonatal Sepsis

- Abnormal neurologic status: irritability, lethargy, poor feeding.
- Abnormal temperature: hyperthermia or hypothermia.
- Apnea.
- Bleeding problems: petechiae, purpura, oozing.
- Cardiovascular compromise: tachycardia, hypotension, poor perfusion.
- Cyanosis.
- Gastrointestinal symptoms: abdominal distention, emesis, diarrhea.
- Jaundice.
- Respiratory distress: tachypnea, increased work of breathing, hypoxemia.
- Seizures.

Blood Culture

The gold standard for the detection of bacteremia in newborns with suspected sepsis is a positive blood culture result. The sensitivity of detecting positive blood culture results has increased with the introduction of semi-automated blood culture detection instruments that determine the presence of growth from bacterial carbon dioxide production detected by the internal computer of the instrument every minute. An important variable that influences the sensitivity of detecting bacteremia is the volume of blood placed in the culture bottles. It was previously considered standard practice to obtain 0.5 mL of blood for neonatal blood cultures. However, studies reported that approximately 25% of neonates with bacteremia may have low levels of bacterial load in their blood (≤4 colony forming units/mL).[64–66] Further evidence suggests that 0.5 mL of blood for culture is insufficient as the sensitivity decreases by 10% to 40% when 0.5 mL is used rather than 1.0 mL.[64,67] Therefore 1 mL of blood is now considered the standard volume for neonatal blood cultures to prevent false-negative results.[68] With the use of new technology, most positive blood culture results are detected within 24 to 48 hours.[69,70] In a term infant who remains asymptomatic after initiating antibiotic therapy, stopping antibiotic administration if the blood culture results remain negative after 36 hours is recommended.[71–73] However, the decision to discontinue treatment with antibiotics should not rely solely on a negative blood culture.

Blood cultures can be falsely negative due to inadequate blood volume in the blood culture, use of antibiotics in the mother or neonate before culture sampling, or other factors. Thus, when the suspicion of sepsis based on clinical signs and symptoms is high and other causes are unlikely, clinicians may consider continuing antibiotic therapy for a complete course despite negative blood culture results.[74] As a caveat, studies have demonstrated that healthcare providers cannot reliably differentiate sepsis from other conditions that occur in ill newborns, especially premature neonates where hemodynamic instability, respiratory distress, and temperature instability are common.[75] Recent literature suggests that antibiotics in the neonatal period may have a deleterious effect on long-term outcomes. Therefore careful consideration of discontinuing antibiotics when possible is an important component of antibiotic stewardship.[76–79]

Urine Culture

The frequency of positive urine culture results in infants with early-onset sepsis is low (<2%), and it is rare to find bacteriuria in infants with negative blood culture results.[80] Therefore it is generally not recommended to obtain urine specimens in the first 72 hours after birth as part of the EOS evaluation.[81]

Cerebrospinal Fluid

In any neonate with signs of sepsis, a lumbar puncture should be considered. Because meningitis is less common in EOS than in late-onset sepsis, lumbar punctures are not always routinely performed during sepsis evaluations occurring within the first 72 hours after birth. However, if a bacterial culture returns positive, a lumbar puncture should be performed as bacteremia can lead to hematogenous seeding of the meninges. Lumbar punctures are deferred in infants with clinical instability or uncorrected bleeding disorders. The details of the examination of cerebrospinal fluid (CSF) and the diagnostic approach for examining CSF is discussed below.

White Blood Cell Count and Neutrophil Indices

White blood cell (WBC) counts, differential, immature; total neutrophil ratio (I/T ratio); and absolute neutrophil count (ANC) vary based upon time since birth and gestational age. None has proven singularly useful for predicting the majority of cases of neonatal sepsis.[82,83] For instance, in a study evaluating over 30,000 complete blood counts of premature neonates, neonates born <28 weeks had peak neutrophil concentrations at approximately 24 hours of age, and those born at ≥28 weeks gestation peaked at 4 to 6 hours of age.[84] Furthermore, maternal and neonatal factors can impact the WBC count and ANC; the presence of labor or being of female sex at birth is associated with higher values. Total WBC counts have a poor positive predictive value for neonatal sepsis, while neutropenia has greater specificity.[85] A number of clinical conditions affect the total neutrophil count. Prolonged crying, meconium aspiration syndrome, maternal fever, and asphyxia are all associated with an increase in the total neutrophil count, and there may be an increase in the total immature neutrophil forms, as well as an increased I/T ratio. Maternal hypertension is associated with a decrease in the total neutrophil count.[86–88]

A large study that evaluated 166,092 neonates found 60% of neonates with positive blood cultures had normal WBC counts (between 5000 and 19,000/mm^3), 87% did not have neutropenia (ANC ≥1500/mm^3), and 31% had an I/T ratio considered normal (<0.2).[89] The sensitivity was quite low for all measures of the complete blood cell (CBC) count (0.3% to 54.5%). Thus, the utility of a CBC, including evaluation of WBC count, differential, ANC, or I/T ratio, to evaluate for EOS is extremely limited. Using CBC results to define an infant as high risk for sepsis poses both a significant risk of missing cases of neonatal sepsis and a risk of unnecessarily prolonging antibiotic administration.

Platelet Counts

Platelet counts are not sensitive for predicting or diagnosing EOS. One study demonstrated that 82% of neonates with positive blood cultures had a normal platelet count of between 150,000 and 400,000/mm^3.[89] The same study demonstrated a sensitivity of predicting EOS with a platelet count of <100,000/mm^3 of 4.0%.[89] While it is presumed that accelerated platelet destruction and possibly depressed production caused by bacterial products on the bone marrow are the underlying mechanisms for thrombocytopenia in some infected infants, it does not occur in all neonates with sepsis. Platelet counts are neither helpful for predicting neonatal sepsis nor evaluating response to effective antimicrobial treatment.[90,91]

Acute-Phase Reactants

No single biomarker has demonstrated superiority in predicting or diagnosing EOS. Moreover, variations in the timing that biomarkers naturally peak, their duration of elevation, and their half-lives make choosing the optimal timing of testing difficult. Here, we will evaluate several biomarkers that have been studied in the quest to improve diagnostic assessment and identification of EOS. Most of these biomarkers are derived from components of the complex inflammatory response to an invading pathogen and are considered acute phase reactants.

Procalcitonin (PCT) and C-reactive protein (CRP) are the most studied acute-phase reactants in neonatal sepsis.[92–94] PCT is the precursor of calcitonin, normally synthesized in the C cells of the thyroid gland. PCT is induced by systemic inflammation resulting from bacterial sepsis and is produced by cells such as hepatocytes, nephrons, and monocytes. The physiologic function of PCT is unknown. In bacterial infections, plasma PCT concentrations increase from 0.001 to 0.01 ng/mL at the baseline to values ranging from 1 to 1000 ng/mL. PCT concentrations rise much faster than CRP concentrations (6 to 8 hours vs. 48 hours for maximum levels). In healthy newborns, plasma PCT concentrations increase gradually after birth, reaching peak levels at approximately 24 hours of age (range 0.1 to 20 ng/mL) and then decreasing to normal values of less than 0.5 ng/mL by 48 to 72 hours of age.[95] This natural increase soon after birth makes PCT a less sensitive method of detecting EOS as compared to LOS. Studies on the use of PCT as a marker of neonatal sepsis have yielded contradictory results regarding its utility for clinical decision-making in both diagnosis and length of antibiotic therapy. In a study of 121 newborns with suspected early-onset sepsis, serial PCT concentration determinations could be used to shorten the duration of antibiotic therapy.[95] However, noninfectious conditions that commonly affect neonates, such as respiratory distress syndrome, infants of diabetic mothers, or hemodynamic instability, can also lead to elevations of PCT levels. Larger studies are needed to determine the value of PCT in diagnosis and therapy.

Monitoring CRP levels has been widely promulgated as an aid to diagnosing neonatal infection and adjusting the duration of antibiotic therapy in infants with suspected versus proven sepsis.[96,97] CRP is produced by the liver in response to stimulation by the pro-inflammatory cytokine IL-6, which is produced by both T and B cells.[92,93] CRP concentration increases within 6 to 8 hours and peaks around 24 hours after infection.[98,99] However, the use of CRP in diagnosing EOS is limited by its natural rise over the first 3 days after birth.[92,94] Specifically, one study evaluated 176 neonates >1500 g at birth and demonstrated a negative predictive value of CRP to be 99% in EOS.[100] Another study demonstrated two CRP measurements <1 mg/dL obtained 24 hours apart between 8 and 48 hours after presentation had a 99.7% negative predictive value for EOS and a 98.8% negative predictive value for late-onset sepsis.[100,101] However, obtaining a CRP at the time of initial infectious evaluation did not prove sensitive for predicting sepsis, and the negative predictive value did not improve when combined with later CRP measurements done at least 8 hours after the initial value. Therefore some suggest using serial CRP measurements that are normal (<1 mg/dL) to help determine early discontinuation of antibiotics rather than as a guide to starting antibiotics.[101]

Because exposure of the host to bacterial products results in a substantial and rapid increase in IL-6 concentrations, IL-6 may be a more useful marker than CRP during the early phase of infection. In one study, the IL-6 concentration had a sensitivity of 89% versus 60% for CRP at the onset of clinical suspicion of neonatal infection.[93] Other studies have demonstrated a sensitivity of 78% and a specificity of 79% for detecting neonatal sepsis.[102] With IL-6 levels and kinetics differing based upon gestational age, further investigations are needed to determine the utility of IL-6 in EOS testing and prediction strategies.[103] IL-6 use is not currently widely recommended outside of experimental testing.

High-sensitivity CRP (hsCRP) measurement has been shown to provide increased sensitivity for detecting neonatal infection.[104] Not all diagnostic laboratories can provide hsCRP values in a timely fashion. In addition, the optimum diagnostic cutoff levels for CRP and hsCRP are debatable. Serum soluble intercel-

lular adhesion molecule 1 (slSAM-1), hsCRP, soluble E-selectin (sE-selectin), and serum amyloid A, individually and in combination, have been studied for the diagnosis of sepsis in a neonatal intensive care unit.[104] In one study, all four measurements had some diagnostic value for neonatal infection; however, slSAM-1, hsCRP, and sE-selectin demonstrated the highest negative predictive value individually (slSAM-1, 84%; hsCRP, 79%; and sE-selectin, 74%).[104] Use of a combination of these measurements enhanced the diagnostic value, with sensitivities of 90.3% and a negative predictive value of 91.3%.[104] However, the application of this set of diagnostic markers is not available for most facilities, and more investigative work is needed to confirm their role in excluding early-onset infection.

To date, similar to the CBC, various cytokine determinations lack clear efficacy and sensitivity in detecting or predicting EOS and therefore have not been recommended for widespread use in EOS evaluation.

Bacterial Polymerase Chain Reaction (PCR)

Other molecular technologies to diagnose neonatal sepsis by the rapid identification and differentiation of gram-negative and gram-positive bacterial bloodstream infections are also being studied. Polymerase chain reaction (PCR), using universal bacterial primers, targets conserved regions of the 16 S ribosomal RNA gene common to all bacteria but not found in other organisms.[105] In one study, universal primer PCR was performed in newborns with clinically suspected sepsis. PCR was performed before antibiotic therapy was started and was repeated at 12, 24, and 48 hours after drug therapy had started.[105] The sensitivity, specificity, and positive and negative predictive values of universal primer PCR were 96.2%, 96.3%, 87.7%, and 98.8%, respectively. Two patients were blood culture positive but 0-hour PCR negative and seven patients were 0-hour PCR positive, but the blood culture result was negative. Of the patients with a 0-hour PCR-positive result, seven remained positive at 12 hours, but none remained positive at 24 and 48 hours after starting antibiotic therapy. Although universal bacterial primer PCR may be a useful test for diagnosing an early episode of culture-proven sepsis, it cannot be used for diagnosis if the patient has been exposed to antibiotic therapy for 12 hours or more.[105] Larger studies are required before this assay can be recommended for routine clinical use in newborns suspected of having sepsis.

Diagnostic Approach to Neonates with Suspected Sepsis

All symptomatic newborns must be carefully evaluated for the possibility of bacterial sepsis and treated with antibiotics, if necessary. Although the presence of various risk factors should increase the suspicion of sepsis, the absence of risk factors in the symptomatic infant does not indicate that sepsis risk can be dismissed. In adjusting to postnatal life, some newborns exhibit abnormal signs transiently, such as tachypnea, before becoming asymptomatic. However, in any newborn who has other findings or is still symptomatic 4 to 6 hours after birth, consideration of including a sepsis evaluation is imperative; a diagnostic evaluation with a blood culture and, as appropriate, a chest radiograph should be strongly considered. Antibiotic therapy can be stopped when the clinical suspicion of sepsis is low, the physical findings are normal, and the screening results for sepsis, including the blood culture results, remain negative. If the blood culture is positive or there are persistent clinical signs of sepsis, the newborn should be treated with an appropriate course of antibiotics. Furthermore, if a lumbar puncture was not done initially and the blood culture was positive, a lumbar puncture should be performed due to potential hematogenous seeding of the brain from bacteremia.

Deciding to evaluate and/or treat asymptomatic neonates for EOS is a difficult task. Combining the objective risk factors found to be most highly associated with neonatal sepsis with the newborn physical examination over the first 12 hours led to the development of a Neonatal Early-Onset Sepsis Calculator to guide evaluation and therapy of the late preterm (≥34 weeks' gestation at birth) and term infant who has maternal risk factors for sepsis.[19,106] The current version of the neonatal EOS calculator (found at https://neonatalsepsiscalculator.kaiserpermanente.org/) includes definitions of clinical status, including three specific categories: clinical illness, equivocal symptoms, and well-appearing. Combining clinical status with maternal risk factors, the calculator provides a numerical estimate of the risk of sepsis and a clinical recommendation for management and monitoring. Several studies have demonstrated the benefit of using this EOS calculator in reducing unnecessary antibiotic exposures.[107,108] A systematic review reported a 44% decrease in empiric antibiotic utilization with the implementation of the EOS calculator.[109]

For preterm neonates born at ≤34 weeks' gestation, the decision to evaluate and treat empirically for EOS is even more challenging. Notably, the EOS calculator does not include neonates born below 34 weeks' gestation, and with decreasing gestational age, there is an increased association with sepsis. Furthermore, very preterm neonates often have temperature instability, respiratory distress, and hypotension that are independent of infection, making the determination of whether symptoms are due to clinical infection or simply prematurity difficult. Preterm neonates who are born to a mother with cervical incompetence, pre-labor rupture of membranes (PROM), chorioamnionitis, intraamniotic infection, preterm labor, or otherwise unexplained etiology for a nonreassuring fetal status are considered high-risk for EOS and should have a blood culture and empiric antibiotics initiated immediately after birth. Preterm infants considered to be at lower risk for EOS include neonates born to mothers who had preterm delivery via (1) cesarean delivery, (2) due to an obstetric indication such as pre-eclampsia or noninfectious medical illness, and (3) no labor, rupture of membranes, or induction of labor occurred prior to delivery, and they may be monitored clinically without evaluation or treatment for EOS.[110] If the neonate is born via vaginal delivery or cesarean, but the mother had induction of labor or rupture of membranes prior to birth, and the delivery was due to noninfectious maternal etiologies, the neonate can still be monitored clinically, or a blood culture can be obtained. In these latter two low-risk situations, antibiotics can be initiated promptly if the neonate develops signs and symptoms of sepsis after birth.[110]

Diagnostic Approach for Neonates at Risk for GBS Early-Onset Disease (GBS-EOD)

The AAP has specific recommendations for the prevention of early-onset neonatal GBS sepsis that overlap with the AAP EOS clinical guidelines. Per the AAP recommendations, evaluation for GBS-EOD is based upon gestational age categories: those born ≥35 weeks' gestation versus those born <35 weeks' gestation. The Neonatal Early-Onset Sepsis Calculator (previously discussed) is a multivariate risk assessment that includes all causes of

EOS, including GBS, and can be used for those infants born ≥35 weeks' gestation. Another option for determining proper evaluation methods for GBS-EOD in those born ≥35 weeks' gestation is through a risk assessment based on the newborn's clinical condition. Of neonates with GBS-EOD, nearly 95% develop symptoms within the first 48 hours after birth.[17] Using this data, for those who develop signs of illness within the first 48 hours after birth, empiric antibiotics are initiated.[111–113]

For those born <35 weeks' gestation, the decision to empirically treat neonates from birth depends on the risk factors discussed above and the indication for delivery. For neonates born by cesarean section to mothers who had preterm birth due to noninfectious medical illness of the mother (e.g., pre-eclampsia, gestational hypertension, placental insufficiency, severe intrauterine growth restriction, multiples), or birth that occurred with no labor, it is reasonable to consider whether to perform laboratory evaluation due to the low-risk status for GBS EOD or other causes of EOS and to defer use of empiric antibiotics. However, if the neonate demonstrates signs of hemodynamic instability after birth, empiric antibiotics may be initiated. Preterm infants may be considered low risk for sepsis if they are born due to noninfectious maternal or fetal indications but had an induction of labor. For these infants, close observation is recommended as long as the mother received adequate IAP for GBS, if indicated. If not, the infant should be considered high-risk. High-risk infants born <35 weeks' gestation are those born preterm due to cervical insufficiency, preterm labor, chorioamnionitis, PROM, or other unexplained onset of non-reassuring fetal heart rate tracings. In these high-risk infants for both GBS-EOD and EOS, obtaining a blood culture and starting empiric antibiotics is recommended.

Treatment of Early-Onset Bacterial Infections

Antimicrobial Therapy

The choice of antibiotic for an infant with suspected early-onset sepsis depends on the predominant bacterial pathogens and the antibiotic susceptibility profiles for the microorganisms causing early-onset disease in a particular geographic region. Any decision to discontinue antimicrobial therapy should be based on the level of suspicion for sepsis when treatment was begun, the culture results, laboratory test results, and the clinical behavior and course of the infant. If sepsis is highly suspected in an infant, antibiotics should be considered for a full course even if the culture results are negative.

Empiric therapy for early-onset sepsis generally consists of combinations of antibiotics effective against the most common gram-positive pathogens (e.g., GBS, *L. monocytogenes*) and gram-negative pathogens (e.g., *E. coli*) associated with EOS. The two combinations most commonly used are (1) ampicillin with an aminoglycoside, usually gentamicin, or (2) ampicillin with a third-generation cephalosporin, usually cefotaxime. Cefotaxime has minimal toxicity and is well tolerated by newborns. However, the third-generation cephalosporins are associated with the induction of various β-lactamase–producing gram-negative bacteria, including extended-spectrum β-lactamase–producing organisms that are resistant to all β-lactam antibiotics and frequently to other antibiotics.[114] Cephalosporins and vancomycin, a glycopeptide antibiotic, are also associated with the development of vancomycin-resistant enterococci.[115] Another disadvantage of cephalosporin antibiotics is the lack of effectiveness against enterococci or *L. monocytogenes*. Moreover, studies have demonstrated an association between empiric third-generation cephalosporin use and invasive candidiasis in preterm neonates.[116] Therefore ampicillin and an aminoglycoside are the primary antibiotics recommended for empiric EOS treatment, depending upon local antibiotic resistance patterns. A third-generation cephalosporin is warranted in empiric treatment if there is suspicion of underlying meningitis. In infants with bacteremia and sepsis caused by GBS or *L. monocytogenes*, the combination of gentamicin with ampicillin or penicillin is used during the first few days, and the remainder of the course of therapy is completed with ampicillin or penicillin monotherapy once sterility of blood, CSF, and/or urine is achieved.

When the likelihood of infection is very low, antibiotic therapy should be stopped as soon as possible. In most hospitals using modern blood culture instrumentation, 36 hours is sufficient to determine whether a blood culture result is negative, assuming that no antibiotics were given before the culture was obtained.[117] One recent study reported that all pathogenic blood cultures collected before antibiotic implementation in EOS were positive within 24 hours, and other studies have demonstrated 92% to 100% culture positivity by 24 hours after collection.[70,118–121] Therefore the duration of empiric antibiotics may become shorter pending future clinical studies.

Experimental Immunologic Adjuvant Therapies

Various adjunctive therapies have been proposed to improve the immune status of the newborn to reduce neonatal sepsis mortality. Some of these therapies, such as intravenous immunoglobulin (IVIG), have been conclusively ruled out as potential preventative or therapeutic options for neonatal sepsis. A Cochrane review and a large clinical trial that enrolled 3493 infants reported that IVIG administration did not reduce mortality, the severity of illness, or major disability during hospital stay or by 2 years of age.[122]

Other immunomodulating factors such as granulocyte colony-stimulating factor (G-CSF) have been studied to both prevent and treat neonatal sepsis. A Cochrane meta-analysis concluded that prophylaxis with G-CSF does not significantly reduce mortality in all infants, although in premature infants with neutropenia (ANC <1750), mortality may be reduced.[123] Another meta-analysis reported G-CSF as an adjunct treatment for neonatal sepsis significantly decreased mortality with the most benefit for preterm neonates, those with baseline neutropenia, and those with low birthweight.[124] Further studies, such as a large randomized clinical trial, are warranted to investigate G-CSF's adjuvant effects in the treatment of neonatal sepsis.

Late-Onset Neonatal Bacterial Infections

LOS occurring after the first 72 hours of birth is a significant cause of morbidity and mortality among neonates worldwide. LOS attributed to nosocomial, or healthcare-acquired, infections is discussed in Chapter 37. Here, we examine the literature surrounding non-nosocomial LOS.

Pathogenesis and Epidemiology of Late-Onset Neonatal Bacterial Infections

While vertical transmission from mother-to-neonate before or during delivery is the cause of most cases of EOS, LOS occurs predominantly through horizontal transmission. Horizontal infection refers to contact with environmental sources or care providers after birth rather than directly related to the birthing

process. The neonate becomes colonized by potentially pathogenic bacteria within their environments, including skin and gut microbiomes. The causative pathogen associated with LOS is commonly found in abundance in the affected neonate's gut microbiome, which has implications for other aspects of neonatal management.[125] Processes that disrupt the skin barrier, such as the use of intravenous catheters, scalp electrodes, or even forceps delivery, predispose infants to develop LOS.[125] Other risk factors associated with LOS are delayed early enteral feeding with breast milk or underlying cardiac or pulmonary disease.[126–130] However, the most important risk factor for LOS is the same as for EOS—extreme prematurity, with the incidence of LOS inversely related to gestational age. One study reported approximately 36% of neonates born <28 weeks of gestational age had at least one episode of LOS, which decreased to 30% between 29 and 32 weeks, 18% between 33 and 36 weeks, and 17% for term neonates.[128]

Late-Onset Bacterial Pathogens

Coagulase-Negative Staphylococci

CONS can create biofilms, especially on central lines, and is a predominant etiology for nosocomial infections; therefore it is primarily discussed in Chapter 37.

Gram-Negative Bacteria

Gram-negative bacilli are also commonly associated with LOS and include *E. coli*, *Enterobacter* spp. *Klebsiella* spp, and *Pseudomonas* spp. When *E. coli* infection occurs after 6 days of age, an evaluation for galactosemia should be considered. Fungi such as *Candida* spp. are also a significant contributor to LOS and vary based upon geography, and are discussed in Chapter 36.[127,131]

Bacterial Pathogens Causing Both EOS and LOS: GBS and *L. monocytogenes*

While most bacterial pathogens can cause both early- and late-onset sepsis, GBS and *L. monocytogenes* are common etiologies for both time periods. Within this chapter, we have already discussed in detail GBS and *L. monocytogenes*.

Prevention Strategies for Late-Onset Neonatal Bacterial Infections

Several strategies can be implemented to reduce the risk of LOS. Early introduction of enteral feeds, specifically with breast milk, is an evidence-based strategy to prevent LOS. Breast milk contains antibodies, lactoferrin, human milk oligosaccharides, nutrients, and other beneficial factors that promote nutrition, growth, passive immune protection, and the promotion of the neonate's own immune functions.[132–134] Starting human milk feeding within the first 72 hours after birth is associated with a significant 3-fold reduction in the risk of LOS.[135]

Lactoferrin, a protein in human milk that is a part of the innate immune system in preventing infection, has been found to decrease the risk of LOS compared to placebo.[136–138] A Cochrane review reported that low-quality evidence exists demonstrating enteral lactoferrin supplementation decreases LOS but not NEC, all-cause mortality, or neurodevelopmental outcomes at 24 months of age in preterm infants. Therefore, the routine use of lactoferrin is not recommended.

Probiotics are another strategy to potentially reduce LOS. The gut microbiome of neonates undergoes profound change immediately after birth. It is thought that an abnormal gut microbiome can lead to alterations in the intestinal barrier and bacterial translocation from the gastrointestinal tract to the bloodstream, leading to sepsis. Probiotics containing potentially "beneficial" strains of bacteria such as *Bifidobacterium* or *Lactobacillus* spp. are believed to facilitate colonization of the gut microbiome with these "healthy" commensal bacteria as opposed to pathogenic bacteria such as CONS. A systematic review and meta-analysis reported that probiotics significantly reduce the incidence of LOS in exclusively human milk-fed preterm infants.[139] However, some studies have demonstrated probiotic-associated neonatal sepsis as well as recalls of certain probiotics due to contamination with *Salmonella*, *Rhizopus*, or *Penicillium* species. Thus, the American Academy of Pediatrics cautions against the routine use of probiotics at this time.[140] Further studies are needed to clarify the specific strains, dosing, and frequency of probiotics to potentially optimize their effectiveness in the prevention of LOS.

Evaluation of a Neonate with Potential Late-Onset Bacterial Sepsis

Neonates with late-onset sepsis admitted from the community are at lower risk for multidrug-resistant bacteria than neonates who have been admitted to the hospital since birth. UTIs and meningitis are more likely to occur in LOS than in EOS, and therefore, blood, urine, and CSF cultures should be performed in neonates presenting with signs of sepsis after 72 hours from birth, especially those presenting after 7 days from birth.

In contrast to older pediatric patients and adults, male neonates are more likely than female neonates to have a UTI until approximately 1 year of age. After 1 year of age, females become more likely than males to have a UTI. UTIs rarely occur as part of EOS but are more common after 72 hours of age. Therefore, urine culture by sterile techniques is a routine part of a sepsis evaluation for LOS. The most common pathogen associated with UTIs in neonates is *E. coli*.[141,142] A urine culture should be obtained by sterile catheterization or suprapubic bladder tap. A renal ultrasound should be performed if the neonate has a UTI due to the association with renal abnormalities in 20% to 50% of neonates with a UTI.[141,142] A renal ultrasound can detect hydronephrosis or structural abnormalities, but it cannot detect vesicoureteral reflux, a potential cause of neonatal and infant UTIs. If indicated, a voiding cystourethrogram (VCUG) can be performed approximately 1 to 2 months after the completion of antibiotic therapy.

A lumbar puncture should be performed in any neonate with signs of sepsis, a positive blood culture, or worsening clinical status while on antimicrobial treatment. A lumbar puncture can be deferred to a later time in neonates with life-threatening cardiopulmonary compromise or coagulopathy with a severe risk of bleeding. In clinically unstable neonates and those with coagulopathy, the lumbar puncture should be performed once the infant has stabilized.

Treatment of Late-Onset Neonatal Bacterial Infections

Neonates with LOS admitted from home are at lower risk for multidrug-resistant bacteria than neonates who have been in the hospital since birth. Therefore treatment strategies will be different for a neonate with a community-acquired infection versus those with a high risk of nosocomial infection. This latter group

will be discussed in a separate chapter on nosocomial infections. In general, treatment with ampicillin and an aminoglycoside (generally gentamicin) is an appropriate empiric treatment for LOS. The addition of an extended-spectrum cephalosporin (cefotaxime, ceftazidime, or cefepime) is warranted if there is a concern about underlying meningitis. Dosing of ampicillin and gentamicin depends upon gestational age and days since birth. If the site of infection is skin, bone, or joints, then nafcillin or vancomycin (if high rates of methicillin-resistant *S. aureus* are common in the community) rather than ampicillin should be substituted to treat for potential pathogens commonly associated with neonatal osteomyelitis and septic arthritis—*S. aureus*, GBS, *E. coli*.[143,144] Finally, if the infection is suspected to be from the gastrointestinal tract, such as with necrotizing enterocolitis, an antibiotic with more robust anaerobic bacterial coverage—such as clindamycin or metronidazole—can be added to the empiric treatment regimen.

Neonatal Bacterial Meningitis

Neonatal bacterial meningitis is associated with higher mortality and morbidity. Meningitis is associated with the same pathogens that cause bacterial sepsis, with GBS and *E. coli* accounting for approximately 70% of all cases, and *L. monocytogenes* accounting for an additional 5% in the first week of life. On occasion, it is possible to isolate *S. pneumoniae* and *H. influenzae*. In newborns older than 1 week and residing in neonatal intensive care units, coagulase-negative staphylococci are common isolates. The underlying pathogenesis of bacterial meningitis is a seeding of the meninges during bacteremia, and bacterial meningitis occurs in approximately 15% to 25% of neonates with bacteremia. This high occurrence rate highlights the importance of a lumbar puncture if a blood culture is positive and a lumbar puncture has not been performed.[145]

GBS meningitis usually presents as a late-onset disease. The most common GBS serotype identified is III.[146] Ansong et al. reported that GBS meningitis complicated 22 of 145 episodes (15%) of early-onset GBS sepsis and 13 of 23 episodes (57%) of late-onset GBS sepsis.[147] GBS meningitis has a mortality approaching 30% and morbidity of 50% and can occur in the presence of negative blood culture results. In one study, 20% of infants with GBS meningitis had negative blood culture results.[147]

Approximately 80% of all serotypes of *E. coli* that cause meningitis in newborns possess the K1 capsular antigen. The K1 capsular polysaccharide antigen is considered one of the primary virulence factors of this capsular type of *E. coli* because antibody against K1 antigen has been shown to be protective in neonatal rat models of infection. Mortality rates for neonatal *E. coli* meningitis range from 20% to 60%.[148]

Pathology and Clinical Manifestations of Neonatal Bacterial Meningitis

At autopsy, infants who die of meningitis often have purulent exudates on the meninges and the surfaces of the ventricles associated with inflammation. Historically, hydrocephalus and nonspecific encephalopathy were demonstrated in approximately 50% of infants who died of bacterial meningitis.[149]

The signs and symptoms of neonatal meningitis are not easy to distinguish from those of sepsis. Temperature instability, including hypo- or hyperthermia, is considered one of the most common findings and is seen in nearly 60% of newborns with bacterial meningitis.[150,151] Neurologic signs such as irritability, tremors, twitching, lethargy, and seizures are also quite common. Irritability has been reported to occur in approximately 60% of cases, and seizures in 20% to 50%.[150] Notably, seizures are more common in gram-negative bacterial meningitis than gram-positive pathogens. Seizures occur due to direct central nervous system inflammation or by metabolic abnormalities such as hypoglycemia or hyponatremia.[152] Other common findings include feeding intolerance, including emesis (50%), respiratory distress (50%), and apnea (10% to 30%).[150] A bulging fontanel may occur, but this is usually a late manifestation.

Diagnosis of Neonatal Bacterial Meningitis

The gold standard for the diagnosis of meningitis is the analysis of the CSF, including the WBC count, glucose and protein levels, Gram stain, and culture. The interpretation of CSF cell counts in newborns may be difficult.[153,154] There can be significant overlap in the WBC count, glucose, and protein levels between neonates with bacterial meningitis and those without meningitis. The CSF WBC count slowly decreases in term newborns during the first week after birth but may remain high or even increase in premature newborns. There is no change in CSF WBC counts or protein content with gestational age, but there is a significant decrease with postnatal age.[155] The 95th percentile for CSF WBC count for neonates is reported to be 19/μL, but individual infants with no proven infection had higher WBC counts ranging from 75 to 100/μL.[156,157] CSF WBC counts of >21 cells/μL have both a sensitivity and specificity of approximately 80% in predicting culture-positive bacterial meningitis.[153] However, it is important to note that culture-proven bacterial meningitis with normal CSF parameters occurs in 1% to 10% of infants with proven meningitis.[153,154,158] Finally, CSF glucose and protein values are highly variable and have significant overlap between those with meningitis and those without meningitis.[153,159–161]

A Gram stain of CSF must be carefully examined in every infant with suspected meningitis. Approximately 20% of newborns with proven meningitis are reported as showing "no bacteria seen." Although an increase is expected in the number of neutrophils with bacterial meningitis, one may see a predominance of lymphocytes before a conversion to polymorphonuclear leukocytes. With *L. monocytogenes*, examination of the CSF shows a mononuclear cellular response. In clinical care units, repeating the CSF examination and culture 2 to 3 days after the initiation of antibiotic therapy can be helpful to demonstrate the effectiveness of the antimicrobial regimen and associated sterility of the CSF. This examination is especially important if the patient has not responded clinically and is experiencing seizures or continued fever. At times, it is difficult to eradicate the organism from the CSF, and consideration can be given to examining the inhibitory and bactericidal concentrations in CSF. It is especially important to repeat the CSF examination before antibiotic therapy is stopped in patients with more complicated courses and in cases of enteric gram-negative bacterial meningitis.

Treatment of Neonatal Bacterial Meningitis

Infants with bacterial meningitis are frequently ill and should be monitored in the intensive care unit. These patients may require mechanical ventilation, complex fluid management to attenuate the effects of cerebral edema and of secretion of inappropriate antidiuretic hormone, seizure control, vasopressor support, and

cardiopulmonary monitoring. The choice of appropriate antibiotic therapy is based on several factors, including the achievable CSF levels of drugs that have in vitro efficacy against the microorganism. In gram-positive bacteria, the use of penicillin and ampicillin will achieve 10- to 100-fold higher concentrations than the minimal inhibitory concentrations needed to inhibit the bacteria, and there will be rapid sterilization of the CSF. In contrast, aminoglycosides, such as gentamicin and tobramycin, achieve only 40% of peak serum levels in the CSF and may not achieve minimal inhibitory concentrations more than those equal to or slightly greater than those found in vitro for gram-negative bacteria.

Generally, initial empiric antimicrobial therapy selection for presumed early-onset or community-acquired bacterial meningitis includes ampicillin and an aminoglycoside (typically gentamicin) along with a third- or fourth-generation cephalosporin (e.g., cefotaxime, ceftazidime, cefepime). This regimen is effective against common bacterial pathogens associated with early-onset and late-onset sepsis and meningitis (GBS, *E. coli, L. monocytogene*). The addition of an expanded-spectrum cephalosporin increases coverage against *S. pneumococci* and ampicillin-resistant strains of *E. coli*. Ampicillin-resistant bacteria are known to be a common cause of neonatal meningitis, and one study found nearly 80% of infants with meningitis had ampicillin-resistant strains of bacteria as the underlying etiology.[162] Thus, ampicillin and gentamicin are effective therapy against GBS and Listeria, and the addition of a higher generation cephalosporin treats ampicillin-resistant gram-negative bacterial pathogens that are of increasing prevalence. Of note, ceftriaxone should not be used in neonates as it is known to displace bilirubin from albumin and increase the risk for kernicterus and bilirubin-induced neurologic dysfunction (BIND). Furthermore, ceftriaxone is contraindicated when calcium infusing intravenous fluids are also being administered due to the risk of calcium precipitation.

In neonates who have been in the hospital since birth and may have had central lines or other risk factors for coagulase-negative staphylococcus or methicillin-resistant *Staphylococcus aureus* (MRSA), there should be a consideration for vancomycin as part of the empiric treatment regimen for suspected late-onset bacterial meningitis. Importantly, ampicillin should be added if there is a concern for GBS or *L. monocytogenes* such as a Gram stain, culture, or history suggesting a high risk for these pathogens. Although used in the past, neither intrathecal nor intraventricular administration of antibiotics has been found to reduce the morbidity or mortality associated with gram-negative bacterial meningitis.[163]

Once the microorganism has been identified and the antibiotic susceptibility results are available, either a single drug or a combination of drugs found to be effective should be prescribed. GBS is uniformly susceptible to ampicillin and penicillin; therefore, after an initial course of dual therapy, monotherapy with Penicillin G is generally the definitive course of treatment. GBS meningitis is treated for 14 to 21 days. Due to evidence that a dual therapy regimen is more efficacious than ampicillin monotherapy, Listeria is traditionally treated with dual therapy of ampicillin and gentamicin until CSF sterility is documented. Once CSF sterility is achieved, monotherapy with ampicillin can be prescribed to complete a treatment duration of 14 to 21 days. For *E. coli* infections, ampicillin is the preferred treatment for strains that are ampicillin susceptible. For ampicillin-resistant strains, including other gram-negative enteric bacteria with ampicillin resistance, an extended-spectrum cephalosporin (i.e., cefotaxime, cefepime, or ceftazidime) plus an aminoglycoside (generally gentamicin) is the initial therapy until CSF sterility is achieved. At this point, the aminoglycoside can be discontinued, and monotherapy with the cephalosporin can be continued to complete a minimum of 21 days of treatment duration. In settings where multi-drug-resistant pathogens are the etiology of bacterial meningitis, consultation with the hospital microbiology laboratory and the infectious disease team is necessary to determine the best treatment strategy and duration. For gram-positive organisms, a 14-day treatment course is generally sufficient. For gram-negative organisms, a 21-day course is the minimum.

A Cochrane review of two trials of adjuvant corticosteroids in neonatal bacterial meningitis suggested a reduction in death and hearing loss with steroid therapy, but there was no reduction in neurologic sequelae at 10 weeks.[164] However, a clinical trial evaluating the outcomes of neonates exposed to dexamethasone plus antibiotic therapy versus only antibiotic therapy demonstrated no differences in rates of neurologic deficit, mortality, or hearing loss at 2 years of age.[165] Therefore adjunctive steroid use is not currently recommended for the treatment of neonatal bacterial meningitis.

It may be prudent to repeat a CSF examination and culture after the initiation of therapy, especially if the clinical response is less than satisfactory. If organisms are seen on Gram-stained smears of the fluid, modification of the therapeutic regimen should be considered. In general, approximately 3 days or more are required for an antibiotic regimen to sterilize the CSF in infants with gram-negative meningitis. In infants with gram-positive meningitis, sterilization is usually seen within 36 to 48 hours. Neuroimaging should be considered for all neonates with meningitis to exclude parameningeal foci and abscess formation and to assist in assessing the infant's prognosis. Neuroimaging is required in infants with signs of neurologic complications or failure to improve clinically after 24 to 48 hours of appropriate antibiotic treatment.

Outcomes of Neonatal Bacterial Meningitis

Mortality from neonatal meningitis occurs in approximately 10% of cases.[166–172] Meningitis survivors are at high risk for neurodevelopmental impairment. Approximately 35% of survivors go on to have mild disability, and 20% develop moderate or severe disability.[169,171,172] Complications from neonatal meningitis include brain abscess, communicating or non-communicating hydrocephalus, subdural effusions, ventriculitis, deafness, and blindness. An infant who had meningitis may appear relatively healthy at the time of discharge, and only after careful follow-up do perceptual difficulties, reading problems, or signs of brain damage become apparent. Infants who survive neonatal meningitis should have regular audiology, language, and neurologic evaluations until they enter school.[173,174]

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Viral Infections of the Fetus and Newborn

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KEY POINTS

- Viral infections of the fetus and newborn are common problems in neonatology practice.
- Fetal (congenital) viral infections should be considered in the differential diagnosis of newborns with intrauterine growth retardation, physical examination and laboratory abnormalities, and illness in the newborn period.
- Perinatal viral infections involve transmission during the birth process and can result in severe neonatal disease both due to high inoculum and relative lack of protective maternal immunity.
- Postnatal viral infections are common and can cause a diverse range of clinical illness from isolated fever to severe pneumonia to viral sepsis with shock.
- The diversity of virus-caused disease is vast. Laboratory studies focused on virologic detection, driven in most cases by molecular assays such as polymerase chain reaction (PCR), are much more precise and reliable than serologic diagnosis.
- Antiviral therapies are now available for many viral pathogens, underscoring the importance of making a specific and timely diagnosis.

Introduction

Viral infections of the fetus and newborn are common and underrecognized (Box 34.1). Given the life-threatening nature of invasive bacterial infection in the neonate, the identification of viral infections is often assumed to be a matter of secondary importance. However, identifying viral infections is a matter of great urgency because an increasing number of antiviral therapeutic agents are available. Thus, a high index of clinical suspicion in identifying neonatal viral infections can be lifesaving. Moreover, identification of viral disease in the neonate may provide important prognostic information, particularly for viruses associated with a high risk of neurodevelopmental issues. Accordingly, making a diagnosis of a viral infection can help to direct and focus long-term management by the child's pediatrician. This chapter reviews the epidemiology, pathogenesis, diagnosis, and clinical management of many common congenital and perinatal viral infections encountered in neonates.

Of central importance in the evaluation, treatment, and prevention of viral disease in the newborn is identifying the time the infection was acquired. In this chapter, congenital infection is defined as any infection acquired by the fetus *in utero*. Perinatal infections are defined as those acquired during the labor and delivery process, also known as intrapartum. Postnatal infections are defined as infections acquired after delivery (postpartum) through the first month of life. Correctly identifying the timing of acquisition of infection can have substantial implications, not only for the care of the infant but for predicting the long-term prognosis. Fig. 34.1 outlines the most common neonatal viral infections with an emphasis on the relative importance of the risk interval for timing of acquisition. However, as some viral infections can be transmitted at any of these time points, specific disease outcomes based on the timing of infection will be considered on a pathogen-by-pathogen basis.

Several features of maternal and fetal immunity influence susceptibility to viral infection in important ways. Pregnancy is an altered immune state in which there is both immune suppression to promote tolerance of a "foreign" fetus, and heightened immune function, particularly at the maternal-fetal interface via Th2 polarization. While the consequences of this balance are somewhat pathogen specific, pregnant women have been found to be at risk of increased severity of infection with the following viruses: influenza, HSV, hepatitis E, Ebola, SARS-CoV-2, varicella, and measles.[1] While there are clear deleterious consequences of systemic infection regardless of pathogen in pregnancy, ranging from altered neurodevelopmental outcomes[2] to preterm birth,[3] these are generally beyond the scope of this chapter (in part because the risks are generally higher with bacterial as compared to viral infection). In the setting of maternal infection, viral tropism for the maternal-fetal interface is an important determinant of congenital infection; viral replication in maternal decidua or placental trophoblasts has been demonstrated to be important in the following fetal pathogens: cytomegalovirus (CMV), herpes simplex virus (HSV), and Zika virus. Finally, transfer of maternal antibody to the fetus (almost exclusively IgG) occurs via active transplacental transport which begins around 17 weeks of gestation and peaks around 37 weeks.[4,5] Additionally there is uptake from colostrum in the intestine (predominantly IgG) in the first week of life.[6,7] These antibodies typically remain detectable for 6 to 12 months and confer protective immunity in the newborn. Maternal antibody is an important determinant of disease severity particularly in neonatal HSV, VZV, respiratory syncytial virus (RSV), and influenza infections.

BOX 34.1 Viral Pathogens Reported to Cause Congenital/Neonatal Infections

Adenoviridae
- Adenovirus serogroup 3

Arenaviridae
- Lymphocytic choriomeningitis virus
- Lassa fever virus

Bunyaviridae
- Bunyamwera serogroup (Cache Valley virus)
- La Crosse encephalitis virus

Flaviviridae
- Zika virus
- Hepatitis C virus
- Japanese encephalitis virus
- West Nile virus
- St. Louis encephalitis virus
- Yellow fever virus
- Dengue virus

Hepadnaviridae
- Hepatitis B virus

Herpesviridae
- Herpes simplex viruses 1 and 2
- Varicella zoster virus
- Cytomegalovirus
- Epstein–Barr virus
- Human herpesviruses 6 and 7

Orthomyxoviridae and Paramyxoviridae
- Influenza viruses
- Measles virus

Parvoviridae
- Human parvovirus B19

Picornaviridae
- Poliovirus
- Coxsackievirus
- Enteric cytopathic human orphan virus
- Parechovirus
- Hepatitis A virus

Retroviridae
- Human T-lymphotropic viruses 1 and 2
- Human immunodeficiency virus

Togaviridae
- Chikungunya virus
- Rubella virus
- Western equine encephalitis virus
- Venezuelan equine encephalitis virus

General Diagnostic Concepts

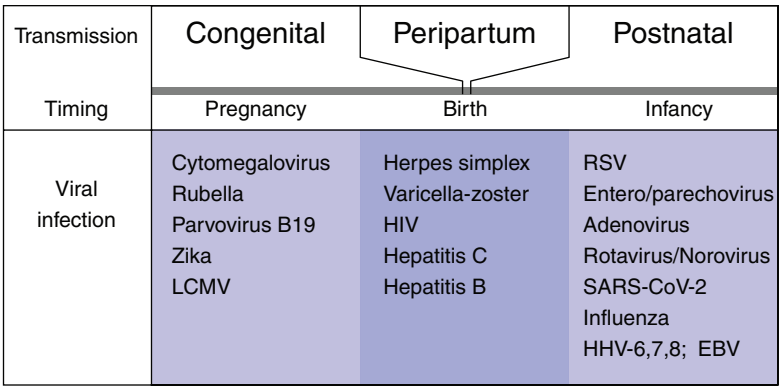
Clinicians caring for newborns have long recognized that there are some common clinical manifestations that suggest the presence of a congenital or perinatal viral infection. These manifestations include intrauterine growth restriction (IUGR), microcephaly, hydrops fetalis, hepatomegaly, splenomegaly, pneumonitis, bone lesions, rashes, and hematologic abnormalities. Because congenital viral infections are commonly encountered in neonatology practice, it is appropriate for clinicians to have a high index of suspicion in any newborn with suggestive signs or symptoms.[8] However, caution should be taken to thoughtfully consider diagnostic possibilities suggested by the history and physical examination, recognizing the specific etiologic diagnoses compatible with an infant's presentation (Table 34.1).

The acronym *TORCH*, first coined by Nahmias et al. in 1971, stands for toxoplasmosis, "other" infections, rubella, CMV, and herpes simplex virus HSV.[9] Numerous variants of this acronym have been suggested in the past 5 decades.[10–14] The use of '*TORCH titers*' in the diagnostic approach to a symptomatic neonate oversimplifies and seriously underestimates the diversity of neonatal viral infections encountered in practice.[15] This acronym has outlived its usefulness and should be discarded from clinical parlance. Unfortunately, numerous clinical laboratories continue to offer the "TORCH panel," typically consisting of serologic tests for toxoplasmosis, syphilis, HSV, CMV, and rubella.

Molecular tools based on polymerase chain reaction (PCR) amplification of viral nucleic acid are now available to identify virtually all pathogenic viruses. PCR tests are more sensitive and specific than serologic tests. Furthermore, measurements of neonatal immunoglobulin G (IgG) antibody titers virtually always reflect transplacentally transferred maternal antibody and provide little information of relevance to the infant's infection status. Also, congenital and perinatal infection can occur with HSV and CMV, even in the face of preconception maternal immunity. Thus, the finding of IgG antibody against these viruses in a TORCH titer is neither diagnostic of infection nor reassuring regarding protection against that infection.

Rather than relying on a large battery of serologic tests, the astute clinician can typically formulate a focused differential diagnosis of a suspected neonatal or congenital viral infection with the history and physical examination, followed by the use of specific diagnostic studies emphasizing virologic methods. Important questions include:
- What is the health and infection history of the mother?
- Did she have any symptomatic infectious illnesses during pregnancy?
- What is her immunization history?
- Has she had chickenpox or other childhood infections?
- What part of the world is she from?
- Is there a recent travel history?
- Are there potential animal exposures (e.g., exposure to cat litter or consumption of undercooked meat might suggest toxoplasmosis; exposure to rodents might suggest lymphocytic choriomeningitis virus [LCMV])?
- Does she have other children, and, if so, what are their ages, overall health status, and histories of group day care attendance?
- Have there been recent illnesses in the household? What time of year is it (e.g., RSV and enterovirus infections have characteristic seasonality in temperate zones)?
- What are her occupational exposures?

The answers to these types of questions, considered in the context of the infant's physical examination, can direct the next steps in establishing a definitive etiologic diagnosis. Some of the classic presentations of the more common perinatal viral infections are provided in Table 34.1. There can be considerable overlap of these clinical features across the different infectious categories listed; for example, the "blueberry muffin" rash of congenital rubella syndrome (CRS) may be indistinguishable from that



• **Fig. 34.1** Overview of viral infections of the fetus and newborn highlighted in this chapter. Individual viruses are listed beneath the timing of transmission that leads to the most significant pathology in the neonate. Some neonatal virus infections (e.g., cytomegalovirus) can be substantial causes of disease whether acquired during gestation or acquired postpartum, whereas others (e.g., RSV) are typically acquired in the postnatal period. *EBV*, Epstein-Barr virus; *HHV*, human herpesvirus; *HIV*, human immunodeficiency virus; *LCMV*, lymphocytic choriomeningitis virus; *RSV*, respiratory syncytial virus; *SARS-CoV*, severe acute respiratory syndrome coronavirus.

TABLE 34.1 Early Clinical Findings Associated With Congenital Viral Infections

Clinical Finding	Congenital Viral Causes (Ranked by Likelihood of Finding in Given Viral Infection)	Nonviral Causes (Including Nonviral Congenital Infections)
Abnormal prenatal ultrasound	CMV (echogenic bowel, brain abnormalities, intrahepatic calcifications), parvovirus B19 (fetal anemia, hydrops), Zika (microcephaly), VZV (limb hypoplasia)	Anatomic (neural tube defects, abdominal wall defects, congenital heart defects), genetic (aneuploidy)
Placental pathology	CMV (chronic villitis), HSV (placental infarcts), Zika (villitis), parvovirus B19, enterovirus, SARS-CoV-2 (acute villitis)	Idiopathic (villitis of unknown etiology), infection (granulomatous villitis: toxoplasma, acute villitis: group B streptococcus, *Escherichia coli*)
Intrauterine growth restriction	CMV, rubella, VZV (limb hypoplasia), HSV	Infection (toxoplasma, malaria, syphilis), genetic (aneuploidy, IGF pathway mutations), placental abnormalities, maternal chronic illness
Congenital contractures/ arthrogryposis	Rubella, varicella, Zika, Coxsackie B	Idiopathic (clubfoot), genetic mutations (distal arthrogryposis), neuromuscular disorders
Skeletal defects	Rubella ("celery stalking" with longitudinal demineralization)	Genetic (osteogenesis imperfecta, achondroplasia), toxin, infection (syphilis)
Microcephaly	CMV, Zika, rubella, LCMV	Genetic (Aicardi-Goutières syndrome, JAM3, NDE1, ANKLE2 mutations), toxins, placental insufficiency, malnutrition
Intracranial calcifications	CMV (periventricular), Zika (parenchymal), LCMV, rubella (basal ganglia)	Infection (toxoplasma), genetic (Aicardi-Goutières syndrome, RNASET2 mutations)
Sensorineural hearing loss	CMV, rubella, LCMV, HSV, VZV, mumps, measles, Zika	Genetic (GJB2, STRC mutations, syndromic), infection (toxoplasma, syphilis), toxins (alcohol, quinine, retinoic acid), maternal thyroid peroxidase antibodies
Congenital cataracts	Rubella, VZV, HSV	Genetic (crystallin, connexin mutations, aneuploidy, galactosemia), infection (toxoplasma, syphilis), medications (long-term glucocorticoids)
Chorioretinitis	Rubella (pigmented retina, cloudy cornea), CMV, VZV LCMV, Zika	Infection (toxoplasma), retinoblastoma
Keratoconjunctivitis	HSV	Postnatal infection (chlamydia, gonorrhea), anatomic (nasolacrimal duct obstruction)
Skin lesions	CMV (purpuric macules of extramedullary hematopoiesis), rubella (purpuric macules of extramedullary hematopoiesis), VZV (cicatricial regions, vesicles), HSV (vesicles)	Erythema toxicum, transient pustular melanosis, milia, acne neonatorum, seborrheic dermatitis, infection (toxoplasma, syphilis, bacterial sepsis, candida)

Continued

TABLE 34.1 Early Clinical Findings Associated With Congenital Viral Infections—cont'd

Clinical Finding	Congenital Viral Causes (Ranked by Likelihood of Finding in Given Viral Infection)	Nonviral Causes (Including Nonviral Congenital Infections)
Thrombocytopenia	CMV, rubella, HSV, HIV	Alloimmune, genetic (Wiskott-Aldrich, thrombocytopenia with absent radius, Bernard-Soulier syndromes, MYH-9 mutations)
Nonimmune hydrops fetalis	Parvovirus B19, CMV, rubella	Infection (toxoplasma, syphilis), genetic (aneuploidy, inborn errors of metabolism, congenital nephrotic syndrome)
Hemolytic anemia	Parvovirus B19, CMV	Alloimmune, genetic (hemoglobinopathies, erythrocyte membrane defects, G6PD deficiency)
Jaundice (conjugated hyperbilirubinemia)	CMV, rubella, HSV, LCMV	Anatomic (biliary atresia), genetic (Alagille syndrome, inborn errors of metabolism), infection (toxoplasma, syphilis)
Hepatosplenomegaly	CMV, rubella, parvovirus B19, LCMV	Genetic (inborn errors of metabolism), tumor, alloimmune hemolytic disease, infection (toxoplasma)
Pneumonitis	CMV, HSV, rubella	Infection (toxoplasma, syphilis)
Cardiac abnormalities	Rubella (PDA, pulmonary artery hypoplasia), parvovirus B19 (hypertrophic cardiopathy)	Idiopathic, genetic (aneuploidy) infection (syphilis)
Myocarditis	Coxsackie B virus, parvovirus B19, enterovirus	Genetic (inborn errors of metabolism, Noonan syndrome), alloimmune, vascular (infarct)

CMV, Cytomegalovirus; *G6PD*, glucose-6-phosphate dehydrogenase; *HSV*, herpes simplex virus; *HIV*, human immunodeficiency virus; *IGF*, insulin-like growth factor; *LCMV*, lymphocytic choriomeningitis virus; *PDA*, patent ductus arteriosus; *SARS-CoV*, severe acute respiratory syndrome coronavirus; *VZV*, varicella zoster virus.

of congenital CMV infection, and both syndromes can include sensorineural hearing loss. The presence of brain calcifications and/or microcephaly, although nonspecific, should always suggest a differential diagnosis that includes CMV, toxoplasmosis, and Zika virus. Because neuroradiology studies cannot reliably distinguish these entities, definitive diagnostic virology is necessary. In this chapter we review specific viral pathogens, their basic virology, the clinical manifestations of diseases they cause in the newborn, management strategies, and prospects for prevention on a pathogen-specific basis.

Cytomegalovirus

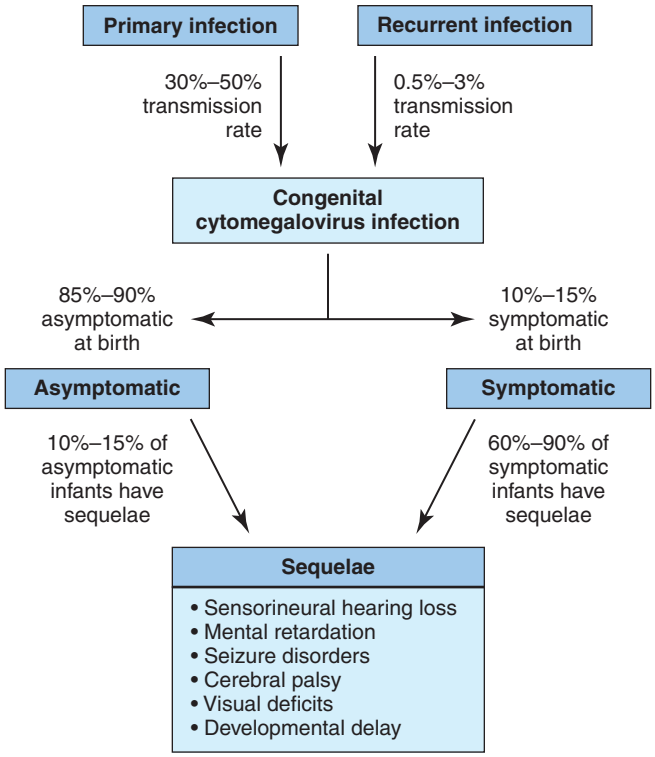
CMV infection is ubiquitous in the general population and usually produces few if any symptoms in the immunocompetent infant, child, or adult. However, CMV-induced illness can be severe in those with impaired, suppressed, or immature immune systems, including newborns. Among the congenitally acquired viral infections in the developed world, CMV infection imposes the largest economic burden and produces the greatest long-term neurodevelopmental morbidity.

Epidemiology

The first description of congenital CMV disease was in 1904,[16] when Ribbert observed the large inclusion-bearing cells that represent the typical histopathologic finding of CMV end-organ disease in a stillborn infant. In 1920 a viral cause was proposed for the "cytomegaly" seen in tissue sections of these inclusion-bearing cells,[17] but it would be several more decades before the ubiquitous nature of this virus and the depth and breadth of the disease it produces would be elucidated. In the developed world, CMV transmission occurs in 0.5% to 1% of all successful pregnancies, making CMV infection the most common congenital viral infection.[18–23] Seroprevalence rates for CMV differ significantly globally, but in general increase with age and are inversely correlated with socioeconomic status.[19] Although the lifetime risk of acquiring CMV infection is high, approaching 90% by the eighth decade of life,[24] seroprevalence is substantially lower among women of childbearing age. Seronegative women are therefore at risk of acquiring primary infections during pregnancy. Among pregnant women, the overall estimated annual seroconversion rate in the United States is 2.3%.[25] Acquisition of CMV occurs via contact with infectious secretions, including blood, saliva, urine, semen, vaginal fluid, and breast milk.[26,27] Primary sources for maternal CMV acquisition are through sexual activity and exposure to young children.[28]

Young children in group day care settings have particularly high rates of salivary CMV shedding,[29–31] thus day care providers and parents of young children in day care are at elevated risk of acquisition of primary CMV infections.[25,32–35] Health care providers in contrast are not at increased risk of acquisition of a primary CMV infection.[25,36]

The risk of intrauterine transmission after primary infection is estimated to be 30% to 50%, [37,38] with increasing rates of fetal infection at later stages in pregnancy.[38,39] However, the risk of symptomatic congenital CMV is highest for women with primary CMV infection during periconception and the first trimester, with acquisition during the second or third trimester rarely leading to adverse fetal outcomes.[38,40,41] In women with preexisting immunity, the risk of congenital infection is significantly lower (1% to 3%). However, reinfection with a different CMV strain, or reactivation, can lead to symptomatic infant disease and sequelae (Fig. 34.2).[42–45] Paradoxically, therefore the highest rates of congenital CMV are seen in populations with higher CMV seroprevalence.[46–49]



• **Fig. 34.2** Profiles of congenital cytomegalovirus (CMV) epidemiology, infection, and outcome. The rates of transmission to the fetus are highest in the setting of primary maternal infection (up to 50%), although 0.5% to 3% of women with preconception immunity may nonetheless transmit CMV because of reinfection or reactivation of latent infection. Among all infants with congenital CMV infection, regardless of maternal immune status during pregnancy, approximately 10% to 15% have symptoms or signs at birth (e.g., microcephaly, chorioretinitis, hepatosplenomegaly, petechiae, purpura, thrombocytopenia, hepatitis, seizures, pneumonitis). Symptomatic infants have the highest risk of neurodevelopmental sequelae, although any infant with congenital CMV infection is potentially at risk of sequelae. Among asymptomatic congenitally infected infants with sequelae, the most common manifestation is sensorineural hearing loss, which may not be present at birth.

Postnatal Acquisition of Cytomegalovirus: Implications for the Premature Infant

In addition to congenital infection, CMV can be acquired in the postnatal period. Postnatal CMV infection in healthy term infants is typically asymptomatic, with no convincing evidence of any adverse neurodevelopmental sequelae.[50,51] In contrast, primary CMV infection in preterm infants can lead to significant morbidity. Potential routes of neonatal infection include exposure to infectious cervicovaginal secretions during delivery,[27,52] ingestion of breast milk, contact with saliva, and via blood transfusion. Of these, the most common is via breast milk.[50,53–55] Detection of CMV by PCR or culture in breastmilk samples of CMV positive women is reported between 63% and 97%[27,50,56] and approximately 20% (range 6% to 59%) of premature infants fed breastmilk from CMV-infected mothers will become infected.[50] Risk for severe illness is greatest in very low birth weight (VLBW) infants, in whom rates of symptomatic illness or death are as high as 17% to 18%.[57,58] Symptoms in these infants include hepatopathy, neutropenia, thrombocytopenia, pneumonitis, and sepsis-like deterioration. The Centers for Disease Control and Prevention (CDC) has estimated that annually up to 4.5% of VLBW and premature infants, or an estimated 2000 infants, in the United States may develop CMV sepsis syndrome because of breast milk-acquired CMV infections.[21] Acquisition of CMV infection in the premature infant may contribute to the development of chronic lung disease.[59,60] The neurodevelopmental consequences of postnatal CMV infection in preterm infants are unclear, with some studies finding a detrimental effect[61] and others not.[51,62]

Proposed efforts to reduce the infectivity of breast milk from seropositive mothers have included freezing breast milk at −20°C, Holder pasteurization, and short-term pasteurization.[54] Of these methods, freezing is the most studied and most likely to maintain the salutary immunologic properties of breast milk. Although freezing of breast milk may lower the incidence of postnatally acquired CMV infection, it does not eliminate the risk.[63,64] Further evidence is necessary to make recommendations regarding what, if any, interventions are appropriate in low birth weight, preterm infants receiving breast milk from CMV-seropositive mothers.

Transfusion-associated CMV infections were at one time a major problem in the neonatal intensive care setting.[65–67] Two approaches are currently used to decrease the risks of transfusion-associated CMV infection: leukocyte reduction and directed transfusion of CMV-negative blood products.[68] A prospective, multicenter birth-cohort study conducted at three neonatal intensive care units (NICUs) in the United States found no evidence of transfusion-associated CMV infection when leukocyte-reduced blood was used for transfusion of VLBW infants.[58] These data support the practice of transfusing leukocyte-reduced blood products to premature infants, including blood from CMV-seropositive donors, to prevent transfusion-associated CMV infection in the NICU.

Pathophysiology

The mechanisms by which CMV injures the fetus involve a complex interplay of viral gene products, maternal immune response, and placental biology. The pathogenesis of disease associated with acute CMV infection has been attributed to lytic virus replication, with end-organ damage occurring either secondary to virus-mediated cell death or from pathologic host immune responses targeting virus-infected cells.[69,70] The factors that contribute to fetal injury include the timing of infection relative to the gestational age of the fetus,[71] the maternal immune status,[72] the extent of associated placental injury,[73] the magnitude of the viral load in the amniotic fluid,[74] the induction of host genes occurring in response to infection,[75] and possibly the genotype of the particular strain of CMV infecting the fetus.[76,77] Much of the injury that CMV produces in the newborn may be caused by placental insufficiency.[78] CMV infection of the placenta might also contribute to IUGR and fetal injury via induction of proinflammatory cytokines and modulation of normal trophoblast gene expression.[79–82] The developing fetal brain is also highly susceptible to CMV-induced injury.[83–85] The pathogenesis of CMV infection in the central nervous system (CNS) seems to be strongly related to perturbations in neural migration, neural death, cellular compositions, and the immune system of the brain.[86,87] In infants with symptomatic congenital CMV infection, histopathologic evidence of viral dissemination can be found in the brain, ear structures, retina, liver, lung, kidney, and endocrine glands.[88–90] The pathogenesis of sensorineural hearing loss (SNHL) is related to an inflammatory labyrinthitis.[91–93] CMV-infected cells can be seen in the semicircular canals, vestibular membrane, cochlea, and other structures of the ear.[89,93]

Clinical Presentation

Signs and symptoms are apparent at birth in 10% to 15% of all children with congenital CMV infection. Infection in the symptomatic infant can involve any organ and manifests itself along a spectrum from mild illness to severe disseminated multi-organ system disease (see Table 34.1). A wide spectrum of disease can be observed, including hemolysis, bone marrow suppression, hepatitis, pneumonitis, enteritis, and nephritis. Infants with symptomatic disease are often premature and small for their gestational age. Clinical features include jaundice, hepatosplenomegaly, lethargy, respiratory distress, seizures, and petechial rash. Common laboratory abnormalities include thrombocytopenia, anemia, and elevated transaminase and conjugated bilirubin levels.

CNS disease is common in symptomatic infants: signs and symptoms may include hypotonia, lethargy, seizures, and microcephaly.[94] Imaging by cranial ultrasound may reveal intracranial calcifications, periventricular cysts, ventriculomegaly, cerebellar hypoplasia, or lenticulostriate vasculopathy.[90,95,96] Additional findings such as white matter abnormalities and neuronal migration disorders may be identified by MRI.[90,96–98]

Ophthalmologic abnormalities are seen in 20% to 40% of symptomatic infants, but rarely in asymptomatic infants.[99,100] Common findings include retinal scars, optic atrophy, and chorioretinitis. SNHL is either present at birth or develops in 40% to 70% of symptomatic infants[94,101,102] and 10% to 15% of infants who are otherwise asymptomatic.[101,103–105] SNHL can be progressive and fluctuating in both asymptomatic and symptomatically congenitally infected infants.[106] It ranges in severity from a unilateral, mild hearing deficit to severe, bilateral, profound deafness. CMV-induced SNHL may be present at birth or can appear later in childhood. Delayed-onset hearing loss usually presents in the first few years of life[101,107–110] but has been reported to evolve and progress through 5 years of age and beyond.

Mortality due to symptomatic congenital CMV disease in the neonatal period is uncommon, likely less than 5%.[111,112] However, long-term neurodevelopmental disabilities are observed in 50% to 75% of children who are symptomatic at birth[102,113] and can include motor deficits (paresis or paralysis), cerebral palsy, intellectual disability, seizures, vision impairment, hearing loss, and learning disabilities.[86,114] In contrast, several studies have suggested that the intellectual development of asymptomatic congenitally infected infants appears to be normal.[115,116]

Diagnosis

Prenatal Diagnosis

Primary infection during pregnancy can be identified by serologic testing, although testing for CMV immunity is not currently standard practice during pregnancy. Recommended testing includes CMV IgG and IgM antibody levels with IgG avidity testing for women in whom CMV IgM is detected. While the presence of CMV IgM is a sensitive marker for primary infection, the specificity for recent primary infection is lower as IgM can persist for months after infection. The additional IgG avidity testing can improve the specificity; a low IgG avidity index is consistent with recent primary infection.[117]

Prenatal ultrasonography provides clues to the possible diagnosis of fetal CMV infection; however, ultrasound has low sensitivity.[118] Findings suggesting possible congenital CMV include echogenic bowel, IUGR, brain abnormalities (microcephaly, ventriculomegaly, periventricular calcifications, periventricular hyperechogenicity), polyhydramnios, pericardial effusion, fetal hydrops, hepatosplenomegaly, intrahepatic calcifications, and placental enlargement.[118–122] Abnormal prenatal findings on ultrasound examination are associated with increased risk of sequelae,[123] while normal cranial imaging by ultrasound or MRI is reassuring for low risk of adverse postnatal outcome.[124–127] When in utero CMV acquisition is suspected due to detection of maternal primary infection in early pregnancy and abnormalities identified by fetal ultrasound, amniotic fluid can be tested by PCR to determine fetal infection. Testing is recommended to be done at least 6 weeks after maternal infection and after 20 weeks' gestation for the highest sensitivity.[117,122,128] This allows sufficient time for transplacental passage, replication in the kidneys, and excretion into amniotic fluid.[129]

Postnatal Diagnosis

Congenital CMV infection is best diagnosed by detection of virus in samples collected from the neonate in the first 2 to 3 weeks of life. The timing of collection of these samples is important because viral isolation beyond 3 weeks of age may represent infection acquired in the birth canal or more likely after exposure to breast milk[130] and not congenital infection. Urine and saliva are the clinical samples of choice for virus detection. Although the "gold standard" for CMV diagnosis has traditionally been viral shell vial culture, PCR has better sensitivity,[131–134] and fewer laboratories are offering viral culture. Thus, CMV culture has largely been replaced by PCR.

Serologic tests are of limited utility in the diagnosis of congenital CMV. Unlike IgG which can reflect maternal antibodies, the presence of IgM antibodies to CMV in cord or neonatal blood represents fetal antibody response. However, IgM serologic tests lack sensitivity and cannot be relied on for diagnosis of congenital CMV infection.[135]

A retrospective diagnosis of congenital CMV in an infant determined to be CMV infected after the first 3 weeks of life can be done by CMV DNA PCR testing of the dried blood spot (DBS) samples collected for newborn screening. The sensitivity of DBS testing depends on the characteristics of the PCR assay performed and is reported to be between 70% and 95%.[23,136,137] Because DBSs are stable in storage for several years, retrospective diagnosis of congenital CMV infection can be made in older infants with identified SNHL.[138,139]

Infants diagnosed with congenital CMV should receive a comprehensive evaluation to identify unapparent signs of symptomatic disease as these may affect treatment recommendations and prognosis. This would include a complete blood count, liver function tests, hearing evaluation, eye examination, and cranial imaging.[140] CMV DNA PCR from blood should be considered at baseline as viral levels in the congenitally infected infant may be associated with long-term outcome.[141–144] Because the finding of CNS disease is a potential harbinger of permanent sequelae, diagnostic CNS imaging is warranted in all suspected cases of congenital infection.[83,84,90,95,145–147] Ultrasonography is an appropriate initial study and is particularly sensitive in detecting periventricular calcifications and lenticulostriate vasculopathy. For symptomatic infants and those with abnormal findings on cranial ultrasound, MRI may provide important additional information. Therefore, the staged sequential use of an initial cranial ultrasound followed by MRI is probably the preferred approach to CNS imaging in this setting. Ophthalmologic evaluations are particularly important for symptomatic infants. Asymptomatic

infants with normal eye exams are unlikely to develop later abnormalities.[99,100]

All congenitally infected infants, regardless of the results of functional hearing assessment at birth, should be monitored prospectively for SNHL.[101,140,148] Children with congenital CMV, whether symptomatic or not, should have neurodevelopmental assessment and follow-up with early intervention if indicated.[140,148]

Mothers of infants with congenital CMV infection should be counseled regarding future pregnancies. For infants with symptomatic congenital CMV infection born to women with low CMV IgG avidity antibodies,[149] some authorities recommend monitoring for the emergence of high-avidity antibody before future pregnancies are contemplated.

Management

The benefits of ganciclovir in neonates with symptomatically congenital CMV have been demonstrated in controlled trials. Neonates with virologically confirmed symptomatic congenital CMV disease involving the CNS who received a 6-week course of IV ganciclovir therapy had a statistically higher likelihood of normal or improved hearing at 6 months of age compared with untreated neonates.[150] Infants in this trial who received IV ganciclovir therapy also had fewer developmental delays at 6 and 12 months, as assessed by the Denver Developmental Screening Test, compared with untreated infants.[151] A subsequent study compared 6 weeks versus 6 months of oral valganciclovir in neonates with symptomatic congenital CMV disease. In this study, all participants received valganciclovir for 6 weeks. Participants were then randomized to receive either continued valganciclovir therapy or placebo for 4.5 months. Hearing was more likely to be improved or to remain normal at 12 and 24 months in the 6-month treatment group than in the 6-week treatment group, and this group also had better neurodevelopmental scores on the Bayley Scales of Infant and Toddler Development at 24 months.[152] Six months of oral valganciclovir therapy (Table 34.2) should thus be considered for all neonates with symptomatic congenital CMV infection with any evidence of CNS involvement (microcephaly, abnormal CNS imaging, CSF positive for CMV DNA, chorioretinitis, or evidence of SNHL). Careful monitoring for neutropenia, the major side effect of valganciclovir therapy, is essential.

Ganciclovir therapy should also be used in any infant with severe or life-threatening end-organ CMV disease (see Table 34.2), whether acquired via congenital infection or by a postnatal route, such as via breastfeeding in a low birth weight premature infant.[153] CMV chorioretinitis, if present, should be managed in consultation with an ophthalmologist and infectious diseases expert and may require prolonged ganciclovir treatment.[154]

TABLE 34.2 Antiviral Agents Commonly Used in Neonatology Practice

Antiviral Agent	Indication	Dose, Route of Administration, Duration of Therapy	Comments
Acyclovir	Neonatal HSV infection	20 mg/kg/dose every 8 h intravenously; 21 days for disseminated or CNS disease; 14 days for SEM disease	Monitor CBC twice weekly; adjust dosage for renal insufficiency.
	Oral suppression following neonatal HSV infection	300 mg/m²/dose, three times a day; duration of therapy, 6 months	Neutropenia observed in half to two-thirds of infants
	VZV infection	10 mg/kg/dose every 8 h for a minimum of 5–7 days	Longer courses may be needed for severe end-organ disease (pneumonia, hepatitis)
Trifluridine, 1%	HSV ophthalmic disease	Apply as eye drops; one drop every 2 h to the affected cornea while awake; maximum nine drops per day	Treat in consultation with an ophthalmologist.
Ganciclovir	Symptomatic congenital CMV infection unable to take oral therapy; acquired symptomatic CMV infection	6 mg/kg/dose every 12 h intravenously; duration of 14–21 days for serious end-organ disease—6 weeks for prevention of hearing loss in infants with congenital disease or until able to take oral valganciclovir	Efficacy against CMV-associated hearing loss in controlled trial; benefits of shorter courses of therapy unknown; neutropenia observed in 63% of patients in controlled trial; adjust dose for renal insufficiency; consider use of G-CSF if continued therapy desired in the setting of neutropenia
Valganciclovir	Symptomatic congenital CMV infection	16 mg/kg/dose, twice daily for 6 months	Valine ester (prodrug) of ganciclovir; toxicity profile similar to that of ganciclovir; theoretical concerns of carcinogenesis, gonadal toxicity
Oseltamivir	Influenza A virus infection, influenza B virus infection	Treatment: Infants <9 months: 3 mg/kg/dose oral twice daily for 5 days (maximum 30 mg) Infants 9–12 months: 3.5 mg/kg/dose oral twice daily for 5 days (maximum 30 mg) Prophylaxis: same doses as above given once daily for 10 days	Dose adjustments required for premature infants

CBC, Complete blood cell count; *CMV*, cytomegalovirus; *CNS*, central nervous system; *G-CSF*, granulocyte colony-stimulating factor; *HSV*, herpes simplex virus, *SEM*, skin, eye, or mucous membranes; *VZV*, varicella–zoster virus.

Although ganciclovir/valganciclovir improves hearing and neurodevelopmental outcomes for symptomatic infants when initiated within the first month of life, it is not yet clear whether valganciclovir holds the promise of improving outcomes in infants with asymptomatic congenital CMV infection, or for infants presenting outside the neonatal period with isolated SNHL. A clinical trial is ongoing to determine whether valganciclovir therapy could prevent progression of hearing loss in older infants with isolated SNHL.[155]

Ganciclovir has been demonstrated to cross the placenta, and therefore could theoretically be used to treat CMV infection in utero,[156] however there are concerns for potential teratogenicity.[157] There have been case reports of treatment of congenital CMV infection in utero with valganciclovir,[158,159] but no systematic trials. High-dosage oral valacyclovir therapy (8 g/day) in recent studies has been found to be safe and to decrease the risk of fetal CMV infection and symptomatic congenital disease.[160,161]

Passive immunization with CMV human immunoglobulin (HIG), a pooled, high-titer immunoglobulin preparation, has been studied for in utero treatment and prevention of congenital CMV infection. Reduction in congenital infection and symptomatic neonatal disease has been seen in CMV HIG-treated pregnant women in several uncontrolled unblinded studies.[162–164] However, other observational studies[165] and a randomized, blinded placebo-controlled trial of CMV HIG for the treatment and prevention of congenital CMV infection failed to demonstrate a benefit.[166] A National Institutes of Health-funded multicenter randomized, placebo-controlled trial of CMV HIG during pregnancy in the setting of primary maternal infection was recently stopped early due to futility with no significant difference in transmission between the treatment and placebo arms of the study.[167] Therefore, it remains unclear if CMV HIG is useful in prevention of viral transmission or sequelae.

Prevention

One important strategy for addressing the problem of congenital CMV infection is the education of women of childbearing age about the risks of CMV transmission and strategies for prevention. Because most maternal CMV infections are asymptomatic, a major goal is education of all women of childbearing age on hygienic practices.[168–170] Hygienic strategies are important because the saliva and urine of infected children are significant sources of CMV infection among pregnant women. Strategies include washing hands whenever there is contact with a child's saliva or urine, not sharing food, utensils, or cups, and not kissing a child on the mouth or cheek.[171–173] In several surveys, only 14% to 30% of women had previously heard of CMV.[174,175] Therefore, educating women on methods to prevent CMV transmission may be effective to decrease seroconversion during pregnancy.[173,176–178]

Prenatal maternal screening for CMV antibodies is controversial. Women who are CMV immune can be infected with new strains with subsequent fetal infection and developmental sequelae.[42,43,45,179] A positive preconception titer result for CMV IgG antibody may provide a false sense of reassurance and decrease a pregnant woman's motivation to engage in careful hygienic practices.

Ultimately the control of congenital CMV infection could be realized by the development of an effective vaccine. No CMV vaccines are currently licensed; however, multiple candidate vaccines are in various stages of development including in active clinical trials.[180]

Rubella

Rubella virus is an enveloped, single-stranded, positive-sense RNA virus belonging to the family *Togaviridae*. Humans are the only known natural host for rubella virus. Rubella usually results in a mild illness with an accompanying exanthem in adults and children; however, rubella infection during pregnancy can produce serious fetal anomalies. An ophthalmologist, Norman Gregg, offered the first description of congenital rubella syndrome (CRS) in 1941 while investigating an epidemic of neonatal cataracts.[181,182] Not until the global pandemic of 1964 to 1965, however, were the multiple teratogenic manifestations of CRS, including permanent neurodevelopmental consequences, fully appreciated. The introduction of rubella vaccine in 1969 led to an immediate reduction in the incidence of CRS in the United States and other developed countries[183] and now most cases occur in women who contracted rubella in countries without widespread vaccine coverage.[184–187]

Epidemiology

The annual incidence of rubella cases has decreased dramatically from 58 per 100,000 population in 1969 to only 10 cases reported in the US in 2012.[188,189] CRS cases in the United States have demonstrated a similar dramatic decline, and in 2004 the CDC concluded that endemic rubella had been eliminated from the United States.[190–192] Rubella is still an important and potentially preventable cause of birth defects globally, with more than 100,000 cases of CRS occurring annually in developing countries in the last decade (Table 34.3).[193] This number is likely lower now as significant progress toward elimination of rubella has been made worldwide, although there are still countries with suboptimal national vaccination programs.[194–198]

The frequency of congenital infection after maternal rubella is 70% to 85% if infection occurs during the first 12 weeks of gestation, 30% to 54% during the first 13 to 16 weeks of gestation, and 10% to 25% at the end of the second trimester.[199,200] The classic findings of congenital rubella are most typically associated with the onset of maternal infection during the first 11 weeks of gestation,[199] and the risk of any teratogenic effect is extremely low after 17 weeks' gestation.[201]

Pathogenesis

Rubella virus is transmitted via respiratory droplets. Viral replication begins in the upper respiratory tract and nasopharyngeal lymphoid tissue, followed by contiguous spread to regional lymph nodes and hematogenous dissemination to distant sites. Fetal infection is believed to occur as a consequence of maternal viremia. The mechanism by which fetal rubella infection leads to teratogenesis has not been fully determined, but the cytopathology in infected fetal tissues suggests necrosis, apoptosis, or both, as well as inhibition of cell division of precursor cells involved in organogenesis.[201,202] Macrovascular fetal endothelial cells have been found to be highly permissive for rubella virus replication, suggesting that the vascular diseases in CRS are triggered by persistent rubella virus infection of endothelial cells.[203,204]

Clinical Presentation

The typical illness in adults and children with acquired rubella consists of an acute generalized maculopapular rash, fever, and arthralgias, arthritis, or lymphadenopathy. Conjunctivitis

TABLE 34.3 Clinically Important but Uncommon Causes of Congenital Infection

Virus	Epidemiology	Pathology	Clinical Presentation (Neonates)	Diagnosis of Fetal/Infant Infection	Management	Prevention	Outcome
Coxsackie B	Congenital infection quite rare	Direct viral myocardial injury	Newborns often asymptomatic, develop heart failure in first week	PCR	Supportive care	None available	Persistent myocardial dysfunction in 33%–66%
HHV-6	Generally acquired prior to age 2, but *in utero* transmission very rare	Can be chromosomally integrated	Asymptomatic in newborns; roseola infantum, febrile seizures	PCR	Supportive care	None available	Possible neurodevelopmental concerns with germline transmission
LCMV	Very rare	Viral- and immune-mediated brain injury	Chorioretinitis, microcephaly, hydrocephalus, neural migration defects	Infant serum, CSF PCR; maternal serology	Supportive care	Avoid rodent exposures in pregnancy	Mortality 35%, neurologic sequelae in 63% of survivors
Parvovirus B19	~1% seroconversion during pregnancy; 25%–50% fetal infection with 3%–12% adverse fetal outcome	Cytotoxic to fetal red blood cells	Risk for fetal hydrops	Amniotic fluid, cord blood PCR	Serial prenatal ultrasounds and IUT for fetal anemia/hydrops	None available	Mortality 15%–25% for severe fetal hydrops even with IUT, slight risk for neurodevelopmental abnormalities in infants requiring IUT
Rubella	Rare in the United States; CRS remains an important cause of birth defects in countries without adequate maternal immunization	Infection of fetal tissues	See Table 34.4	Serology, PCR	Supportive care	Vaccine	Neurologic/neurodevelopmental abnormalities, SNHL, autoimmune disorders
Varicella	CVS—very rare	Destruction of neural tissue	Cicatricial skin lesions, limb hypoplasia, eye abnormalities	Amniotic fluid PCR	Supportive care	Maternal vaccine pre-conception; VariZIG for maternal exposure during pregnancy	Mortality CVS 30%, developmental delay common
Zika	6%–17% of affected pregnancies develop congenital anomalies; 14% have fetal loss	Viral perturbation of neural development	Microcephaly, intracranial calcifications, retinal abnormalities	Infant PCR and IgM	Supportive care	Avoid travel to endemic countries, insect repellent	Visual and/or hearing impairment frequent; developmental delay common even if asymptomatic at birth

CVS, Congenital varicella syndrome; *HHV*, human herpesvirus; *IUT*, intrauterine transfusion; *LCMV*, lymphocytic choriomeningitis virus; *PCR*, polymerase chain reaction.

is also common. Viremia can be detected as early as 9 days prior to the onset of rash. Up to 50% of primary infections are asymptomatic.

Infants with congenital rubella are usually born at term but are often small for their gestational age. Transient manifestations may include skin lesions described as resembling a blueberry muffin which represent extramedullary dermal hematopoiesis,[205–208] thrombocytopenia, hyperbilirubinemia, and leukopenia, hepatosplenomegaly, jaundice, pneumonia, and meningoencephalitis. Radiographic findings include a large anterior fontanel, linear areas of radiolucency in the long bones (i.e., celery stalking), increased densities in the metaphysis, and irregular provisional zones of calcification.[209,210] Permanent findings include SNHL, heart defects, eye abnormalities, neurologic defects, and developmental delay (Table 34.4).[196,208] The most common isolated finding of congenital rubella is SNHL.[199,211] Cardiac lesions are noted in 45% to 70% of infants with the most common abnormalities being patent ductus arteriosus, peripheral pulmonic stenosis, and valve abnormalities.[212–214] Ocular findings include cataracts, pigmentary retinopathy, microphthalmia, glaucoma, and strabismus. The triad of deafness, cataracts, and congenital heart disease constitutes the classic CRS.[215]

The clinical manifestations of CRS differ to some extent depending on the timing of fetal infection. In a prospective study following up pregnant women with confirmed rubella by trimester, a full range of rubella-associated defects (including congenital heart disease and deafness) were observed in nine infants infected before the 11th week. Thirty-five percent of infants (9 of 26) infected between 13 and 16 weeks' gestation had deafness alone.[199]

Evaluation

Diagnosis of congenital rubella syndrome is based on both clinical and laboratory findings. In the first year of life, laboratory evidence of congenital rubella infection can be demonstrated by a positive rubella IgM titer; infant rubella IgG level that persists at a higher level and for a longer period than expected from passive transfer of maternal antibody; isolation of rubella virus from nasal, blood, throat, urine, or CSF samples; or detection of viral nucleic acid by PCR from the throat, CSF, or lens (obtained during cataract surgery). An infected infant can shed the virus for many months after birth despite the presence of neutralizing antibody and thus may pose a hazard to susceptible individuals.[216]

Women who are exposed to rubella during pregnancy should be screened for evidence of previous immunity. If rubella IgG is detectable at the time of exposure, the fetus is probably protected. If no antibody is detectable, additional samples for rubella IgG and IgM should be obtained at 2 to 3 weeks after exposure and again at 4 to 6 weeks after exposure. These samples can be run concurrently with the first serum sample to ascertain whether infection has occurred (i.e., seroconversion).[48]

TABLE 34.4 Manifestations of Congenital Rubella

Transient	Permanent	Delayed Onset

• Low birth weight
• Extramedullary dermal hematopoiesis
• Thrombocytopenia
• Hyperbilirubinemia
• Leukopenia
• Hepatosplenomegaly
• Pneumonia
• Meningoencephalitis
• Linear radiolucent areas in the long bones
• Metaphyseal densities
	• Sensorineural hearing loss
	• Heart defects
	  • Patent ductus arteriosus
	  • Peripheral pulmonic stenosis
	  • Valve abnormalities
	• Eye defects
	  • Cataracts
	  • Pigmentary retinopathy
	  • Microphthalmia
	• Microcephaly
	• Developmental delay
		• Abnormal dental development
		• Autoimmune diseases
		  • Diabetes
		  • Thyroid disease
		• Immunologic abnormalities
		• Progressive panencephalitis
		• Ophthalmologic
		  • Glaucoma
		  • Uveitis

Management

There is no specific antiviral therapy for congenital rubella. Initially the infant may need general supportive care, such as administration of blood transfusion for anemia or active bleeding, seizure control, and phototherapy for hyperbilirubinemia.

Outcomes

Long-term care requires a multidisciplinary approach consisting of occupational and physical therapy, close neurologic and audiologic monitoring, and surgical interventions as needed for cardiac malformations and cataracts.[217] Over half of surviving infants have neurologic and/or developmental abnormalities,[208,218] which may not be diagnosed until the infants are older. Other delayed manifestations include late-onset glaucoma and other ophthalmologic abnormalities,[218,219] progressive SNHL, autism spectrum disorders,[208,220] progressive rubella panencephalitis, abnormal dental development,[221] and immunologic and autoimmune endocrine disorders (see Table 34.3). A higher-than-expected incidence of autoimmune diseases, such as thyroid disorders and diabetes mellitus, has also been reported years after the diagnosis of congenital rubella.[181,213,222,223] Delayed onset immunologic abnormalities include abnormalities in immunoglobulin levels[224–227] and cellular immune responses.[228–230]

Prevention

The critical intervention in prevention of CRS is to ensure that women who are considering pregnancy get appropriately vaccinated. The Advisory Committee on Immunization Practices recommends screening of all pregnant women for rubella immunity and postpartum vaccination of those who are susceptible.[231] Immunity to rubella appears to confer almost complete protection against CRS. Rare cases of documented subclinical maternal reinfection with rubella virus leading to CRS have been reported.[232–235]

Live attenuated rubella virus vaccine is safe and effective, although the duration of immunity is uncertain. Rubella vaccine is currently administered in the United States in a trivalent formulation in combination as measles–mumps–rubella (MMR) vaccine. The vaccine is recommended for children at 12 to 15 months of age and at 4 to 5 years of age. It is also recommended for women of childbearing age in whom the results of both a hemagglutination inhibition antibody test and a pregnancy test are negative. Although no cases of symptomatic congenital rubella have been reported after vaccination during pregnancy in the more than 500 cases monitored, vaccination is not recommended during pregnancy because of the theoretical hazard to the fetus.[236–238]

If a pregnant woman is found to be nonimmune, vaccine should be administered during the immediate postpartum period before discharge. Breastfeeding is not a contraindication to postpartum immunization. Immunization in the postpartum period has rarely produced polyarticular arthritis, neurologic symptoms, or chronic rubella viremia.[239] Immune globulin does not prevent rubella infection after exposure and is not recommended for routine postexposure prophylaxis in pregnancy.[240,241]

Human Parvovirus B19

Parvovirus B19, a small, single-stranded DNA virus, is the only member of the parvovirus family that causes human disease. Primary infection with parvovirus B19 is commonly known as *fifth disease* or *erythema infectiosum* and is classically described as a childhood exanthem with a "slapped cheek" appearance.[242] The spectrum of parvovirus B19-associated disease continues to expand and includes neurologic disease, arthritis, autoimmune disease, hematologic disease, and other dermatologic manifestations.[243–247]

Epidemiology

Parvovirus B19 infection is common in childhood and continues at a low rate throughout adult life. The virus is spread primarily by respiratory droplets[248] but can also be transmitted by blood products[249] and transplacentally.[250,251] Seroprevalence in the population varies by age and geographic location, but averages about 40% to 60% in women of childbearing age.[251–254] Seroconversion during pregnancy is reported to be about 1% with increased rates noted during periodic epidemics.[253]

It is estimated that one-fourth to half of maternal parvovirus B19 infections result in transmission of infection to the fetus.[255–257] The spectrum of diseases associated with parvovirus B19 infection remains incompletely defined, as there is no routine surveillance during pregnancy; however, most pregnancies are unaffected. The overall risk of adverse fetal outcome is between 3% and 12%.[250,257,258] The risk of adverse fetal outcome is increased if maternal infection occurs before 20 weeks' gestation.[255,257–261]

Pathophysiology

Fetal infection most likely occurs transplacentally during maternal viremia. Parvovirus B19 binds to a glycolipid receptor (P-antigen or globoside) present on erythroid progenitor cells[262,263] and placental trophoblasts.[264] P-antigen expression by villous trophoblasts is gestation dependent and is highest in the first trimester.[264] It has been postulated that parvovirus B19 infection leads to cytotoxicity and subsequent anemia by inducing apoptosis of infected red blood cells.[265,266] The virus targets the erythroid lineage cells in the fetal liver, the primary site of erythrocyte production in the fetus.[267] The fetus is especially susceptible to adverse consequences of red blood cell infection secondary to the intrinsic short fetal erythrocyte life span and rapidly expanding blood volume, especially during the second trimester. The P-antigen is also expressed on fetal cardiac myocytes, enabling parvovirus B19 to infect myocardial cells,[268] leading to myocarditis.[269,270] Myocarditis induced by parvovirus B19 can contribute to high-output cardiac failure, and the myocardial inflammation and subendocardial fibroelastosis may also contribute to fetal hydrops.[271,272]

Clinical Presentation

Parvovirus B19 infection causes *erythema infectiosum* in normal hosts, aplastic crisis in patients with hemolytic disorders, and chronic anemia in immunocompromised hosts. A substantial proportion of infected adult women may also have arthropathy in association with parvovirus B19 infection.[273] Once the virus establishes infection, viremia occurs, accompanied by mild systemic symptoms such as fever and malaise. Viremia is short-lived, lasting only 1 to 3 days, and the characteristic immune-mediated rash develops 1 to 2 weeks later. Once the rash appears, an individual is no longer infectious. Infection with parvovirus B19 infection during pregnancy may be asymptomatic more than half of the time.[255,256]

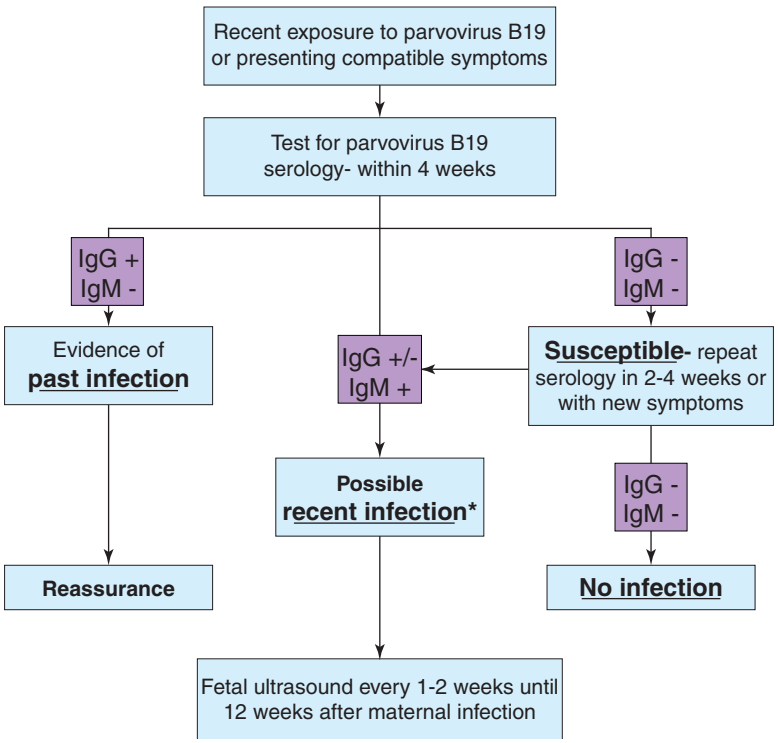
The major clinical presentation of parvovirus B19 infection in the fetus is nonimmune hydrops fetalis (NIHF). Various estimates suggest that human parvovirus B19 infection contributes from 10% to 27% of cases of NIHF.[274–276] The risk of NIHF secondary to maternal parvovirus B19 infection is between 4% and 12% in most reports and is highest for women who acquire parvovirus B19 infection between 13 and 20 weeks' gestation.[250,251] NIHF usually develops 2 to 8 weeks after maternal parvovirus B19 infection.[257] The role that parvovirus B19 plays in intrauterine fetal demise in the absence of hydrops fetalis is incompletely defined. Some studies show increased risk of fetal loss, spontaneous abortion, and stillbirth,[277–279] while others do not.[280,281] The estimated risk of fetal loss in infants infected with parvovirus B19 prior to 20 weeks gestation is 13% and decreases to 0.5% after 20 weeks' gestation.[282]

Parvovirus B19 has been implicated in some cases of congenital anemia.[283–286] Investigation for parvovirus B19 infection should be included in the evaluation for congenital anemias. Thrombocytopenia is common in parvovirus B19-infected fetuses with NIHF. In one series, 46% of fetuses with parvovirus B19-associated NIHF had severe thrombocytopenia.[287]

Evaluation

In children and adults, the diagnosis of human parvovirus B19 infection is most commonly made clinically through recognition of the characteristic rash. Women exposed to parvovirus B19 during pregnancy should be tested for virus-specific IgG and IgM antibodies. Those who are IgG positive and IgM negative are immune and therefore not at risk for infection. Women who are IgM and IgG negative should be monitored for seroconversion by repeat serologic testing in 4 weeks. Women who develop anti-parvovirus B19 antibodies or who demonstrate parvovirus B19 IgM at initial testing should be monitored for potential fetal infection (Fig. 34.3).[288] Parvovirus B19 DNA can be detected in plasma of infected women early in the course of infection and is more sensitive than serology for diagnosis of acute infection. A combination of serology and PCR can be used to improve the accuracy of diagnosis.[251,255,289]

Pregnant mothers with evidence of acute infection should be counseled regarding risks of fetal transmission, fetal loss, and hydrops, and serial ultrasound examinations should be performed every 1 to 2 weeks, up to 12 weeks after acute infection, to detect development of anemia and hydrops.[251,282,288] Monitoring should include assessment for ascites, placentomegaly, cardiomegaly, hydrops fetalis, and impaired fetal growth. Middle cerebral artery Doppler imaging for peak systolic velocity should



• **Fig. 34.3** Management algorithm for parvovirus B19 exposure during pregnancy. *If IgG–, IgM+ can confirm with repeat serology in 1 to 2 weeks or maternal plasma polymerase chain reaction. (Adapted from Crane J, Mundle W, Boucoiran I, et al. Parvovirus B19 infection in pregnancy. J Obstet Gynaecol Can. 2014;36[12]:1107–1116.)

be performed as this is an accurate predictor of fetal anemia which can be detected with this technique before fetal hydrops is evident.[290] Maternal parvovirus B19 infection should also be considered when prenatal ultrasounds show evidence of fetal anemia, cardiomegaly, or hydrops fetalis. As fetal manifestations of parvovirus B19 infection occur weeks after maternal infection, absence of maternal parvovirus B19 IgM or plasma B19 DNA does not exclude infection. In one study,[289] women tested due to suspicion of fetal infection were IgM positive 62.5% and DNA PCR positive 87.5%. Detectable parvovirus B19 DNA by PCR in amniotic fluid or fetal blood establishes the diagnosis in the fetus.[288,289]

Management

Spontaneous resolution of fetal anemia without hydrops has been reported in about 50% of fetal infections, however, spontaneous resolution of hydrops is less common (5% to 30%).[291,292] Because most fetuses do not recover without intervention, fetal transfusion is usually recommended.[288,293,294] The earlier intrauterine transfusion (IUT) is attempted, the more likely it is to be successful. Cordocentesis allows precise assessment of the magnitude of fetal anemia, which can then be corrected by blood transfusion, typically using packed red blood cells. With this approach, outcomes have been favorable in most reported series, even among severely anemic fetuses. Because of the frequency of concurrent thrombocytopenia, platelet transfusion is also frequently required at the time of IUT.[251,295,296] Small studies suggest a survival rate of 76-85% for fetuses with severe hydrops fetalis who receive IUT, compared to 100% mortality for non-transfused fetuses.[259,297] Although high-dose IVIG has been used in the setting of acute infection to attempt to prevent hydrops fetalis during pregnancy,[298] treatment with this modality in the pregnant woman has not been shown to improve fetal outcomes.

Outcomes

Results from several long-term prospective studies of infants born to mothers with documented primary parvovirus B19 infection during pregnancy found no evidence of increased risk of long-term morbidity, development delay, or death in childhood.[299–301] Increasingly, however, there have been reports of neurologic morbidity in infants surviving severe anemia or NIHF due to parvovirus B19. Structural brain abnormalities have been seen in infants with severe anemia or NIHF,[292,302] including small cerebellum,[302] polymicrogyria,[303,304] and heterotopia.[303] Neurologic abnormalities have been noted on follow-up in approximately 10% of infants surviving IUT for B19-associated NIHF.[292,305]

Prevention

There has been limited progress in the development of a candidate parvovirus B19 vaccine. At the current time, routine screening of pregnant women for parvovirus B19 is not recommended.[288] If a pregnant woman has a significant exposure to an infectious case of parvovirus B19, counseling should be provided regarding the potential risk of infection and testing and follow-up as described above should be recommended.

Pregnant healthcare providers should be counseled about the potential risks to their fetus from parvovirus B19 infections. They should consider not caring for immunocompromised patients

with chronic parvovirus B19 infection or patients with parvovirus B19-induced aplastic crises, or should, at a minimum, follow strict infection control procedures including standard droplet precautions.[306]

Zika Virus

Zika virus is a single-stranded RNA virus of the Flavivirus family that was first isolated in 1947 from the Zika forest of Uganda.[307,308] For many years Zika remained endemic in central Africa and was known to cause a mild febrile illness. In the early 2000s another lineage of the virus in Asia and the South Pacific caused several outbreaks, and this Asian lineage Zika virus has been associated with Guillain-Barré syndrome.[309] It was during a global outbreak centered in Brazil and the Americas in 2015–2016 that Zika was identified as a fetal pathogen.[310] Infection by Zika virus of either lineage in infants, children, and adults is generally mild. In contrast, maternal infection with the Asian lineage can cause severe fetal anomalies including intracranial calcifications, microcephaly, and intrauterine demise (see Table 34.3).

Epidemiology

Like many flaviviruses, Zika is spread through mosquito vectors, in this case primarily *Aedes aegypti*. The virus is therefore limited to the equatorial range of this mosquito, although climate change is predicted to enlarge this range.[311] The primary reservoirs are thought to be humans and non-human primates. In addition to blood, Zika virus can be isolated from seminal fluid (as long as 69 days after initial infection) and, to a lesser extent, from saliva, vaginal fluid, and breast milk. Sexual transmission has been well documented. Although it likely only contributes to a small percentage of overall cases, sexual transmission can contribute to new cases in non-endemic areas.[312] Transmission via breast milk has been proposed but documented evidence is quite rare.[313,314] Zika transmission is seasonal. Although cases can occur any time during the year, the majority occur during summer months. Serological studies have demonstrated a relatively high prevalence in endemic areas: 10% in Southeast Asia[315] and 13% in West Africa.[316] At the peak of the outbreak in the Americas in the first half of 2016 seroprevalence reached levels as high as 63%.[317] While new cases in the Americas have decreased sharply since 2016, local spread continues in endemic areas including West Africa, the Caribbean, and Southeast Asia. Fetal anomalies have only been associated with the Asian lineage of Zika and have been documented in the Americas, Southeast Asia,[318,319] and Africa.[320] Up-to-date epidemiologic data is available through the World Health Organization (WHO).[321]

Pathophysiology

Initial inoculation with Zika virus is followed by an incubation period estimated to last 6 days (range: 3 to 14), followed by a symptomatic phase that corresponds to viremia and typically lasts for 5 to 6 days.[322,323] In a pregnant mother, the virus can gain access to the fetus via transplacental spread. Zika has a high degree of tropism for placental trophoblasts and the fetal central nervous system, establishing infection in these tissues that may persist for months.[324–326] For Zika infection, as for most congenital infections that cause fetal anomalies, the consequences for fetal development are worst when infection occurs late in the first or early in the second trimester.[327,328] Congenital Zika syndrome (CZS) includes a heterogeneous set of anomalies that reflect the virus' tropism for neural tissue and harmful consequences on brain development: microcephaly, thin cerebral cortex with subcortical calcifications, retinal abnormalities, congenital contractures, and early hypotonia.[329] The mechanism underlying these findings remains the subject of active investigation, but the pattern of injury mimics fetal brain disruption sequence, a syndrome in which loss of cells in the brain parenchyma results in collapse of the fetal skull during development.[330] Viral infection of neural progenitor cells is thought to be central to CZS, leading to disruption of the fetal brain either by directly causing cell death, altering developmental programs, or inducing a type I interferon response.[331–336] Epidemiological studies have only linked CZS to Asian lineage Zika virus. There is speculation that this is because congenital infection with African lineage viruses results in severe pathology and ultimately loss of pregnancy, whereas pathology resulting from Asian lineage virus infection is less severe resulting in a viable pregnancy, albeit with abnormal development.[337,338]

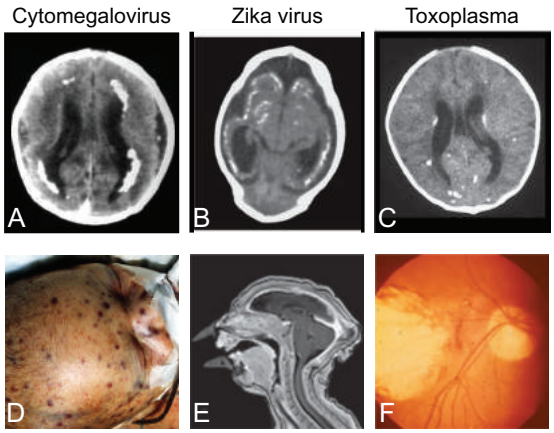
Clinical Presentation

It is important to recognize that severe microcephaly frequently depicted in the media is at one end of the CZS spectrum, which ranges from asymptomatic to intrauterine demise. Among pregnancies affected by maternal Zika infection, an estimated 6% to 17% develop congenital anomalies, while fetal loss may occur in as many as 14%.[339–342] Neonatal mortality in the first week of life may be as high as 4% to 7%.[341,342] Identified risk factors associated with death in CZS include low birth weight, prematurity, and 5-minute APGAR less than 7.[343] Microcephaly is by far the most common clinical finding described in CZS, occurring in as many as 91% of affected infants.[344] Additionally, the spectrum of structural neurologic abnormalities includes (in order of decreasing frequency): intracranial calcifications, increased ventricular and extra-axial fluid spaces, cortical thinning with abnormal gyral patterns, hypoplasia of the corpus callosum, decreased myelination, and cerebellar hypoplasia.[329] Notably, intracranial calcifications in CZS are typically subcortical and not periventricular as in CMV (Fig. 34.4). Even in infants with normal neuroimaging, as many as 10% may have functional neurologic impairment (see outcomes, below).[345] Eye abnormalities are also frequently seen (24% to 55% of cases). Microphthalmia, focal pigmentary mottling, and chorioretinal atrophy are the most common, but cataracts, intraocular calcifications, and optic nerve findings have been described.[346–348] Sensorineural hearing loss appears to be less common, affecting approximately 6% of infants with microcephaly.[349] Congenital contractures have been reported in approximately 10% of microcephalic CZS cases and may be related to loss of upper motor neurons or pathology within spinal cord and nerve roots.[350,351]

In children and adults, acute Zika virus infection is symptomatic in only 20% to 50% of cases.[352] Symptoms are generally mild and include headache, arthralgia, myalgia, non-purulent conjunctivitis, erythematous (and often pruritic) rash, and lower back pain.[341] Perinatal and postnatal acquisition of acute Zika virus infection in infants has only rarely been described, but case reports and animal data suggest very young children may be at higher risk for neurologic complications.[353]

Evaluation

Laboratory diagnosis of acute Zika virus infection relies on PCR of serum or urine, which should be obtained within 7 days of



• **Fig. 34.4** Intracranial calcifications and associated findings in select congenital infections. **(A–C)** Axial CT of intracranial calcifications. While the distribution of calcifications has some correlation with specific infectious etiologies, significant overlap prevents definitive diagnosis on the basis of imaging. **(A)** Periventricular calcifications in an infant with cytomegalovirus (CMV) infection. **(B)** Subcortical calcifications in congenital Zika infection. **(C)** Periventricular and basal ganglia calcifications in an infant with congenital toxoplasmosis. **(D)** A newborn with congenital CMV infection with a purpuric rash consisting of both petechiae and foci of extramedullary hematopoiesis. **(E)** Parasagittal T1-weighted MRI of the same infant with Zika infection in **(B)** showing scalp rugae, occipital bone prominence, and severe microcephaly consistent with fetal brain disruption sequence. **(F)** Acute chorioretinitis in an infant with congenital toxoplasmosis, a finding seen in approximately 90% of fetal infections with this parasite. *CT*, Computed tomography; *MRI*, magnetic resonance imaging. (A, Noyola DE, Demmler GJ, Nelson CT, et al; Houston Congenital CMV Longitudinal Study Group. Early predictors of neurodevelopmental outcome in symptomatic congenital cytomegalovirus infection. *J Pediatr*. 2001;138(3):325–31; B, de Oliveira-Szejnfeld PS, et al. Congenital brain abnormalities and Zika virus: what the radiologist can expect to see prenatally and postnatally. Radiology. 2016;281:203–218. C, Campbell AL, Sullivan JE, Marshall GS. Myelitis and ascending flaccid paralysis due to congenital toxoplasmosis. Clin Infect Dis. 2001;33:1778–1781. D, courtesy Larry I. Corman; E, de Oliveira-Szejnfeld PS, et al. Congenital brain abnormalities and Zika virus: what the radiologist can expect to see prenatally and postnatally. Radiology. 2016;281:203–218. F, Centers for Disease Control and Prevention. Parasite Image Library. Available at: www.cdc.gov/dpdx. Accessed on June 23, 2021.)

symptom onset. Serologic testing (particularly IgM) is unreliable due to cross-reactivity of Zika and dengue antibodies, and persistence of Zika IgM beyond the initial infectious period. Laboratory abnormalities in the setting of acute Zika virus infection are often nonspecific and may include leukopenia, thrombocytopenia, and elevated hepatic function tests.[354] Interestingly, hematologic, hepatic, or renal abnormalities have not been documented in infants born with congenital Zika infection.[329]

The CDC has issued guidance on testing for pregnant women who may have had Zika virus exposure, including a screening tool for providers to help evaluate the risk level for infants who may have been exposed to Zika virus in utero.[355] In general, risk of exposure is defined as travel to Zika-endemic areas or sex with a partner with travel or residence in these areas. Testing of asymptomatic patients (including pregnant women) is not recommended even with Zika virus exposure. For pregnant women who do have symptoms, testing for Zika virus and dengue virus by PCR can be considered. For women who have Zika exposure and a fetus with ultrasound findings consistent with CZS, PCR from maternal serum and urine and IgM are recommended; testing of amniotic fluid or placental and fetal tissues may be considered depending on level of concern.

For infants with findings suggestive of CZS, or documented maternal infection with Zika virus during pregnancy, a comprehensive physical examination (including growth parameters) and additional laboratory evaluation of the newborn is recommended.[356] As of 2017, CDC guidance recommends the following screening evaluation for all infants with in utero Zika exposure: Zika PCR (from serum and urine) and IgM (serum) obtained perinatally, head ultrasound, comprehensive ophthalmologic examination, and automated auditory brainstem response testing performed before one month of age. In addition, for infants with any findings concerning for CZS, PCR and IgM testing from CSF can be considered. In an infant with findings consistent with CZS, the differential diagnosis should include infectious etiologies including CMV, rubella, toxoplasmosis, syphilis, and HIV. Congenital abnormalities due to infections with related flaviviruses, such as West Nile virus, have been described[357] but appear to be far rarer than CZS.[358,359] Genetic etiologies should also be considered including Aicardi-Goutières Syndrome, and mutations in JAM3, NDE1, and ANKLE2 genes.[329] There has been description of familial fetal brain disruption sequence, although a genetic diagnosis has not been defined. Additional specificity may be obtained by considering the differential for individual findings, as in Table 34.1. For infants with Zika exposure, close follow up is warranted with attention to neurodevelopmental, vision, hearing, and growth parameters. For infants where there is concern for CZS, referral to a neurodevelopmental specialist, early intervention services, and provision of additional family support is recommended.

Management

As of 2021, no specific medication has been developed for the treatment of infection due to Zika virus or any other flaviviruses, despite active research in this area.[360,361] The success of nucleotide analog drugs that are highly effective in treating the closely related hepatitis C virus offers promise, and many of these drugs are being investigated for repurposing to treat Zika infection.[362] Vaccine development is also an active field of Zika research, and several vaccines have entered Phase I and II trials.[363]

Avoidance of travel to high-risk areas currently is the most reliable prevention strategy for men and women of reproductive age. If Zika-endemic areas are unavoidable, the WHO recommends use of an FDA-approved insect repellant (e.g. DEET, IR3535, or Icaridin) as well as the use of personal nets when sleeping or resting.[364] The WHO also has extensive guidelines for limiting transmission and avoiding CZS, which include the following: women should not engage in sex that could lead to conception for 2 months after known or presumptive infection with Zika virus, while men should abstain from sex or use condoms to prevent transmission for 3 months after known or presumptive infection.[365] Mosquito vector control is also a widely discussed aspect of limiting the human consequences of disease. As Zika virus can be transmitted via blood transfusion (even from asymptomatic donors),[366] screening of blood products is an important tool for preventing spread—particularly if the donor pool is impacted by an ongoing outbreak.

Outcomes

Because of the relatively recent appreciation of the role of Zika virus in fetal developmental anomalies, neurodevelopmental surveillance data regarding the long-term outcomes of CZS are somewhat limited. As with other etiologies of microcephaly, the severity of structural abnormality is a strong predictor of the severity of neurologic impairment.[367–369] Microcephaly can progress after birth[370] (perhaps due to persistent viral replication[324]), but resolution of microcephaly and normal neurodevelopment have also been described.[371] Outcomes in normocephalic infants with Zika exposure in utero vary. Some studies have found that infants without overt CZS findings have normal neurodevelopment,[372] while most studies find some abnormalities (albeit less severe than those with overt CZS).[368,373,374] Across neurodevelopmental domains, motor function appears to be the most consistently impaired, with receptive language ability relatively spared in many infants.[368,373] Long-term sequelae suffered by infants who experience CZS may include neurodevelopmental delay, hypertonia and contractures, seizures, ophthalmic abnormalities and visual impairment, hearing loss, cryptorchidism, and endocrine dysfunction.[371,374–376]

Lymphocytic Choriomeningitis Virus

LCMV is a negative-strand RNA virus in the family *Arenaviridae* that is associated with congenital anomalies. Rodents are the primary reservoir, particularly mice and hamsters. Sequelae of human infection range from asymptomatic to flu-like illness to encephalitis, while congenital infection can result in severe developmental anomalies. LCMV was first described as a cause of congenital infection in 1955 in a 12-day-old infant.[377] Because primary infection may be mild or asymptomatic and testing not commonly available, LCMV is likely underdiagnosed as a cause of congenital infections.[378]

Epidemiology

Human seroprevalence ranges between less than 1% and 10% worldwide and differs extensively with geographic region, primarily related to the degree of contact with rodent reservoirs.[379–382] Studies conducted in the 1940s–1970s found as many as 11% of cases of aseptic meningitis and encephalitis were associated with LCMV infection,[383] but this incidence has decreased significantly in more recent studies.[384,385] In temperate climates, human exposure is more common during the fall and winter, when rodents move indoors. Outbreaks have been detected in individuals with contact with hamsters and mice as laboratory animals[386,387] or as pets.[388–390] Awareness of LCMV as a congenital pathogen increased in the 1990s–2000s with several case series published,[378,390–392] however the true frequency of congenital LCMV infection is unknown because there is no active surveillance. Maternal exposure to rodents during pregnancy is only identified in approximately one-fourth to one-half of congenital cases.[392] The diagnosis of LCMV infection should be considered in all cases of infant hydrocephalus.

Pathophysiology

Humans acquire LCMV infection from aerosolized particles, bites, or fomite contact with virus excreted from rodents.[393] Human-to-human horizontal transmission by organ transplant has been documented.[394] LCMV is well-studied in rodents but its pathophysiology in humans is relatively poorly understood. Like other arenaviruses, LCMV replicates either at the site of infection or in draining lymph nodes; this localized replication is followed by viremia which leads to viral spread to parenchymal organs and the CNS.[395] Pathologic findings include lymphocytic infiltration and extramedullary hematopoiesis. The pathogenesis of LCMV-mediated brain injury is likely a combination of virus-mediated lysis and immune-mediated secondary damage due to infiltrating $CD8^+$ T-cells and pathogenic cytokine release.[395–397] In some settings LCMV appears to establish persistent infection, which may be facilitated by viral inactivation of the host innate type 1 IFN response,[398] or by a high rate of spontaneous mutation.[399]

Clinical Presentation

The typical presentation of LCMV infection is a nonspecific flu-like illness. Symptoms include fever, malaise, nausea, vomiting, myalgias, headache, pharyngitis, cough, and adenopathy; it is estimated that asymptomatic or mild LCMV infections occur in approximately one-third of patients infected. CNS disease typically develops as a second phase after resolution of constitutional symptoms. Neurologic manifestations occur in approximately one-fourth of infectious episodes and range from simple meningitis to fulminant meningoencephalitis; transverse myelitis, Guillain–Barré syndrome, and deafness have also been reported. Severe non-neurologic complications may also include pneumonitis, arthritis, myocarditis, parotitis, and dermatitis. Recovery may take months but usually occurs without lasting sequelae.[400]

Congenital anomalies are most severe when maternal infection occurs in the first or second trimester, but only 50% to 60% of mothers of infants with a diagnosis of congenital LCMV infection recall having symptoms.[401] Neurologic findings dominate the developmental anomalies caused by fetal LCMV infection, and chorioretinitis, hydrocephalus, and intracranial calcifications are the most common findings (see Table 34.3).[401,402] Chorioretinitis is nearly universal, present in more than 90% of cases. Other ocular findings include chorioretinal scars, optic atrophy (usually bilateral), nystagmus, esotropia, exotropia, leukocoria, cataracts, and microphthalmia.[392,403,404] Microcephaly and macrocephaly are both common (30% to 40% each) and neuropathology may be severe, including porencephalic cysts, periventricular infection, and cerebellar hypoplasia.[400] Flattened gyri, lissencephaly, and schizencephaly have been reported,[392] suggesting LCMV infection results in fetal neuronal migration defects.[395]

Most affected infants are born at term, and birthweights are generally appropriate or large for gestational age. Systemic symptoms are rare, although hepatosplenomegaly and jaundice have been noted. Other individual case report findings include pes valgus, dermatologic findings resembling staphylococcal scalded-skin syndrome,[401] spontaneous abortion,[388] and intrauterine demise secondary to hydrops fetalis.[403,405,406] It is important to note that because systemic symptoms are typically minimal at birth, the diagnosis of congenital LCMV infection may not be considered until an affected infant is a few months old, when microcephaly, macrocephaly, visual loss, or developmental delay are noted.

Evaluation

While the virus can be detected by PCR in serum or CSF during acute infection,[407] maternal and/or infant serology is often the more feasible method to diagnose LCMV disease, particularly when congenital infection is suspected.[392] Because of the low

baseline population seroprevalence, a positive LCMV antibody test is much more useful for diagnosis than is detection of antibodies to other potential congenital infections such as CMV. There is a commercially available immunofluorescent antibody test that detects both IgM and IgG for LCMV, although enzyme-linked immunosorbent assay antibody testing (performed by the CDC) is more sensitive.[408–410] Some studies have found antibody as late as 30 years after suspected exposure.[411]

Information about routine laboratory data in patients with a diagnosis of congenital LCMV infection is minimal, but thrombocytopenia and hyperbilirubinemia have been reported. CSF findings are variable. Up to half of cases demonstrate a mild increase in white blood cell count (up to 64 cells per microliter in one case series of 18 infants), the serum protein concentration may be normal or mildly elevated, and the serum glucose concentration may be normal or mildly decreased.[401]

Management

No approved treatments exist for LCMV infection. Ribavirin has been used for management of other arenavirus infections and inhibits LCMV growth in vitro.[412] Although novel approaches are being used to develop antivirals against LCMV and other pathogenic arenaviruses, which include Ebola, there are currently no recommendations for the use of antiviral agents against these viruses.[413,414] In an outbreak associated with solid organ transplant, one affected recipient received ribavirin and reduced levels of immunosuppressive therapy and survived.[394]

Prevention

Public health officials and clinicians should be aware that (1) wild, laboratory, and pet rodent exposure can lead to intrauterine infection with LCMV and (2) congenital infection has been associated with potentially devastating ophthalmologic and neurologic sequelae. Pregnant women should be educated about the risks of exposure to infected rodent excreta and instructed to avoid rodents and rodent droppings. Obstetricians and neonatologists should seek a history of pet or wild rodent exposure for counseling purposes and to aid in the evaluation of infants with unexplained CNS diseases. There are currently no vaccines approved by the FDA for the prevention of arenavirus disease, although candidate multivalent arenavirus vaccines capable of providing T cell-mediated protection against a variety of pathogenic arenaviruses are currently in development.[415] Attenuated LCMV is itself being used as a vector for vaccine development for heterologous pathogens.[416]

Outcomes

The proportion of asymptomatic infected infants is unknown. Chorioretinitis is the most common clinical finding, and the incidence of LCMV-caused retinal disease may be markedly underestimated.[404,417] In a review of 26 serologically confirmed infant cases, 9 infants (35%) died and 10 (63%) of the 16 survivors had severe neurologic injury.[401] Neurologic sequelae reported in the setting of congenital LCMV infection include spastic quadriparesis, mental retardation, developmental delay, seizures, sensorineural hearing loss, and visual impairment.[392,400,418,419] The combination of microcephaly and periventricular calcifications are associated with poor long-term prognosis.[402]

Herpes Simplex Viruses

HSV-1 and HSV-2 are highly related viruses. Although classically HSV-1 has been identified as a cause of oral infections (gingivostomatitis and pharyngitis), and HSV-2 has been implicated as the most common virus associated with genital herpes, in recent years these distinctions have become blurred. The greatest risk for the newborn is in the context of a first-time episode of maternal genital HSV infection occurring during pregnancy.

Epidemiology

In the United States, the reported incidence of neonatal herpes ranges from 1 in 2000 births to 1 in 10,000 births,[420–424] likely depending on the local prevalence of genital HSV and specificity of reporting. The incidence of neonatal HSV infection in the US has increased over the past decade.[424] The greatest risk of transmission to the fetus and the newborn occurs when an initial maternal infection is contracted in the second half of pregnancy.[425] Primary genital herpes infection in a pregnant mother results in an estimated attack rate of 33% to 60% for her infant, whereas recurrent maternal infection results in a 1% to 3% attack rate.[426–429] Approximately 85% of infants with neonatal HSV infection acquire the infection from the maternal genital tract at the time of delivery, but only 15% to 30% of mothers who give birth to neonates with neonatal HSV infection have a known history of genital HSV infection.[430] It is estimated that 22% of pregnant women are infected genitally with HSV, but most of these women are unaware of their infection.[429,431] Approximately 2% of women acquire primary HSV infections during pregnancy.[428] The majority of neonatal herpes simplex infections were caused by HSV-2 in early reports,[432,433] however, genital HSV infections in the US are increasingly caused by HSV-1, leading to an increased risk for neonatal infections due to HSV-1.[434,435]

Most HSV infections in newborns are acquired peripartum due to exposure to infected genital secretions.[427,436] Other modes of infection from maternal transmission include maternal viremia near the time of delivery[437] and post-natal acquisition during breast feeding from HSV lesions on the breast.[438–440] Intrapartum interventions that risk penetrating fetal skin, such as scalp electrode monitoring, increase the risk of transmission to the infant.[441,442] Post-natal acquisition of HSV from an individual other than the mother, such as from a recurrent oropharyngeal lesion[443–445] or a complication of ritual circumcision[446–448] has been reported.

Pathophysiology

The degree of genetic relatedness of HSV-1 and HSV-2 is approximately 45%, and the genome structures and morphology of the virion (virus particle) are virtually identical.[449] Both are acquired predominantly at mucosal surfaces and require intimate contact for transmission. After primary infection in epithelial cells, intraaxonal trafficking of viral DNA to the dorsal route ganglia results in the establishment of latency.[450–452] A variety of triggers, including ultraviolet radiation, stress, and immunosuppression, can cause the virus to reactivate at the level of the dorsal route ganglia and lead to the production of infectious virus, which can traffic via the axon to the cutaneous surface or ocular surface, producing lesions.[453] The recrudescence of HSV lesions, usually manifested as vesicular or ulcerative lesions at the site of primary infection,

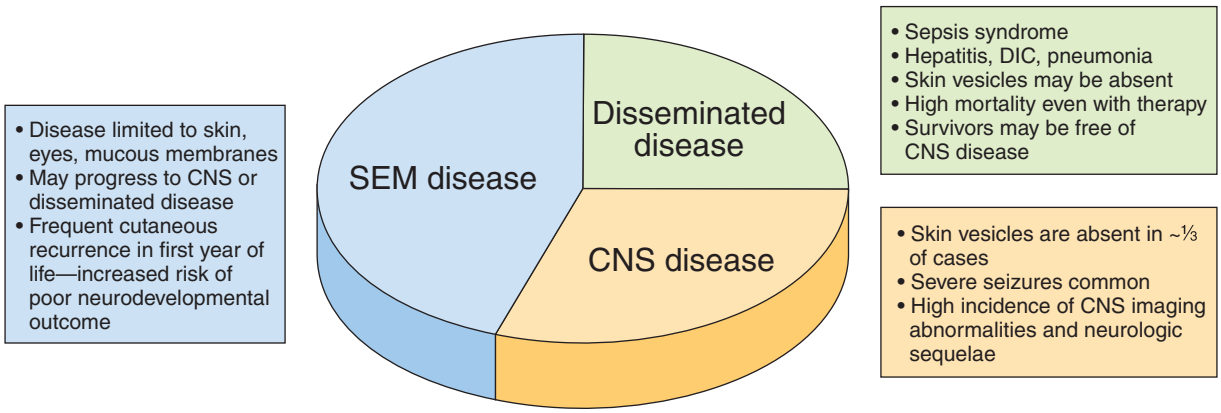
can in turn lead to person-to-person transmission, including maternal–fetal and maternal–infant transmission.

A wide variety of disease syndromes are associated with primary and recurrent HSV infection. HSV-1 is the primary causal agent of herpetic gingivostomatitis, herpes labialis, and keratoconjunctivitis. HSV-2 is almost always a genital pathogen, although genital herpes is associated with both HSV-1 and HSV-2. Exposure to an individual (including a healthcare provider) with any of these cutaneous manifestations of HSV infection could put a newborn at risk of acquisition of infection if care is not taken to protect the newborn. Typically, symptomatic disease manifests itself with a mixture of vesicles, ruptured vesicles with resulting ulcers, and crusted lesions on the genital area, buttocks, or both. Systemic flulike symptoms such as headache, fever, and swollen glands can accompany an outbreak of genital herpes, particularly during primary infection. Other symptoms include dysuria, urinary retention, vaginal or penile discharge, genital itching, burning, tingling, and groin sensitivity. Primary genital herpes during pregnancy can be associated with disseminated maternal disease, including severe hepatitis, disseminated intravascular coagulation (DIC), and CNS infection.[454–458] It has been recognized in recent years that many individuals with genital herpes are asymptomatic and unaware of their status.[459,460] Therefore, a negative maternal history of HSV *should not* dissuade the clinician from considering the possibility of neonatal herpes in an infant with compatible signs and symptoms. Although a history of maternal cervical, vaginal, or labial lesions should be sought when neonatal HSV infection is being considered in the differential diagnosis, overt herpetic disease in the maternal genital tract is evident in only approximately one-third of patients.[461] In the remaining two-thirds of cases, infection occurs in the context of asymptomatic maternal genital tract shedding of HSV.

Clinical Presentation

Neonatal herpes can have devastating long-term consequences, making early recognition of paramount importance. Most newborns with perinatal or postnatal HSV infection are normal at birth. Illness typically develops after 3 days of age; therefore, the presence of skin lesions, oral ulcers, and other signs and symptoms in the first 72 hours of life can suggest diagnoses other than HSV. Premature infants appear to be at greater risk, possibly because of reduced transplacental transfer of protective antibody. Approximately one-third of affected infants are of less than 36 weeks' gestational age.[432,462]

Infection can manifest itself in newborns in one of three forms: disease limited to the skin, eye, or mucous membrane (SEM disease); disease involving the CNS; or disseminated HSV infection, frequently manifesting itself as a sepsis-like syndrome, with pneumonia, hepatitis, and viremia (Fig. 34.5).[463,464] There can be overlap in these syndromes; for example, an infant with disseminated disease may initially have only skin lesions. The relative proportion of infants with disseminated disease has been declining in recent years, probably because earlier recognition and treatment of SEM disease have resulted in more timely intervention with antiviral therapy. Disseminated disease usually begins toward the end of the first week of life. Skin vesicles may be an early sign, but they are entirely absent in almost half of patients. The scalp should be inspected carefully, particularly near the site of insertion of fetal scalp electrodes, because such lesions are easy to overlook. Systemic symptoms, although initially insidious in onset, progress rapidly. Poor feeding, lethargy, and fever may be accompanied by irritability or seizures if the CNS is involved. These symptoms are followed rapidly by jaundice, hypotension, DIC, apnea, and shock. This form of disease is indistinguishable at its onset from both neonatal enterovirus infection and bacterial sepsis. HSV infection should be considered in the differential diagnosis of infants who have fever during the first 2 weeks of life, because fever can herald the onset of systemic disease. Localized disease may begin somewhat later, with most cases appearing in the second to third weeks of life. When the CNS is the primary site of infection, the skin or eyes may or may not be involved: importantly, up to one-third of infants with documented neonatal HSV CNS disease will never have skin lesions during their clinical course.[465] These infants are lethargic, irritable, and tremulous, and seizures are common and often difficult to control.



• **Fig. 34.5** Characteristic presentations of neonatal herpes simplex virus (HSV) infection. Approximately 45% of neonatal HSV infections manifest themselves as skin, eye, or mucous membrane disease (SEM disease), 25% as disseminated disease, and 30% as central nervous system (CNS) disease. Characteristic features of each subtype of neonatal HSV are listed. Disease may span categories; for example, infants with SEM disease may progress to disseminated or CNS disease, and infants with CNS disease may develop skin vesicles later in the hospital course, although up to one-third of infants with CNS disease never have cutaneous manifestations. Overall, approximately half of all infants with neonatal HSV disease will have CNS involvement (CNS disease or disseminated disease with CNS involvement). *DIC*, Disseminated intravascular coagulation.

Other less common but potentially localized findings are keratoconjunctivitis, chorioretinitis, and pneumonitis. Pneumonitis can manifest itself as a focal infiltrate or as diffuse bilateral disease. Secondary dissemination from a localized infection is common in the neonate. Acute retinal necrosis has been described in a series of infants with neonatal HSV infection[466] and can also be a late finding associated with recurrence.[467,468] Intracranial hemorrhage, aseptic meningitis, and fulminant liver failure have been described.[469–473] Some of the less common presentations of neonatal HSV infection can include hydrops fetalis,[474,475] laryngitis,[476] and supraglottitis.[477] Intrauterine infection is uncommon but can present with neurologic, ophthalmologic, and cutaneous manifestations.[478,479]

Evaluation

The cornerstone of the diagnosis of neonatal HSV infection is virologic detection; serology is of limited use in the management of suspected infection in the infant, although type-specific serology is useful in evaluation of maternal HSV status in the setting of infants born to women with active genital HSV lesions.[480] HSV-1 and HSV-2 are both easily recovered by culture of clinical samples and culture has been the primary diagnostic modality. However, use of HSV DNA PCR for all sample types is increasing. Although there are no large studies comparing the two modalities, HSV DNA PCR has been shown to be more sensitive than culture for surface swabs in neonates[481,482] and viral culture is becoming less available.[483] Diagnostic evaluation for neonatal HSV infection should include specimens from the mouth, nasopharynx, conjunctiva, anus, and any skin vesicles for culture (if available) and/or HSV PCR; whole blood or plasma for HSV PCR; and CSF for cell count, protein, glucose, and HSV PCR.[241] In addition, blood should be sent for a complete blood count including differential and platelets and transaminases. No single test is sufficiently sensitive to identify all the variable presentations of neonatal HSV. Therefore, is it important to obtain all of the recommended specimens to guide diagnosis and treatment.[482,484] HSV DNA is detectable in plasma/blood in all infants with disseminated disease and many infants with CNS and SEM disease.[481,484,485] Plasma HSV PCR levels correlate with outcome in infants with DIS disease.[484,486] In the absence of other signs of disseminated infection, a positive blood PCR does not define disseminated disease as many infants with SEM disease will be viremic.[481,484–486]

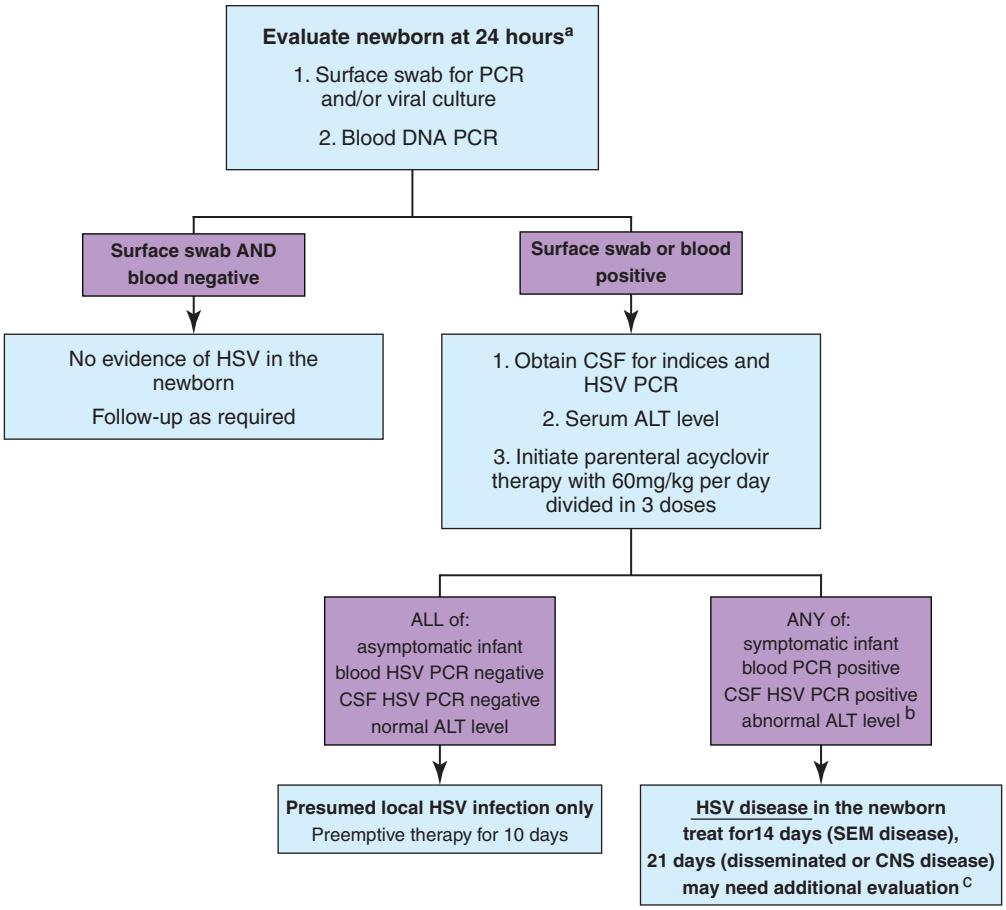
If the CNS is involved, evaluation of the CSF usually reveals a lymphocytosis, red blood cells, normal or high protein level, and low or normal glucose level. HSV DNA is detected only about 70% of the time in infants with clinical CNS disease, therefore absence of HSV by PCR does not rule out CNS disease.[484,486] Some experts recommend a second CSF specimen be obtained for evaluation at the end of antiviral therapy, as persistence of HSV DNA may be a poor prognostic factor and an indication for continuing antiviral therapy.[429,487–489]

Infants with CNS disease should undergo neuroradiographic imaging with computed tomography (CT) or magnetic resonance imaging (MRI). Neurodevelopmental sequelae in infants have been correlated with MRI abnormalities, particularly with diffusion-weighted imaging, which appears to be a valuable prognostic adjunct in neonatal HSV disease.[490] Electroencephalography (EEG) to evaluate for seizures should also be considered in infants with CNS involvement or with disseminated disease. EEG findings will be abnormal in approximately 80% of such patients.[432] Ophthalmologic evaluations are indicated, particularly in any infant with evidence of conjunctivitis.[241]

Management

Intravenous acyclovir at a dosage of 60 mg/kg per day, divided into three doses given every 8 hours is the standard of care for neonatal HSV (see Table 34.2). Treatment should be for 14 days for infants with SEM disease or 21 days for infants with disseminated or CNS disease. Infants with CNS disease should have a repeat lumbar puncture prior to stopping treatment. Those with HSV DNA detected by PCR in the CSF at the end of 21 days of therapy should receive acyclovir for an additional 7 days.[480] There is no role for orally administered acyclovir in the initial management of neonatal HSV, and no role for topically administered acyclovir in neonatal SEM disease. Herpetic keratoconjunctivitis should receive topical ophthalmic antiviral therapy along with parenteral treatment. In addition to antiviral therapy, appropriate supportive care is essential, with anticipatory management targeting complications of neonatal HSV disease such as seizures, pneumonitis, and hepatic insufficiency.

Treatment of asymptomatic neonates born to women noted to have genital lesions at the time of delivery is controversial. Guidelines endorsed by the AAP, as summarized in Fig. 34.6, provide evidence-based guidance on the treatment of neonates born to women with active genital herpetic lesions.[480,491] Implementation of these guidelines requires coordination between the pediatric and obstetric teams and availability of maternal PCR and/or culture of suspect lesions coupled with serologic assessment, along with virologic testing of the newborn at 24 hours of age. The key determination of risk for the infant is the serostatus of the mother. Infants born to women with primary, first-episode genital HSV infection have up to a 60% risk of neonatal HSV infection.[436] On the other hand, term infants born to women with a history of recurrent genital herpes are at low risk (<1%) of infection. For infants born in settings where PCR and type-specific serology may not be readily available, the most important variable informing clinical management is the maternal history. If the maternal HSV history is unclear, the infant is premature, or other obstetric complications or risk factors are present (e.g., prolonged rupture of membranes, maternal fever, signs or symptoms of chorioamnionitis, fetal scalp electrode monitoring), then empiric antiviral therapy is warranted. In settings where PCR and type-specific serology may not be readily available, after the appropriate specimens have been collected and sent for virologic analysis, the infant should receive parenteral acyclovir therapy (60 mg/kg per day) pending the results of diagnostic virology studies. In all cases, the family should be educated on signs and symptoms of neonatal HSV disease, and the infant should be followed closely. For post-natal exposure, which is an uncommon but documented cause of neonatal HSV, a similar decision algorithm for assessment and treatment of the exposed infant has been proposed.[492] There is no established standard of care for HSV testing among neonates evaluated for concerns of neonatal sepsis.[493] Some experts recommend the use of empiric acyclovir therapy in this setting,[494] and others recommend a more selective approach, based on analysis of history, risk factors, and laboratory analyses, such as liver function tests and CSF parameters.[495–497] A retrospective analysis of an empiric acyclovir strategy restricted to newborns with onset of febrile illness at 21 days of age or earlier, who would typically receive empiric parenteral antibiotic therapy, captured 90% of HSV cases.[497,498] Estimated rates of HSV infection among neonates evaluated for serious bacterial



• **Fig. 34.6** Treatment of a newborn born to a woman with presumed active genital herpes simplex virus (HSV) lesions at delivery in the setting of recurrent genital HSV infection. [a]Awaiting period of 24 hours is currently recommended in this setting before HSV surface cultures and/or PCRs are obtained for HSV genome detection from the neonate. This approach aids in differentiation of contamination of neonatal skin from maternal secretions from true infection of the newborn. [b]ALT level greater than 2 times the upper limit of normal. [c]Additional evaluation may be required based on symptoms and response to therapy. *ALT*, Alanine aminotransferase; *CSF*, cerebrospinal fluid; *PCR*, polymerase chain reaction. (Adapted from Kimberlin DW, Baley J, Committee on Infectious Diseases, Committee on Fetus and Newborn. Guidance on management of asymptomatic neonates born to women with active genital herpes lesions. Pediatrics. 2013;131:e635–e646.)

infection is 0.7% to 2%.[497–499] Regardless of the strategy chosen, it is important to be aware that while most infants present with signs and symptoms that suggest HSV infection, a significant minority present with non-specific symptoms.[500,501]

The observation that recurrent skin lesions within the first 6 months of life predicted an adverse neurologic prognosis, possibly because recurrent skin lesions are a surrogate marker for subclinical reactivation events in the CNS,[502] led to the evaluation of the use of oral acyclovir for HSV suppression following the initial IV treatment. A phase III, placebo-controlled trial performed by the Combined Antiviral Study Group (CASG) confirmed that acyclovir suppressive therapy for 6 months after completion of IV therapy for neonatal HSV disease resulted in improved neurodevelopmental outcomes.[503] Infants with CNS disease randomized to receive oral acyclovir therapy had improved neurodevelopmental outcome and fewer frequent recurrences of skin lesions while receiving therapy. The results of this study have led to a recommendation that infants with neonatal HSV infection of any disease classification should receive oral acyclovir therapy for 6 months (see Table 34.2). Absolute neutrophil counts should be monitored at 2 and 4 weeks, and monthly thereafter, after commencement of suppressive therapy.[491]

Prevention

Neither routine HSV screening of pregnant women nor antepartum genital HSV cultures in asymptomatic women with recurrent disease is currently recommended.[504] However, women with recurrent HSV outbreaks during pregnancy or with a first episode of HSV during pregnancy should be offered suppressive acyclovir therapy beginning at 36 weeks of pregnancy. Although there is no evidence that suppressive acyclovir therapy reduces disease in newborns[505] and there are documented cases of neonatal infection in women on suppressive therapy,[506] it has been shown to reduce maternal asymptomatic shedding, risk of clinical recurrence of HSV at the time of delivery, and need for cesarean birth for recurrent HSV.[507] Cesarean delivery of women with active genital lesions at the time of delivery has been shown to reduce,

although not completely prevent, vertical transmission of HSV to the infants. Therefore, cesarean delivery is indicated in women with active genital lesions or prodromal symptoms, such as vulvar pain or burning, at delivery, because these symptoms may indicate viral shedding.[504]

Even with careful histories and meticulous physical examination during labor and delivery, many at-risk deliveries cannot be predicted or identified, because so many HSV-seropositive women are asymptomatic, do not know that they have genital herpes, and may unknowingly shed virus at the time of delivery. Infants in whom HSV infection is known or highly suspected should be isolated with contact precautions, and skin lesions should be covered. Finally, any healthcare provider with active herpetic whitlow or other skin lesions should not have direct patient care responsibilities for neonates.

While there have been multiple clinical trials of HSV vaccines for the prevention of genital HSV, to date none have shown significant success.[508]

Outcomes

Even with timely institution of antiviral therapy, the prognosis following neonatal HSV infection is guarded for infants with disseminated and CNS infections. Mortality rates for infants with disseminated disease have improved with the use of high-dose acyclovir treatment but are still 30% to 40%.[432] Antiviral therapy has decreased neonatal CNS infection mortality from approximately 50% to 6%, but 50% to 70% of survivors have sequelae.[420,432,484,509] Risk factors for increased morbidity and mortality associated with CNS infection include prematurity, seizures on initiation of therapy, and infection with HSV-2.[432,486,502,510] Lethargy at initiation of therapy has been associated with a higher mortality rate in neonates with disseminated HSV infection.[432] Infants with skin involvement often have recurrent crops of skin vesicles for several years, particularly those infected with HSV-2. Recurrences in the first 6 months of life have decreased with the use of oral suppressive acyclovir. In an infant younger than 6 months, readmission to the hospital for evaluation and IV acyclovir is appropriate when cutaneous recurrences are observed. Ongoing diligence in infants with CNS infections is warranted as late recurrences can result in additional CNS morbidity.[511,512]

Varicella-Zoster Virus

VZV is a member of the α-herpesvirus subfamily of the *Herpesviridae*. Like the related HSV-1 and HSV-2, VZV can infect neurons, where it establishes latent infection. Primary varicella infection, commonly known as *chickenpox*, usually results in a fever and a characteristic vesicular exanthem. Reactivation from latency, which can occur years or decades after the primary VZV infection, is referred to as *zoster* or *shingles*. Zoster is characterized by a painful vesicular rash in a dermatomal distribution.

The neonatologist may encounter consequences of maternal VZV infection in two different clinical presentations. In *congenital* varicella, VZV is transmitted to the fetus in the first or second trimester of pregnancy, where it can produce a number of teratogenic consequences.[513–516] In contrast, *neonatal* varicella occurs in the setting of primary maternal varicella acquired late in the third trimester, and the affected infant can exhibit symptoms and signs in the neonatal period. This section reviews both presentations of VZV-related disease in infants.

Epidemiology

Primary VZV infection during pregnancy occurred with a frequency of about 1 per 10,000 pregnancies in a population-based study from 2003 to 2010 in the United States.[517] The major concern in the setting of primary maternal VZV infection early in pregnancy is the risk of congenital varicella syndrome (CVS). Approximately 130 cases of CVS were reported in the literature between 1947 and 2013.[518,519] The risk of symptomatic intrauterine VZV infection after maternal varicella occurring during the first 20 weeks of pregnancy is approximately 1% to 2%.[519–522] Symptomatic disease appears to be more common in female infants.[518] Although rare,[520] CVS following maternal zoster has been reported.[519]

Maternal infection in the third trimester is not associated with CVS, presumably because this falls outside the time frame when VZV is teratogenic to the developing fetus. However, maternal infection just before or after delivery poses a high risk of neonatal varicella. For neonates born to a mother whose illness begins 5 days or less before delivery or up to 2 days after delivery, the neonatal infection rate is 17% to 31%.[523–525]

Pathophysiology

Congenital varicella syndrome results from maternal primary varicella infection occurring during the first or second trimester, and results from transplacental passage of virus during maternal viremia. It has been proposed that some of the congenital malformations associated with CVS may be a consequence of zoster-like virus reactivation in the infected fetus rather than the direct effects of the primary viral infection. The common finding of unusual cicatricial rashes in dermatomal distributions in newborns with CVS is more compatible with zosteriform reactivation events than primary skin infection.[526,527] Immature cell-mediated immune response in the fetus may underlie episodes of reactivation in utero.[526,528] Pathology reports have noted destruction of neural tissue with residual dystrophic calcifications, chronic active inflammation in non-neural tissues surrounding viral inclusions, and evidence of chronic placental villitis.[529–531]

Neonatal varicella is usually caused by maternal chickenpox acquired near term or immediately postpartum. Infection is primarily transmitted by transplacental viremia; it can also be contracted postnatally by the aerosol route or by direct contact with infectious lesions. Neonatal acquisition due to maternal zoster is very rare. Serious postnatal infection acquired from maternal varicella via breastfeeding has not been reported. Transplacentally transmitted infections manifest in the first 10 to 12 days of life, whereas chickenpox after that time is most likely acquired by postnatal infection.

Clinical Presentation

Congenital

Cicatricial skin lesions occur in most infants with CVS. These may appear initially as areas of skin loss which become cicatricial with time.[532] Other common findings include neurologic abnormalities (cortical or spinal cord atrophy, seizures, microcephaly, encephalitis, Horner syndrome), ophthalmologic abnormalities (chorioretinitis, microphthalmia, atrophy, and cataracts), and asymmetric muscular atrophy with limb hypoplasia.[518,524,525]

Intrauterine growth retardation, gastrointestinal, and genitourinary abnormalities have also been reported.[532,533]

Neonatal

When maternal varicella occurs more than 5 days before delivery, neonatal disease usually begins within the first 4 days of life and is typically mild. In contrast, varicella in a newborn whose mother develops varicella from 5 days before until 2 days after delivery is associated with a high risk of morbidity and mortality.[534,535] In this second group, neonatal disease typically begins between 5 and 10 days after delivery, and fatal outcomes have been reported in 23% to 30% of cases.[536,537] When the disease appears in this setting, it closely resembles varicella in the immunodeficient or immunosuppressed host. Recurrent crops of skin vesicles develop over a prolonged period. Typical presenting signs are fever, hemorrhagic rash, and visceral dissemination with involvement of the liver, lung, and brain. Secondary bacterial infection may occur.

Evaluation

Congenital

CVS should be suspected in a newborn with compatible abnormalities whose mother had a history of varicella in the first or second trimester. Confirmation requires evidence of intrauterine varicella infection, which could be done via direct viral detection from newborn samples,[538] or serologically by documenting persistence of VZV IgG beyond 6 or 7 months (the presumed duration of passive transfer of maternal antibodies) or detection of VZV IgM in infant blood. The latter is less useful as only one-fourth of infants reported with classic CVS have positive VZV IgM titer test results.[518,520] VZV can also be detected from skin lesions,[539] blood, or CSF by PCR, although there is no data on the sensitivity of VZV PCR for diagnosis in congenital disease. Development of clinical zoster in early infancy also suggests previous *in utero* infection.[518,520] Similar congenital skin lesions have rarely been associated with congenital herpes simplex virus[540] and coxsackievirus infections.[518] Diagnosis of CVS prenatally requires both documentation of varicella infection and consistent clinical findings by ultrasound.[541] Infection is determined by detection of VZV DNA by PCR in amniotic fluid.[528,542] Reported prenatal ultrasonography findings include polyhydramnios, hydrops, progressive IUGR, microcephaly, limb hypoplasia, and liver hyperechogenicities.[531,543] Abnormal ultrasonography findings may not develop in the fetus for at least 5 weeks after maternal infection.[544–546] Repeat ultrasound is indicated if the VZV DNA PCR is positive but the initial ultrasound is normal.

Neonatal

Neonatal varicella can be diagnosed based on presence of a clinically consistent rash following exposure to varicella and confirmed by VZV DNA PCR of the lesions.[539] Direct fluorescent antibody testing of a vesicle scraping provides a more rapid result, but is less sensitive than PCR.

Management

Although there are no controlled studies of acyclovir in pregnancy, treatment with oral acyclovir or valacyclovir is recommended by some experts especially during the second and third trimesters.[288] IV acyclovir is recommended for pregnant women with serious complications of varicella, particularly pneumonia. There are no recommendations for the use of acyclovir or IVIG for treatment or prevention of CVS. For neonatal varicella, treatment with IV acyclovir, 30 mg/kg per day divided into doses every 8 hours, is recommended, particularly for infants at the highest risk of adverse outcomes (i.e., the infant born to a woman who develops varicella from 5 days before until 2 days after delivery; Table 34.2).

Prevention

Pregnant women who lack a history of VZV infection or immunity should receive varicella zoster immune globulin (VariZIG) as soon as possible following VZV exposure. VariZIG should ideally be given within 96 hours of exposure but can be given up to 10 days after exposure.[288] The incidence of varicella following VariZIG is between 7% and 30%.[547,548] Newborns of mothers in whom varicella develops from 5 days before to 2 days after delivery should receive VariZIG as soon as possible (Box 34.2). VariZIG is given intramuscularly at a recommended dose of 125 units per 10-kg bodyweight (62.5 units for infants ≤2 kg, 125 units for infants >2 kg to ≤10 kg), up to a maximum of 625 units. If VariZIG is unavailable, IVIG (400 mg/kg) can be a substitute. A recent review of the VariZIG expanded access program found the incidence of varicella in exposed neonates after receiving VariZIG was 23.7% with only one infant having disseminated disease.[547] For healthy term newborns exposed postnatally to varicella, including newborns whose mother's rash began more than 48 hours after delivery, VariZIG is not generally indicated. However, some experts would consider administering VariZIG to infants exposed within the first 2 weeks of life whose mothers do not have evidence of immunity to varicella as they may also be at increased risk for severe disease due to their immature T-cell immunity.[241] VariZIG is not indicated for an infant whose mother has zoster. Infants receiving VariZIG should be placed in respiratory isolation for 28 days or until discharge, because administration of VariZIG can prolong the incubation period.

In the event of a significant varicella exposure in a newborn nursery, exposed infants born at less than 28 weeks' gestation or with birth weights less than 1000 g, regardless of maternal history, should receive VariZIG. Premature infants older than 28 weeks' gestation at birth should receive VariZIG if their mothers do not have documentation of immunization, serologic immunity, or a prior history of varicella (see Box 34.2).[241]

The best means of preventing VZV infection in the fetus and neonate is to follow current recommendations for universal varicella immunization of all children at 12 to 15 months of age, as well as vaccination of all susceptible adolescents and adults. There

• BOX 34.2 Candidates for Varicella–Zoster Immunoglobulin After Significant Exposure to Varicella–Zoster Virus Infection in Perinatology and Neonatology Practice

- Pregnant women without evidence of immunity
- Newborn whose mother develops chickenpox within 5 days before delivery or within 48 h after delivery
- Hospitalized premature infant (≥28 weeks' gestational age) whose mother lacks evidence of immunity to varicella
- Hospitalized preterm infants (<28 weeks' gestational age or birthweight ≤1000 g) regardless of maternal immunity

is no evidence that CVS occurs after exposure to varicella vaccine during pregnancy. From March 17, 1995, through March 16, 2005, 981 women were enrolled in a pregnancy registry for women exposed to varicella vaccine.[549–551] Pregnancy outcomes were available for 629 prospectively enrolled women. Among the 131 live births to previously VZV-seronegative women who received vaccine, there was no evidence of CVS. Nonetheless it is recommended that adolescents and women of childbearing age should avoid pregnancy for at least 1 month after immunization.

Outcomes

Overall mortality rates for CVS are estimated at 30%. Deaths occur in the first few months of life, usually secondary to severe pulmonary disease.[518,521] Zoster during early infancy is common[518] and is considered by some experts to be a criterion for diagnosis of CVS. Between 1% and 2% of infants without clinical evidence of CVS, but whose mothers had chickenpox in the second and third trimesters, develop zoster in the first few weeks of life. If zoster occurs, consideration should be given to ophthalmologic evaluations to rule out the possibility of CVS.[518,520]

Prior to the availability of varicella immune globulin and acyclovir, the mortality from neonatal varicella acquired from a mother with varicella contracted around the time of delivery (–5 days to +2 days) was reported as 23% to 30% of cases.[536,537] Approximately 25% to 50% of exposed infants treated with varicella zoster immune globulin may still develop varicella, but the disease is often attenuated, with no reports of mortality.[547,552]

Human Immunodeficiency Virus

Epidemiology

The HIV epidemic has seen dramatic shifts since the first cases were identified in the early 1980s. HIV-2 is endemic in West Africa and is significantly less prevalent than HIV-1. More than 99% of infections in the US are due to HIV-1.[553] In this section HIV will be used to denote HIV-1 unless otherwise indicated. Great strides have been made in diagnosis and treatment which have converted HIV from an almost universally fatal infection to a chronic but manageable disease. Mother-to-child transmission can occur in utero, peripartum, or post-partum via breastfeeding. However, the majority of transmission occurs peripartum. Without treatment the risk of transmission from an infected woman to her infant is around 25%, however, with universal prenatal HIV counseling and testing,[554] maternal antiretroviral (ARV) therapy, scheduled cesarean deliveries for higher-risk women, infant ARV prophylaxis, and avoidance of breastfeeding, the rate of mother-to-child transmission has dropped to 1% to 2% in the United States and Europe.[555,556] Of an estimated 5000 women with HIV giving birth annually in the US,[557] the number of new cases of perinatally-acquired HIV has been less than 100 in recent years.

Through efforts such as the UNAIDS Global Plan,[558] there has also been significant progress toward the global elimination of mother-to-child transmission of HIV, however, there remains much work to be done. In 2021, 81% of pregnant women living with HIV had access to ARVs to prevent maternal to child transmission. One result of increased maternal access to ARVs is the decline in infections among children (52% decline between 2010 and 2019), however, worldwide there were still 160,000 new infections in children in 2021.[559]

Prevention

Antepartum

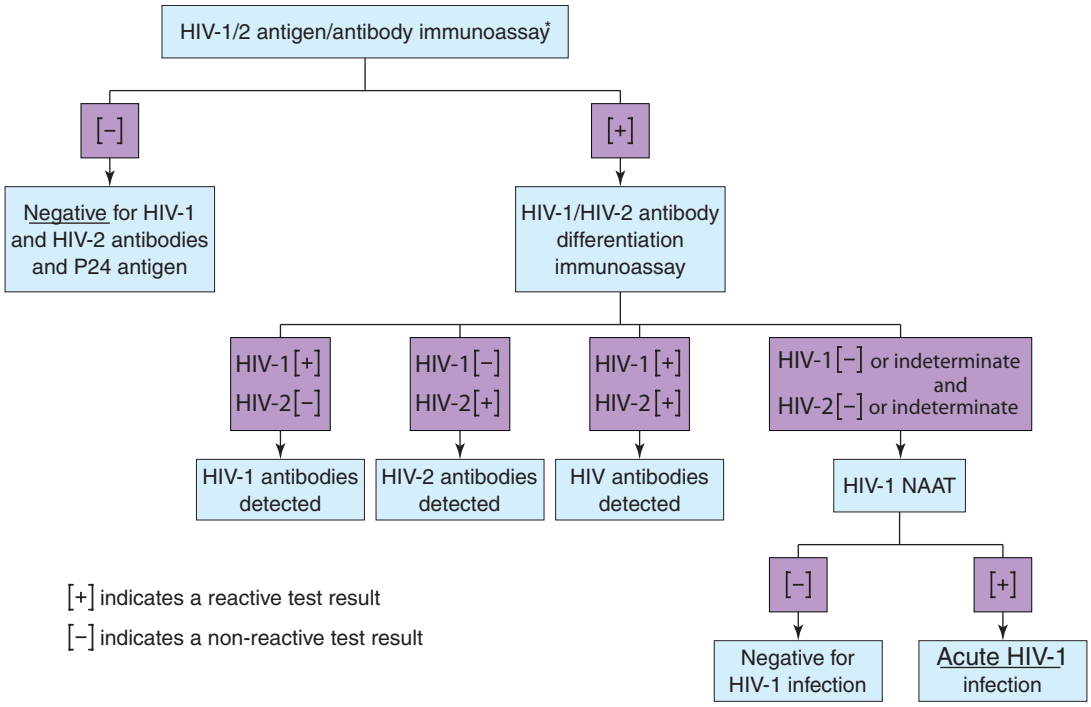
All pregnant women with an unknown HIV status should be tested for HIV with their first routine prenatal screening.[554] The best screening test is an FDA-approved HIV antigen/antibody combination immunoassay. This test detects both HIV-1 and HIV-2 antibodies as well as the HIV-1 p24 antigen. The addition of antigen testing shortens the window to detect infection to about 2 weeks after initial infection compared with the 4-week window of other assays. A reactive antigen/antibody immunoassay should be followed with a supplemental HIV-1/HIV-2 antibody differentiation test. Discordant results should be then tested with an FDA-approved plasma HIV RNA PCR assay (Fig. 34.7) to establish a diagnosis of acute infection vs a false-positive antigen/antibody screen.[560]

Women with an initial negative HIV test who are considered to be high risk should be tested again with an HIV antibody–antigen combination test in the third trimester, ideally before their 36th week of gestation. This includes women with a new sexually transmitted infection diagnosed in pregnancy, with high-risk HIV-associated behaviors, who have signs and symptoms concerning for acute HIV, who are incarcerated or who live in areas or receive care in facilities with an HIV incidence of at least 1 case per 1000 pregnant women per year.[561]

Acute HIV infection during pregnancy or breastfeeding poses an increased risk of mother-to-child transmission of HIV because of the high levels of HIV. Providers must be diligent and quick to test or retest pregnant women who are high risk or who present with a constellation of symptoms suggestive of acute retroviral syndrome such as pharyngitis, lymphadenopathy, myalgia, arthralgia, rash, or fever. A plasma HIV RNA PCR test should be sent in addition to an antigen/antibody combination immunoassay when acute HIV infection is suspected as the virologic assay will detect HIV about 5 days earlier.[562]

Once a pregnant woman has been identified as infected with HIV, antiretroviral therapy (ART) should be started as soon as possible. All pregnant women with HIV-1 infection should receive combination ART regardless of the absolute CD4 count or viral load. ARV drug resistance testing should be performed for women who are ARV-naive or ARV-experienced with a viral load of more than 500 copies/mL (usual threshold for genotypic resistance testing), but ART should be started while the results are pending and adjusted accordingly later because of the importance of viral load suppression.[563–565] Maternal ARVs decrease the risk of infant transmission by decreasing levels of virus in maternal blood and genital secretions as well as by providing ARV prophylaxis to the infant through placental transfer.

The choice of ARV drug combination is based on many factors, but in general ARV-naive pregnant women should receive at least three active ARVs. Preferred ARV regimens change frequently, and a thorough discussion of risks and benefits of potential ARV regimens is beyond the scope of this chapter. Currently recommended preferred regimens can be found online in the regularly updated Department of Health and Human Services Guideline, "Recommendations for the Use of Antiretroviral Drugs in Pregnant Women with HIV Infection and Interventions to Reduce Perinatal HIV Transmission in the United States,"[562] referred to in this chapter as the "DHHS Perinatal ARV guidelines" or by calling the National Perinatal HIV Hotline (1-888-448-8765).



• **Fig. 34.7** Human immunodeficiency virus (HIV) testing diagnostic algorithm. *For serum or plasma. *PCR*, Polymerase chain reaction. (Adapted from Centers for Disease Control and Prevention and Association of Public Health Laboratories. *Laboratory Testing for the Diagnosis of HIV Infection: Updated Recommendations. 2014.*)

The goal of ART is to maintain a viral load below the limit of detection throughout pregnancy. To that end, viral load should be tested several times during the pregnancy with a plasma HIV RNA PCR. Testing should be performed at the initial visit, 2 to 4 weeks after initiation or change of ART, monthly until viral load is undetectable, and at least every 3 months after suppression is achieved. An additional test must be done in all cases at 34 to 36 weeks' gestation to ensure viral suppression at delivery and to plan for the mode of delivery.

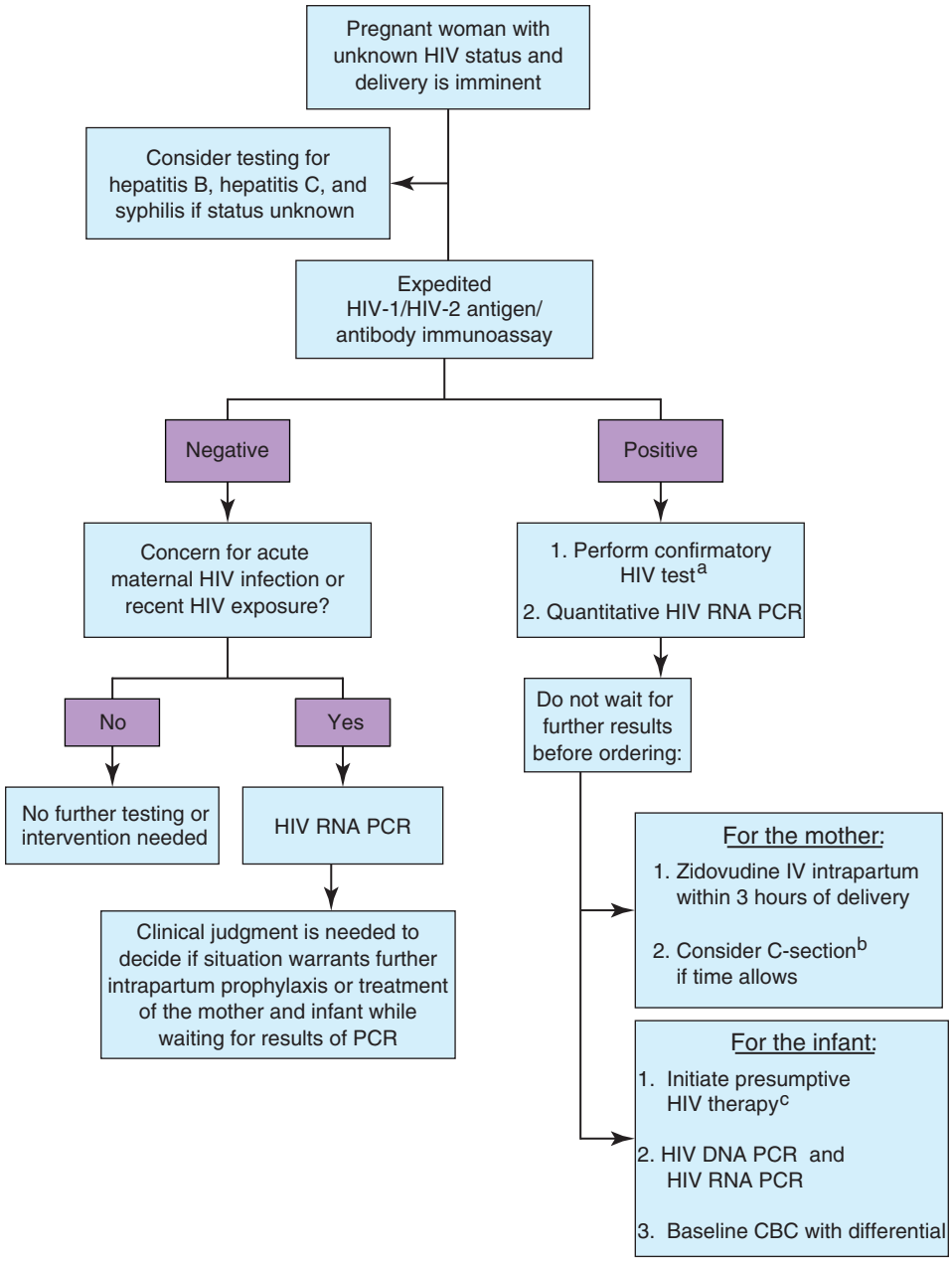
Antiretroviral therapy initiated prior to pregnancy or within the first trimester may be associated with an increased risk of preterm birth and low birth weight. However, this risk is outweighed by the benefits of ARV treatment during pregnancy to prevent infant HIV infection.[562]

Guidelines on the prevention of HIV-2 (in contrast to HIV-1) transmission during pregnancy are based largely on expert opinion. Data suggest that the rates of perinatal transmission of HIV-2 are low regardless of intervention (0% to 4% reported). There are no randomized clinical trials investigating optimal ARV prophylaxis during pregnancy for women infected with HIV-2. Treatment with ARVs known to be active against HIV-2 should be initiated for women who are pregnant or trying to conceive.[562] Breastfeeding should also be avoided by women living with HIV-2.

Intrapartum

The first study to demonstrate the effectiveness of ARVs in preventing maternal-to-child HIV transmission was Pediatric AIDS Clinical Trials Group (PACTG) protocol 076 which provided oral zidovudine (ZDV) to women during pregnancy, IV ZDV during labor, and oral ZDV to the infants.[566] Following these results, IV ZDV during labor for women with HIV became standard. However, now with more effective combination ART which achieves viral suppression, IV ZDV during labor is no longer universal. The French Perinatal Cohort group demonstrated that the difference in transmission for women with HIV RNA levels below 1000 copies/mL at delivery was not significantly different between those who received IV ZDV and those who did not.[567] IV zidovudine therapy should be given to HIV-infected women in labor with HIV RNA loads greater than 1000 copies/mL or with unknown viral loads. It is not required for HIV-infected women receiving ART who have documented viral loads of less than 50 copies/mL near delivery and for whom there are no concerns about adherence to their ARV regimen.[562] IV ZDV can be considered for women with HIV RNA levels between 50 and 1000 copies/mL as the transmission rate is slightly higher in this situation than it is for women with HIV levels less than 50 copies/mL at delivery.[568,569]

Women presenting in labor without a documented HIV result available or who are at increased risk of HIV acquisition and were not retested in the third trimester should be tested as soon as possible, ideally with an HIV 1/2 antigen/antibody combination immunoassay. If these results would not be available in less than 1 hour, then testing should be done with the most sensitive expedited test available.[562,570] If the initial screening test is positive, confirmatory testing should be done and intravenous ZDV should be initiated pending these results. See Fig. 34.8 for a suggested approach for a pregnant woman with unknown HIV status with delivery imminent. IV zidovudine should be started at least 3 hours prior to delivery and is given as a 2 mg/kg continuous infusion during the first hour and then 1 mg/kg IV continuous infusion each hour thereafter until the cord is clamped.



• **Fig. 34.8** Proposed algorithm for management of a woman presenting for delivery with unknown HIV status. A rapid turnaround time (<1 hour) HIV 1/2 antigen/antibody combination immunoassay should be performed. If an expedited HIV 1/2 antigen/antibody combination immunoassay is not available, initial testing should be performed by the most sensitive expedited test available. If the result is positive, confirmatory testing should be sent and maternal IV zidovudine therapy should be started immediately. The recommended dosing is 2 mg/kg IV continuous infusion during the first hour followed by 1 mg/kg IV continuous infusion each hour thereafter until the cord is clamped. This should begin at least 3 hours before delivery. Presumptive HIV therapy should be started in the newborn. This can be discontinued if the follow-up maternal testing does not confirm HIV positivity. [a]See fig. 34.7 for HIV diagnostic testing algorithm. [b]C-section should be considered if labor has not started. [c]Recommendations for presumptive therapy (which also serves as infant prophylaxis) are available in the Pediatric Antiretroviral Guidelines: Antiretroviral Management of Newborns with Perinatal HIV Exposure or HIV Infection available at Antiretroviral Management of Newborns with Perinatal HIV Exposure or HIV Infection | NIH. *CBC*, complete blood count; *C-section*, cesarean section; *HIV*, human immunodeficiency virus; *IV*, intravenous; *RNA*, ribonucleic acid; *PCR*, polymerase chain reaction.

Women on stable ART with viral loads less than 1000 copies/mL near the time of delivery should continue their oral ARVs during labor and delivery and be offered standard vaginal delivery. There is a potential increased risk of transmission with some obstetric procedures, including artificial rupture of membranes, invasive fetal monitoring such as with fetal scalp electrodes, and use of forceps or vacuum-assisted delivery. Hence, these should be avoided if possible, particularly for women with viral loads ≥50 copies/mL.

HIV-1-infected pregnant women who have plasma viral loads with more than 1000 copies/mL or unknown viral loads near the time of delivery should have a scheduled cesarean delivery at 38 weeks. This timing is recommended to decrease the possibility of the onset of labor or rupture of membranes before delivery can occur. Studies in 1999 (both a multicenter randomized controlled trial[571] and a metaanalysis[572]) showed a decreased risk of transmission by 50% to 80% when cesarean deliveries were performed at this juncture. However, cesarean section is not recommended for the sole purpose of prevention of perinatal HIV transmission in women on ART with viral loads less than 1000 copies/mL near the time of delivery as there is minimal additional benefit in this situation[568,573] and there is increased risk for complications from cesarean section in women with HIV.[574]

Postpartum

Mothers with HIV-1 infection should continue their ARV regimen after delivery, as ART is now recommended for all HIV-1-infected individuals. The unique challenges of labor, delivery, and motherhood may increase the barriers to adherence, and so the mother should receive additional support to continue therapy. Contraception options should be discussed early. As the mother will no longer receive obstetric care after her postpartum visit, careful attention should be made to ensure that she is following up with an HIV care provider.

Breastfeeding by HIV-infected mothers is not recommended in the United States and other developed countries where replacement feeding (formula) is affordable, the water supply is safe, and the risk of infant death due to diarrheal and respiratory infections is low.[575] Risk of transmission from breastfeeding is 15% to 20% over the first 2 years when neither maternal nor infant ARV prophylaxis is used.[576] Maternal ART continued during breastfeeding significantly decreases the risk of transmission,[577–579] however, infant prophylaxis with ART and maternal postpartum ART cannot completely eliminate the risk of infection. Maternal ARVs decrease the amount of cell-free HIV in breastmilk but have less effect on cell-associated HIV.[580]

There may be times when an HIV-infected mother chooses to breastfeed despite being counseled otherwise due to cultural or other psychosocial issues. In this situation, maternal viral suppression should be documented prior to delivery and throughout breastfeeding. Adherence support should be provided, and maternal viral load monitored regularly during breastfeeding. Exclusive breastfeeding is recommended for the first 6 months. For women on combination ARVs with suppressed virus, infant ARVs beyond the initial 6 weeks are not required, however, some experts recommend continuing infant prophylaxis until 1 to 4 weeks after cessation of breastfeeding (DHHS Perinatal ARV guidelines).[562] Infants should be tested for HIV every 3 months during breastfeeding and then at 4 to 6 weeks, 3 months, and 6 months after weaning.

Another potential mode for mother-to-child transmission is via premasticated food.[581,582] Healthcare providers should be aware of this risk and counsel mothers and other HIV-infected caregivers to avoid this practice.

Management

Management of the HIV-Exposed Infant

Antiretroviral prophylaxis should be started as soon as possible and ideally within 6 to 12 hours of birth in all HIV-exposed newborns. Recommendations for infant ARV prophylaxis vary depending on the risk of transmission to the newborn. Infants born to women who have been on effective ART with viral loads less than 50 copies/mL near the time of delivery are at low risk of transmission and should receive oral zidovudine for 4 weeks. See Table 34.5 for recommended zidovudine dosing based on gestational age. A complete blood count with differential should be performed before zidovudine therapy is started. Anemia, and less commonly neutropenia, are the primary complications seen in infants on a prophylactic zidovudine regimen. If the infant also had in utero exposure to maternal combination ART, there may be more anemia and/or neutropenia than found in infants only treated postnatally with zidovudine.[583] Many experts will check a complete blood count with differential again when they are also drawing blood for a nucleic acid amplification test or if the infant has symptoms of anemia.

TABLE 34.5 Zidovudine Prophylaxis Dosing for Human Immunodeficiency Virus-Exposed Infants

	Dosing	Duration
Zidovudine	≥35 weeks' gestation at birth: 4 mg/kg per dose orally* twice daily ≥30–<35 weeks' gestation at birth: 2 mg/kg per dose orally advanced to 3 mg/kg per dose orally at age 15 days <30 weeks' gestation at birth: 2 mg/kg body weight per dose orally advanced to 3 mg/kg per dose every 12 h after age 4 weeks	Start as soon as possible after birth, preferably within 6–12 h of delivery—continue for 4–6 weeks†

Modified from Panel on Treatment of Pregnant Women with HIV Infection and Prevention of Perinatal Transmission. *Recommendations for Use of Antiretroviral Drugs in Transmission in the United States*. Available at https://clinicalinfo.hiv.gov/sites/default/files/guidelines/documents/Perinatal_GL.pdf

*If unable to tolerate oral medicines the IV dose is 75% of the oral dose provided at the same interval

†Duration of 4 weeks recommended for infants at low risk of perinatal transmission (see text). 6 weeks is recommended for infants at high risk of perinatal HIV transmission along with additional antiretroviral agents (see guidelines).

Newborns considered to be at higher risk of HIV transmission include those born to mothers who did not receive any antepartum or intrapartum ART, received only intrapartum ARVs, received ARVs during pregnancy but did not achieve viral suppression to below 50 copies/mL near delivery, or had primary or acute HIV during pregnancy. These infants have a higher risk for both in utero and peripartum transmission and should begin *presumptive* HIV therapy as soon as possible after delivery, which also serves as prophylaxis against HIV acquisition.[562] Infants born to women with unknown HIV status during pregnancy but with a positive expedited HIV test at the time of delivery should also begin presumptive therapy which can be discontinued if the maternal infection is not confirmed (see Fig. 34.8). Current recommendations for presumptive HIV therapy include a three-drug regimen—zidovudine, lamivudine, plus nevirapine or raltegravir. Dosing recommendations are based on gestational age and birth weight. Information on and availability of new antiretroviral agents for neonates change frequently, and recommendations for

preferred regimens and doses are updated regularly in the DHHS pediatric ARV guidelines.[584] Length of therapy depends on level of risk and results of infant HIV testing but is usually 4 to 6 weeks in the absence of any positive HIV test results.

Nucleic acid amplification tests should be used to determine HIV status in exposed newborns, as antibody testing will reflect the mother's serologic status until the infant is older than 18 months. Testing can be done with either HIV RNA PCR or HIV DNA PCR assays. Testing should generally be done at birth, 14 to 21 days, 1 to 2 months, and 4 to 6 months. Birth testing can be omitted for low-risk infants for whom there are no concerns regarding access to follow-up. Additional testing should be done 2 to 6 weeks after discontinuing presumptive HIV therapy in high-risk infants who discontinue therapy.

Infants are considered presumptively HIV uninfected if they have had two negative nucleic acid amplification test results at age ≥14 days and at age ≥4 weeks or one negative result at ≥8 weeks. HIV infection can definitively be excluded with negative testing at ≥1 month and ≥4 months of age in exposed infants who are not breastfed. If the HIV status is unknown or not at least presumptively excluded by the time the prophylaxis course is complete at 4 to 6 weeks, infants should start prophylaxis for *Pneumocystis jirovecii* pneumonia.

HIV-infected mothers may have an increased incidence of coinfection with other pathogens that can be transmitted from mother to child. Pathogens to consider include HBV, HCV, *Treponema pallidum, Mycobacterium tuberculosis*, HSV, CMV, and *Toxoplasma gondii*. Infants may need additional testing depending on any known coinfections in the mother and the clinical history of the mother, including evidence of any of these diseases during the perinatal period.

Management of the HIV-Infected Infant

Early diagnosis and treatment initiation are important due to the benefits of early therapy in infants and young children. Infants with asymptomatic HIV aged 6 to 12 weeks were randomized to early versus delayed ART in a clinical trial in South Africa.[585] There was a 75% reduction in mortality in the infants randomized to early therapy. Infants treated early also had improved growth and neurodevelopmental outcomes.[586] Early initiation of treatment for infants diagnosed at birth also results in more rapid viral suppression and reduces the size of the viral reservoirs, which may have implications for future control of viral replication.[587–589] Managing antiretroviral therapy in neonates is challenging and recommendations for dosing of specific antiretrovirals in young infants are updated regularly as new data are available.[584] Infants infected with HIV should be referred to a pediatric HIV expert.

If the result of a nucleic acid amplification test is positive, the test should be repeated for confirmation. Viral genotypic resistance testing should be performed, and the ART regimen adjusted as needed. Genotype testing reflects the actual genetic mutations that may or may not confer clinically important resistance. With the more rapid turnaround of nucleic acid amplification tests, diagnoses are made earlier than previously experienced and increasingly there is more information available on dosing and safety of ARVs in the early newborn period. ARVs used during pregnancy, labor, and breastfeeding and for infant prophylaxis have dramatically reduced mother-to-child transmission of HIV. In addition, improvements in HIV diagnosis and treatment have resulted in decreased morbidity and mortality in infants and children infected with HIV. To capitalize on these advances, vigilance is necessary to ensure that all women are tested and treated appropriately during pregnancy.

Guidelines for the treatment of women during pregnancy, ART, infant prophylaxis, and pediatric ART change rapidly. We recommend readers access the US Department of Health and Human Services HIV management website at clinicalinfo.hiv/gov for the most up to date guidelines and information.[590]

Hepatitis Viruses

There are six distinct viruses known to cause viral hepatitis—hepatitis A virus (HAV), hepatitis B virus (HBV), hepatitis C virus (HCV), hepatitis D virus (delta agent) (HDV), hepatitis E virus (HEV), and hepatitis G virus (HGV). The main features of each virus type are listed in Table 34.6.[591–593] HAV is passed by fecal–oral transmission and is a rare cause of neonatal disease, although nosocomial transmission in the NICU setting has been reported. HEV is similar to HAV in its mode of transmission and clinical manifestations, except for an increased mortality in pregnant women infected with HEV.[594,595] There are no data regarding perinatal transmission of HEV. HDV may cause only coinfection or superinfection with HBV. The only clinical significance of HDV is that HBV infection may become more severe when HDV is present. Perinatal transmission has been described[596] but is uncommon. HGV, also known as *GB virus type C*, has been associated with acute and chronic hepatitis, and HGV infection is usually noted as a coinfection with HBV or HCV. Perinatal transmission can occur in 60% to 80% of infants born to HGV-viremic mothers, but there has been no report of clinical hepatitis attributed to HGV infection in this setting[597–599] and the clinical significance of HGV infection is still poorly understood. HBV and HCV are both transmitted vertically and are of the greatest importance to the care of newborns. In this section we focus on HBV and HBC.

Hepatitis B Virus

Epidemiology

The incidence of acute HBV infection decreased in the United States by 90% after the introduction of HBV vaccine, from 9.6 cases per 100,000 population in 1982 to 1 case per 100,000 in 2018.[600] Estimates of the number of people with chronic HBV in the United States vary from 850,000 to over 2 million, with the majority coming from countries where HBV is endemic.[600] In the United States, approximately 20,000 infants are born annually to mothers who are chronic HBV carriers,[601] with about 20 perinatally infected children identified each year.[602] Globally about 1% of children are chronically infected with HBV.[603] There were an estimated 257 million people infected with hepatitis B worldwide in 2015 with 900,000 deaths. Most deaths are a result of cirrhosis or hepatocellular carcinoma (HCC) in adults as a result of infection acquired in childhood.[603]

Most maternal to child transmission of HBV occurs during labor and delivery, however, in utero infections can occur which accounts in part for infant infection despite immunoprophylaxis.[604–607] In the absence of prophylaxis, infants born to women who are hepatitis B surface antigen (HBsAg)- and hepatitis B e antigen (HBeAg)-positive have a 70% to 90% chance of acquiring infection. In contrast, the risk of an infant acquiring HBV infection if born to an HBsAg-positive but HBeAg-negative mother is significantly lower (0% to 30%).[606,608–611]

TABLE 34.6 Viral Hepatitis Types A, B, C, D, E, and G: Comparison of Clinical, Epidemiologic, Immunologic, and Therapeutic Features

Feature	Hepatitis A	Hepatitis B	Hepatitis C	Hepatitis D	Hepatitis E	Hepatitis G
Virus	Hepatitis A virus	Hepatitis B virus	Hepatitis C virus	Hepatitis D virus (hepatitis delta virus)	Hepatitis E virus	Hepatitis G virus (GB virus type C)
Family	*Picornaviridae*	*Hepadnaviridae*	*Flaviviridae*	Unassigned	*Hepeviridae*	*Flaviviridae*
Genome	RNA	DNA	RNA	RNA	RNA	RNA
Incubation period	15–40 days	50–180 days	1–5 months	2–8 weeks	2–9 weeks	Unknown
Mode of Transmission						
Oral (fecal)	Usual	No	No	No	Usual	No
Parenteral	Rare	Usual	Usual	Usual	No	Usual
Perinatal	Rare	Yes	Yes	Only with hepatitis B virus	Unknown	Yes
Other	Food-borne or waterborne	Sexual contact	Sexual contact less common	Sexual contact less common	Waterborne transmission in developing countries	Sexual contact; probably less common
Sequelae						
Carrier state	No	Yes	Yes	Yes	No	Yes
Chronic disease	No cases reported	Yes	Yes	Yes	No cases reported	Yes; controversial
Interventions						
Immunoglobulin	Yes	Yes	No	No	No	No
Vaccine	Yes	Yes	No	No	No	No
Antiviral therapy	No	Yes	Yes	No	No	No

From Krugman S. Viral hepatitis: A, B, C, D and E—infection. *Pediatr Rev.* June 1992;13(6):203–212; DOI: 10.1542/pir.13-6-203

Pathophysiology

HBV is a DNA virus that localizes primarily in hepatic parenchymal cells but circulates in the bloodstream, along with several viral proteins or subviral antigens. Several distinct genotypes have been identified, and these subtypes show biologic variability in transmission and disease progression.[612–615] Innate immune responses in the trophoblast appear to play a key role in defense against transplacental transmission, particularly Toll-like receptors 7 and 8.[616] Immunoglobulin against HBV also plays a role in prevention of transmission at the placental level: antibody deposition in Hofbauer cells that serves as an immune barrier to transmission between the mother and the fetus.[617] HBeAg plays a role in immune tolerance to infection in the newborn. HBeAg is the only HBV antigen to cross the placenta, and its presence leads to tolerance of the viral capsid protein by helper T cells in the newborn.[614] Transplacental leakage of HBeAg-positive maternal blood is a potential source of intrauterine infection[618] as is infection of the ovarian follicles and placenta.[605,619–621] Most infants born to mothers infected with HBV have a negative test result for HBsAg at birth but in the absence of prophylaxis are at risk of becoming HBsAg-positive during the first 3 months of life, suggesting that transmission is primarily peripartum and not transplacental.[591,622–624] High maternal HBV DNA levels are the most important risk factor for perinatal transmission and failure of post-natal immunoprophylaxis.[625–629]

Clinical Presentation

Infants with HBV infection do not show clinical or chemical signs of disease at birth. In some infants the infection becomes clinically manifest, with jaundice, fever, hepatomegaly, and anorexia in the first few months of life, followed by either recovery or chronic active hepatitis. Fulminant hepatitis in infancy occurs more commonly in infants who acquire infection from mothers who are HBeAg negative and HBeAb positive.[630–633]

Perinatally infected children typically initially experience an immune-tolerant phase with high levels of virus, but minimal or no hepatic inflammation. This phase can last for years, eventually transitioning into an immune active phase with increases in liver enzyme levels and evidence of hepatic inflammation. The immune active phase can be prolonged, leading to hepatic fibrosis. With time the majority will develop into an inactive carrier state with normalization of their liver enzymes, decreased inflammation, and HBeAg seroconversion.[634–637] Chronic HBV infection develops in up to 90% of perinatally infected infants.[638]

Evaluation

Diagnosis of HBV infection is by detection of subviral antigens in serum. HBsAg appears early, usually before liver disease is evident and persists in those who become long-term carriers. HBeAg, HBeAb, aminotransferases, and HBV DNA PCR are important in confirming diagnosis and monitoring disease activity.

Prevention

In the United States, in the past three decades a comprehensive immunization strategy[639] has been implemented consisting of four components:

1. Universal immunization of all infants beginning at birth.
2. Prevention of perinatal infection through routine screening of all pregnant women and appropriate immunoprophylaxis of infants born to HBsAg-positive women (or women whose HBsAg status is unknown) (Table 34.7).
3. Routine immunization of children and adolescents who have not been immunized previously.
4. Immunization of nonimmunized adults at increased risk of infection.

The doses and recommended options for administration of HBV vaccines currently licensed in the United States are provided in Table 34.8.[639] Infants who received hepatitis B immunoglobulin and HBV vaccine at birth, followed by two or three additional

TABLE 34.7 Hepatitis B Virus Immunoprophylaxis by Maternal HBsAg Status

Maternal HBsAg Status	Infant Birthweight (g)	Birth		Follow-up*
HBsAg positive	≥2000	HBV vaccine†HBIG	≤12 h	Complete a 3 dose HBV vaccine series
	<2000	HBV vaccine†HBIG	≤12 h	Complete a 4 dose HBV vaccine series
HBsAg negative	≥2000	HBV vaccine†	≤24 h	Complete a 3 dose HBV vaccine series
	<2000	HBV vaccine†	Hospital discharge or age 1 month	Complete a 3 dose HBV vaccine series
HBsAg unknown	≥2000‡	HBV vaccine†	≤12 h	Complete a 3 dose HBV vaccine series
	<2000	HBV vaccine†HBIG	≤12 h	Complete a 4 dose HBV vaccine series

Adapted from Schillie S, Vellozzi C, Reingold A, et al. Prevention of hepatitis B virus infection in the United States: recommendations of the Advisory Committee on Immunization Practices. *MMWR Recomm Rep*. 2018;67(No. RR-1):1–31. DOI: http://doi.org/10.15585/mmwr.rr6701a1

HBsAg, hepatitis B surface antigen; *HBIG*, hepatitis B immunoglobulin; *HBV*, hepatitis B virus

*4-dose vaccine series acceptable for infants with birth weight ≥2000 g when using combination vaccines to complete the series.

†single-antigen vaccine.

‡mother with unknown status should be tested for HBsAg status as soon as possible after delivery; if found to be HBsAg positive, infant should receive HBIG as soon as possible, no later than 7 days of age.

TABLE 34.8 Licensed Monovalent and Combination Hepatitis B Vaccines

	SINGLE-ANTIGEN VACCINES		COMBINATION VACCINES		
Age group	Recombivax Hepatitis B Dose§	Engerix-B Dose	Twinrix* Dose	Vaxelis† Dose	Pediarix‡ Dose
Newborns, children and adolescents aged <20 years	5 µg (0.5 mL)	10 µg (0.5 mL)	NA	10 µg (0.5 mL)	10 µg (0.5 mL)
Adults aged >20 years	10 µg (1.0 mL)	20 µg (1.0 mL)	20 µg (1.0 mL)	NA	NA

Adapted and updated from Schillie S, Vellozzi C, Reingold A, et al. Prevention of hepatitis B virus infection in the United States: recommendations of the Advisory Committee on Immunization Practices. *MMWR Recomm Rep*. 2018;67(No. RR-1):1–31. DOI: http://doi.org/10.15585/mmwr.rr6701a1

NA, not applicable.

*Twinrix is a combination of Engerix-B (20 µg) and hepatitis A vaccine, licensed for use in people aged 18 years or older in a three-dose series in a 0-, 1-, and 6-month schedule.

†Vaxelis is a combination of diphtheria and tetanus toxoids and acellular pertussis (DTaP), inactivated poliovirus (IPV), *Haemophilus influenzae* type b conjugate, and hepatitis B recombinant vaccines approved for use at 2, 4, and 6 months of age to be used from 6 weeks of age through age 4. Not to be used for the birth dose or for children 5 years and older.

‡Pediarix is a combination of hepatitis B (Engerix-B, 10 µg) with diphtheria and tetanus toxoids and acellular pertussis (i.e., DTaP), and inactivated poliovirus. It is recommended for use at 2, 4, and 6 months of age but should not be administered at birth, before 6 weeks of age, or after 7 years of age.

§Recombivax and Engerix are monovalent recombinant hepatitis B vaccines that can be administered starting at birth. Each vaccine has a pediatric and adult dose formulation. For both vaccines, pediatric and adult doses are administered on a 0-, 1-, and 6-month schedule, although Recombivax can also be administered to adolescents 11–15 years of age as two adult doses on a 0- and 4- to 6-month schedule.

immunizations (see Table 34.7), should be tested for HBsAb and HBsAg at 9 to 12 months of age or 1 to 2 months after the last vaccine. Infants who did not receive postnatal prophylaxis should be tested as soon as they are identified.

Approximately 5% to 10% of exposed infants are infected despite receiving HBIG and HBV vaccine at birth, with infants born to women with high viral loads being the most at risk.[625–627,636] Provision of oral antiviral therapy to highly viremic women in addition to immunoprophylaxis for the infant can almost eliminate the risk of transmission of HBV to their newborns.[640–643] Therefore, the American Association for the Study of Liver Diseases (AASLD) and World Health Organization (WHO) guidelines recommend that pregnant women who do not require HBV treatment for their own infection have HBV DNA levels obtained at 26 to 28 weeks of pregnancy and if greater than 200,000 IU/mL should be started on tenofovir at 28 to 32 weeks of gestation.[603,644] While tenofovir disoproxil fumarate is the recommended antiviral in the AASLD guidelines due to the lack of significant data on tenofovir alafenamide (TAF), subsequent studies have found TAF to be equally efficacious.[641,642] Although HBsAg has been found in breast milk, breastfeeding does not appear to have any influence on the rate of transmission[645] and maternal HBV infection is not a contraindication to breastfeeding.

Management

Treatment is not recommended for children who have normal transaminase levels and are immune tolerant or inactive carriers regardless of HBV DNA levels.[644,646] For children with immune active disease, ALT elevation and measurable HBV DNA, treatment should be considered. Currently preferred agents for treatment of children with chronic HBV and evidence of active disease include interferons (IFN alfa-2b and PEGylated IFN alfa-2a) and the nucleoside/nucleotide analogues entecavir and tenofovir. Choice of agents depends on stage of disease and age.[644,646,647] Infants and children with chronic HBV infection should be referred to a pediatric hepatologist for management.

Outcomes

Infants who become infected with HBV have a 90% risk of chronic infection. Without treatment there is a 15% to 40% risk of developing cirrhosis and/or hepatocellular carcinoma.[634,636,648–651]

Hepatitis C Virus

In 1989, HCV was identified to be the main cause of non-HAV, non-HBV, parenterally transmitted hepatitis. HCV is now a major cause of chronic hepatitis, cirrhosis, and hepatocellular carcinoma in the United States and globally.[652,653] HCV is a small, single-stranded RNA virus that is a member of the family *Flaviviridae;* this family consists primarily of vector-borne viruses including Zika virus and West Nile virus. HCV is an exception in this family because it is not transmitted by insect vectors. Seven genotypes are described, with significant biologic differences in regard to disease progression and responsiveness to therapy.[654]

Epidemiology

In the United States, the number of reported cases of hepatitis C infection has increased every year since 2009, with the highest incidence in people aged 20 to 39 years.[653,655] It is estimated that over 2 million people or ~1% of all adults in the United States are living with HCV.[653] HCV is primarily transmitted through exposure to infected blood with IV drug use being the greatest risk factor in the United States.[655] Transmission in the health care setting can occur through improper infection prevention practices. The risk of HCV infection from transfused blood after the advent of HCV screening is estimated to be less than 1 in 1 million units transfused. Sexual transmission is less efficient, although high-risk sexual behaviors contribute to HCV transmission.[656]

Perinatal transmission is the most common route of infection in children and the leading cause of childhood HCV infection.[657] Although data in pregnancy is incomplete due to lack of universal testing, the prevalence of HCV in pregnant women in the US is reported as approximately 0.4% to 0.7% in recent years,[658] with significant geographic variability.[655] The vertical transmission rate of HCV is approximately 5% in HIV-negative mothers and 10% from mothers co-infected with HIV.[657] An estimated 1700 children are infected with perinatally acquired HCV each year in the United States.[659]

Pathophysiology

Transmission of HCV from mother to child occurs either in utero or more frequently peri-partum.[660] HCV has been shown to productively infect placental trophoblasts in cell culture,[661] although a direct transplacental route of infection remains unproven. Placental innate immunity mediated by natural killer cell responses may prevent direct transplacental transmission of virus in most cases.[662] Infection of and replication in maternal peripheral blood monocytes can also affect the ability of the virus to infect the fetus or newborn.[663] HCV mother-to-infant transmission is highest in women with detectable blood levels of HCV RNA during pregnancy. Although there is no clear level of viremia associated with transmission.[657,664] Transmission by women with undetectable HCV RNA levels is uncommon. Maternal coinfection with HCV and HIV and history of IV drug use are risk factors for transmission because of increased maternal HCV viral load.[665,666]

Although mode of delivery is not demonstrably associated with transmission risk,[664] procedures which increase exposure to maternal blood during delivery such as scalp electrode monitoring and episiotomy, are associated with an increased risk of transmission[667–669] and should be avoided if possible.[670] Rupture of membranes greater than 6 hours is also associated with increased risk of transmission.[664]

Although HCV can be found in breast milk and colostrum,[671,672] studies comparing breastfed and bottle-fed infants born to HCV-infected mothers have not shown a statistically significant difference in rates of transmission.[664,673,674] Maternal HCV infection, therefore, is not a contraindication to breastfeeding;[670] however, it may be prudent for mothers who are infected with HCV and who choose to breastfeed to consider abstaining from breastfeeding if their nipples are cracked and bleeding.[675]

Clinical Presentation

Several studies have found that infants born to HCV-infected women are at higher risk for prematurity and low birth weight.[670] Most neonates perinatally infected with HCV demonstrate little in the way of clinical symptoms; they may have elevated liver ALT

levels either transiently, intermittently, or persistently,[676] most commonly noted between 3 and 6 months of age and peaking in the first 2 years of life. The most common clinical finding is hepatomegaly which develops in approximately 10% of children.[676]

Evaluation

Diagnosis of perinatally acquired HCV can be through detection of anti-HCV IgG after 18 months of age by which time at least 99% will have cleared maternal antibody.[677] Earlier identification of infected infants can be done by obtaining serum or plasma HCV RNA PCR after 1 to 2 months of age as the highest sensitivity of PCR is reported after 1 month of age.[678] Exposed infants whose initial RNA PCR is negative should still have anti-HCV IgG tested at 18 months of age.[241]

Management

Infants confirmed as HCV-infected should be monitored yearly for clinical and laboratory evidence of liver disease[679] and should be referred to a pediatric gastroenterologist for evaluation and treatment. Until recently, only IFN and ribavirin were approved by the FDA to treat adults and children with chronic HCV infection. The development of novel and highly effective direct-acting antivirals (DAAs) such as protease inhibitors, non-structural protein 5a inhibitors, and nucleotide and non-nucleotide polymerase inhibitors for HCV infection has revolutionized the care of infected patients[680,681] and PEG-IFN/ribavirin regimens are no longer recommended.[679,682] Choice of specific combinations of the DAA agents and length of treatment is based on the HCV genotype, previous treatment, age, and disease severity.[683,684] There are now several DAA agents labeled for use in children down to age 3 years, with more under study.[684–688] Efficacy and tolerability of these agents is excellent with sustained virologic response achieved in 90% to 100% of treated children.[684]

Prevention

Although development of an HCV vaccine is a major public health priority,[689] currently no vaccine is available. Like all other infants, infants with HCV should receive routine HBV immunization. In addition, they should receive HAV vaccination at 2 years of age. Parents should be advised to avoid unnecessary administration of medicines known to be hepatotoxic. Standard precautions are recommended for the hospitalized infant.

Outcomes

Chronic hepatitis develops in 60% to 80% of HCV-infected adults, which leads to cirrhosis or liver failure within 20 to 30 years after infection in one third of infected adults.[690] In patients with cirrhosis, the incidence of hepatocellular carcinoma is 2% to 5% per year. In children, 20% to 25% will experience spontaneous viral clearance.[676,679,691] Polymorphisms in the *IL28B* gene, and infection with HCV genotype 3, have been associated with greater chances of spontaneous viral clearance.[692] About 50% of infected children will develop chronic asymptomatic infection with the remaining 25% to 30% developing chronic, active infection.[676] In general, HCV progression is mild or moderate in children with chronic infection, however approximately one-third will develop cirrhosis by the third decade of life.[693] Early treatment with DAAs should prevent progression of HCV-related liver disease.

Enteroviruses and Parechoviruses

Enteroviruses are non-enveloped, single-stranded positive-sense RNA viruses in the family *Picornaviridae* (*pico* means very small in Spanish). Human enteroviruses include polioviruses 1, 2, and 3, coxsackieviruses A and B, and the echoviruses. Parechoviruses will be included here as they are closely related to enteroviruses and cause a similar spectrum of disease. Historically, poliovirus infection has been responsible for the greatest morbidity in infants, causing severe or fatal disease as well as residual paralysis in a high proportion of survivors. Global efforts to extend vaccination coverage have resulted in a significant decrease in polio cases and have effectively eradicated the disease in the developed world. Neonatal diseases associated with non-polio enteroviruses, for which there are no vaccines, remain common and can be associated with serious morbidity and occasional death. Typically acquired postnatally, these agents are associated with a wide range of clinical syndromes in the NICU, including CNS infection, myocarditis, and viral sepsis. Enteroviruses are the most frequently identified etiology in infants undergoing workup for neonatal fever.

Epidemiology

Enterovirus and parechovirus infections are seasonal, occurring most commonly during summer and autumn in temperate climates. The incidence varies from year to year, with outbreaks frequently caused by a single serotype and sometimes by multiple serotypes.[694–696] An estimated one fourth of all symptomatic enterovirus infections occur in children less than 1 year of age.[697] Although mild or asymptomatic disease is the rule even in infants, the incidence and severity of infections are inversely correlated with age.[698] In infants, as in older children and adults, transmission is typically via the fecal–oral route; as non-enveloped viruses, enteroviruses are stable in the environment for days to weeks.[699] Asymptomatic transmission is common; in one study of families of infants with fever and enterovirus or parechovirus infection, approximately 90% of siblings were simultaneously infected, while approximately one-third of these were asymptomatic. The adults in these households had lower rates of simultaneous infection but higher rates (around 85%) of asymptomatic infection.[31] Nosocomial outbreaks can be a significant source of infections: nursery and obstetric clinic outbreaks of both coxsackievirus B[700–702] and echovirus[703–705] infections have been reported with severe, and sometimes fatal, illness.

Enterovirus infections account for at least one-third of admissions of febrile neonates for suspected sepsis and, during the peak enterovirus season, between half and two-thirds of all neonatal admissions.[706,707] A comprehensive study prospectively enrolled over 600 healthy mother-infant pairs in Rochester, NY from June through October and tested for enterovirus from throat or stool cultures.[708] Remarkably, 12.8% of all newborns tested positive within the first month of life, although 79% of these infections were asymptomatic. In the remaining (21%) of infections, lethargy and fever were the most common symptoms. All symptomatic infants were admitted to a hospital for an evaluation to rule out sepsis per established guidelines, leading to a hospitalization rate of 7 per 1000 live births and making enterovirus infection a more common reason for hospitalization than group B streptococcus, HSV, and CMV infections combined. Parechoviruses appear to be similarly common in both febrile and well infants.[709,710]

Pathophysiology

Neonates can acquire enterovirus infections *in utero*, during labor and delivery, or postnatally. The dominant mode of transmission of serious neonatal infection is through contact with maternal blood, fecal material, or vaginal or cervical secretions, most likely during or shortly after delivery.[708,711] Having an older sibling in the household is an independent risk factor for acquisition of neonatal disease.[712] Intrauterine transmission appears to occur via transplacental spread in late pregnancy, secondary to maternal viremia, and has been described in up to 22% of cases of neonatal enterovirus infection when symptoms are present in the first day of life.[713,714] Except for transplacental infections, the portal of entry for enteroviruses is via the oral or respiratory route. Enteroviruses are resistant to the acidity of the stomach and replicate easily in the upper GI tract. After local replication, virus seeds the draining lymphoid tissue and then the blood. Severe enterovirus disease results from viral dissemination to distal tissues, which can include the heart, CNS, liver, pancreas, adrenal glands, skin, mucous membranes, and respiratory tract. Viral infection can cause direct cellular dysfunction, as in polio virus infection of lower motor neurons[715] or coxsackievirus B3 infection of cardiac myocytes.[716] However, in severe or persistent infections an aggressive local inflammatory response, including extensive infiltration by lymphocytes, mononuclear cells, histiocytes, and polymorphonuclear leukocytes, is a prominent feature.[717]

The presence of transplacental maternally derived antibody significantly mitigates the severity of disease,[718] and breastfeeding appears to provide a relative degree of protection against acquisition of infection.[719] Development of antibody is associated with recovery, although virus may continue to be shed in the stool for several weeks. Failure to clear enterovirus infection, particularly from the CNS, should suggest an underlying humoral immunodeficiency such as aggamaglobulinemia.[720,721] Infected individuals shed virus via the gut primarily, but secretion of virus in saliva and respiratory secretions has been shown.

Clinical Presentation

There is little evidence that enterovirus infections in pregnant mothers have adverse developmental consequences of infection on the fetus. While case reports and correlative epidemiological studies have proposed enteroviruses as teratogenic agents,[722–724] these reports are rare and the relatively high incidence of maternal enterovirus infections in normal pregnancies argues against a causal link. Similarly, enteroviruses have been implicated as a cause of fetal demise, but only in isolated cases.[725–727]

When neonatal infection is acquired in utero or perinatally from the mother, infants are typically asymptomatic at birth.[708,713] The mother may have a history of recent high temperatures and GI symptoms. Symptoms develop in the baby after an incubation period of 1 to 5 days, with most affected infants developing symptoms within their first week of life.[698] The most common complaints on initial presentation are fever and poor feeding; one-fourth of infected infants develop diarrhea or vomiting. Many have an erythematous maculopapular rash. Population-based studies find that most enterovirus infections are relatively mild, and overall survival is excellent.[728,729] One large study in Salt Lake City enrolled 1779 febrile infants younger than 90 days who were undergoing sepsis evaluation; 1061 were tested for enterovirus, and 214 (20%) were positive (57% from blood and 74% from CSF). The mean age of infants with enterovirus infection was 33 days; 91% were admitted but only 2% required intensive care.[730] The duration of illness ranges from less than 24 hours to longer than 7 days but generally is 3 to 4 days.

These observations underscore the generally benign and self-limited nature of neonatal enterovirus disease. However, morbidity can be substantial in severe disseminated infection. In infants admitted to the ICU, CNS findings are the most common, followed by respiratory tract disease, with cardiac involvement being less common.[706,731] Many severely affected infants have a viral sepsis syndrome—characterized by DIC, refractory hypotension, and multisystem organ dysfunction. Severe infections are typically acquired in the immediate perinatal period. The mother is commonly symptomatic and may have been empirically treated with broad-spectrum antibiotics for possible chorioamnionitis (a maternal history of suspected chorioamnionitis with negative bacterial cultures should suggest this association, particularly during the typical enterovirus season). If myocarditis is present, congestive heart failure is often severe. Some neonates exhibit primarily pulmonary disease or GI involvement, including diarrhea and necrotizing enterocolitis.[732] In a review of neonatal meningitis seen over a 15-year period in Galveston, Texas, enterovirus was the most common cause of meningitis in newborns older than 7 days.[733] The severity of CNS infection is quite variable, from mild meningitis to severe encephalopathy and periventricular leukomalacia.[734,735] Intracranial bleeding ranging from small to massive, severe hemorrhage has also been reported as a complication of neonatal enterovirus infection.[472,736,737] Other rarely associated findings include disseminated vesicular rash, dermal hematopoiesis, and hemophagocytic syndrome.[518,738,739]

It appears that any species of human enterovirus or parechovirus can cause disease in the newborn, with individual species occasionally associated with specific clinical syndromes. Coxsackie B viruses are frequently associated myocarditis and, less commonly, aseptic meningitis.[740–742] Enterovirus A71 is notable for its etiologic role in epidemics of severe neurologic diseases including brainstem encephalitis, as well as severe pneumonia.[743–745] A 2014 outbreak in the United States highlighted enterovirus D68 as an etiology of severe pneumonia and identified a possible association between this species and acute flaccid myelitis in children.[746–748] Echoviruses are associated with severe nonspecific febrile illnesses including DIC, aseptic meningitis, but can cause fulminant hepatitis.[703,749–751] Parechovirus genotype A3 is the most commonly identified viral etiology of meningitis in infants less than 90 days in some studies.[709,752] Enteroviruses, along with other viruses, have been posited as a potential etiology in sudden infant death syndrome (SIDS), possibly from myocarditis or pulmonary infection,[753,754] but this association has been controversial. In one study of SIDS victims, a comprehensive assessment was undertaken to attempt to identify potential viral infection of the myocardium. Overall, 62 SIDS victims and 11 controls were studied. Enteroviruses were detected in 14 cases (22.5%), adenoviruses in 2 cases (3.2%), EBV in 3 cases (4.8%), and parvovirus B19 in 7 cases (11.2%), whereas control group samples were completely negative for viral nucleic acid.[755] However, another evaluation of histopathologic features and PCR analysis from 24 SIDS cases failed to demonstrate any association with viral infection,[756] leaving this putative linkage unclear.

Evaluation

PCR assays have replaced viral culture as the gold standard diagnostic modality for enterovirus infections.[757] Pan-enterovirus PCR assays, which can be performed on blood, CSF, stool, respiratory, and throat swabs, typically detect all enterovirus species with similar sensitivity, and species identification can be performed by sequencing. In infants, it appears blood PCR has the highest sensitivity for diagnosing enterovirus and parechovirus sepsis,[758,759] although it is also common for non-sterile sites such as the pharynx or GI tract to be the only location where virus is detected despite symptoms involving multiple organ systems.[760] For neonatal enterovirus disease, the dried umbilical cord has been used for PCR-based retrospective diagnosis of in utero infection.[761] Rapid multiplex PCR platforms in widespread use often include a "rhinovirus/enterovirus" target and parechovirus target. Because of genetic similarity within a genus these tests generally cannot provide genotype-level detail but appear to have good sensitivity, including for the detection of enterovirus D68[762] and A71.[763] Serologic tests for enteroviruses exist but are less useful than PCR or culture for initial diagnosis.[764]

Laboratory evaluation is predicated on the clinical syndrome and the end organs involved. In severe systemic enteroviral disease, associated findings may include thrombocytopenia, elevated transaminase levels, hyperbilirubinemia, hyperammonemia, hematologic abnormalities consistent with DIC, anemia, peripheral leukocytosis, and abnormal chest radiographs. Infants with enterovirus meningitis typically have moderate CSF pleocytosis, which can be either lymphocytic or polymorphonuclear but may lack pleocytosis even in the setting of documented CNS infection.[765,766] Accordingly, during periods of active enterovirus infection in the community, CSF should be sent for enterovirus PCR in patients where CNS disease is suspected, even if pleocytosis is absent. When myocarditis is a diagnostic possibility, echocardiogram and electrocardiogram are indicated and may reveal diminished left ventricular function or dysrhythmias. Liver biopsy and histopathologic examination may be warranted in cases of fulminant hepatic failure.[717,737]

Management

The cornerstone of treatment of neonatal enterovirus disease is supportive care. Myocarditis and heart failure can be treated with inotropic support, diuretics, aggressive fluid management, and other supportive measures. DIC should be treated with blood products and other supportive measures as indicated.

The antiviral drug pleconaril was developed specifically to treat picornavirus infections but has failed to obtain FDA approval largely due to an unfavorable side-effect profile and is not available for use in the US. Other small-molecular inhibitors of picornavirus infection are in preclinical development,[767,768] and of these pocapavir has been used on an emergency investigational basis in several cases of neonatal enteroviral infection.[769–771]

IVIG has been reported anecdotally to treat neonatal enterovirus infections with various degrees of success.[772,773] Only one randomized trial has systematically studied its use in infants with enterovirus sepsis. In that 1995 study of 16 infants, which included 9 who were randomized to receive IVIG at a dose of 750 mg/kg, patients given IVIG containing high titers of neutralizing enteroviral-specific antibodies had more rapid resolution of viremia. However, there were no significant differences in other major clinical outcomes, such as the duration of hospitalization, fever, and symptoms of acute illness between treatment and control groups.[774] While IVIG is a relatively common therapy for viral myocarditis in adults and children, individual studies have yielded conflicting results[775] and an ongoing Cochrane Review has not demonstrated evidence of benefit.[776] Similarly, there is no evidence demonstrating corticosteroid or other immunomodulatory therapies are of benefit in treating enterovirus infections.

Prevention

The core tenets of infection prevention—particularly hand washing—are the primary tools of defense against enterovirus infection and spread. Standard contact precautions are typically used for hospitalized infants with known or suspected enterovirus infections, although strict cohorting strategies have been reported to limit nursery outbreaks.[777] Anecdotal reports of the prophylactic use of IVIG in nursery outbreaks to prevent further horizontal transmission of enterovirus infection have produced conflicting results.[778–781] Polio virus vaccine studies have demonstrated the efficacy of vaccination in eliminating enteroviral disease at the population level. In areas of the world where polio infections are still endemic, oral polio vaccination at birth is recommended by the WHO due to evidence supporting the efficacy of this vaccine even in newborn populations. Because there are multiple nonpolio enteroviral serotypes that cause clinical disease, broad immunization against multiple enterovirus species is conceptually difficult. Vaccines against enterovirus A71 have been developed and licensed in China and tested in children as young as two months old.[768,782]

Outcomes

Factors associated with severe neonatal disease include peripartum maternal illness, younger age of neonate, absence of serotype-specific antibody, and absence of fever and irritability.[728] These risk factors, all associated with peripartum infection, support the notion that intrauterine transmission causes particularly severe disease. The highest mortality rates are associated with the combination of severe hepatitis, coagulopathy, and myocarditis. Severe hepatitis caused by enterovirus infection is associated with mortality rates ranging from 30% to 80%.[714,737] By the time DIC has developed, the prognosis is grave; a prothrombin time longer than 30 seconds was an independent predictor of death in one retrospective case review.[737]

Relatively few long-term follow-up studies have been published, but the available information suggests that infants who survive severe enteroviral neonatal sepsis with hepatitis have a complete recovery in most instances.[783] Outcomes of 6 of 11 survivors with follow-up ranging from 9 to 48 months included normal growth and no residual medical problems or liver dysfunction.[737] In contrast, persistent myocardial dysfunction appears to be common (33% to 66%) in survivors of neonatal viral myocarditis.[783,784] Common sequelae include chronic heart failure (ventricular dysfunction), aneurysms within the left ventricle, and mitral regurgitation.

The long-term prognosis following CNS infection is also mixed and appears to depend on virus species. Among picornavirus species, enterovirus A71 and D68 and parechovirus A3 species are associated with severe CNS disease and with lasting neurologic sequelae.[785–788] The presence of seizures or other signs of encephalitis (abnormal CSF, clinical exam, or imaging) are associated with a higher likelihood of long-term neurologic sequelae.[783,789,790] On a

• BOX 34.3 Common Respiratory Viruses Causing Clinical Disease in Infants

- Rhinovirus
- Respiratory syncytial virus
- Coronavirus
- Parainfluenza
- Human metapneumovirus
- Influenza
- Adenovirus
- Bocavirus
- Echovirus

Ranked in descending order of frequency. Data adapted from Tregoning JS, Schwarze J. Respiratory viral infections in infants: causes, clinical symptoms, virology, and immunology. *Clin Microbiol Rev*. 2010;23:74–98.

population level, studies in the 1970s found some impairment of intellectual development in 13% to 42% of infants with enterovirus meningitis compared to carefully selected control groups.[791,792] In contrast, several subsequent studies (in the 1980s and 1990s) found no significant differences in neurodevelopmental outcomes compared to controls.[793,794] Larger and more recent studies provide an overall reassuring picture of normal or near-normal developmental outcomes in most neonates with enterovirus and parechovirus infections involving the CNS.[789,795–797] One plausible explanation is that increased availability of PCR-based diagnostic testing, which is now commonly used in blood and CSF evaluation of febrile infants, has led to the inclusion of more patients with mild disease.

Respiratory Viruses

Most newborns with respiratory viral infections are asymptomatic or only mildly symptomatic. However, any respiratory virus can cause symptomatic disease. Respiratory viral infections in the neonatal intensive care unit (NICU) population are likely more common than generally recognized (Box 34.3) and can result in prolonged hospitalization and adverse outcomes.[798–803]

Respiratory syncytial virus (RSV) is the leading cause of viral pneumonia and bronchiolitis in young infants and has been associated with nosocomial outbreaks in NICUs.[804–809] Pregnant women and young infants are at increased risk of complications from influenza virus infection. Therefore, RSV and influenza are reviewed in the following sections.

Respiratory Syncytial Virus

Epidemiology

In temperate climates RSV causes annual epidemics during the winter and early spring months (typically November to April). During these months, RSV can be responsible for up to 20% of all pediatric hospital admissions.[810] Nosocomial infections are frequent during these times, and illness among hospital staff members is a major factor in its spread from infant to infant. Several nursery outbreaks have been described, and hospital acquired RSV infections in the NICU have been shown to result in increased length of hospitalization, increased use of antibiotics, mechanical ventilation, and mortality.[806,809] In one recent outbreak whole genome sequencing was used to determine the pattern of transmission within the NICU.[808] Two community introductions were identified with seven infected infants. Rapid implementation of enhanced infection control measures limited the spread within the NICU. All infected infants were symptomatic: two with lower respiratory disease and three required increased respiratory support. Compared to infections with other respiratory viruses, RSV-infected infants are more likely to require hospitalization and supplemental oxygen.[811] Risk factors for RSV-associated hospitalization include young age, prematurity, siblings in day care or school, and chronic lung disease.[810,812–817]

Clinical Presentation

RSV infection has been associated with apnea, primarily in infants of younger age and a history of prematurity.[818–820] RSV infection in the first few weeks of life may present with apnea, poor feeding, irritability with minimal respiratory symptoms.

Evaluation

Direct fluorescent antibody and immunoassay rapid diagnostics are available for detection of viral antigen in nasopharyngeal or tracheal aspirate, nasopharyngeal swab, or other respiratory secretions. Recently, rapid antigen assays have largely been replaced by PCR, which is preferred because of its higher sensitivity. PCR is commonly performed as a component of a "multiplex" assay that simultaneously tests for multiple viral pathogens.[821]

Treatment of RSV infection is largely supportive. Ribavirin, a nucleoside analog with in vitro activity against RSV, has been trialed for treatment of RSV bronchiolitis, however, while some studies have shown benefit,[822] most have not.[823–825] Aerosolized ribavirin is not recommended for routine use for RSV respiratory infection in infants due to the lack of clear benefit along with concerns about cost and safety.[241] Corticosteroids are not effective in the treatment of RSV infection.

Prevention

Prevention efforts have focused on passive and active immunization. When given as monthly infusions, RSV-specific immunoglobulin was shown to be effective in preventing lower respiratory tract infections, hospitalizations, days in hospital, and days in the intensive care unit in infants with cardiac disease, bronchopulmonary dysplasia, and prematurity.[826,827] Subsequently, palivizumab, a humanized mouse monoclonal antibody was developed which showed similar protection against severe RSV disease. In a randomized, placebo-controlled trial of 1502 infants with prematurity or bronchopulmonary dysplasia, infants receiving monthly injections of palivizumab had a 55% reduction in hospitalizations due to RSV.[828] Palivizumab is given as an intramuscular injection and it does not interfere with live virus vaccines. Current recommendations by the American Academy of Pediatrics (AAP) for the use of palivizumab are summarized in Box 34.4.[306] Additional preventive measures for high-risk infants include minimizing exposure to tobacco smoke, avoidance of crowds and situations in which exposure to infected individuals cannot be controlled, careful hand hygiene by caregivers, vaccination against influenza beginning at 6 months of age, and restriction of participation in childcare during the RSV season whenever feasible. RSV vaccines for both infant and maternal immunization are under active investigation.[829–832]

• BOX 34.4 Guidelines for Prophylaxis in Preterm Infants at the Start of the Respiratory Syncytial Virus Season

- Infants born at ≤29 weeks' gestation who are younger than 12 months at the start of the respiratory syncytial virus (RSV) season. (Monthly prophylaxis should be discontinued if an infant is hospitalized with documented RSV disease.)
- In the first year of life, palivizumab is recommended for premature infants with chronic lung disease of prematurity, defined as birth at <32 weeks' gestation and a requirement for more than 21% oxygen for at least 28 days after birth.
- Clinicians may administer palivizumab prophylaxis in the first year of life to certain infants with hemodynamically significant heart disease (consultation with a pediatric cardiologist is recommended).
- Clinicians may administer up to a maximum of five monthly doses of palivizumab (15 mg/kg per dose) during the RSV season to infants who qualify for prophylaxis in the first year of life (monthly prophylaxis should be discontinued if an infant is hospitalized with documented RSV disease).
- Palivizumab is not recommended in the second year of life except for children with chronic lung disease of prematurity who continue to require medical interventions such as supplemental oxygen, diuretics, and/or corticosteroids.
- Palivizumab may be considered in infants with pulmonary abnormalities, neuromuscular diseases, or profoundly immunocompromised states (including heart transplant in children younger than 2 years) or in Alaskan Native or other remote Native American populations in which costs associated with medical transport are high. Palivizumab therapy is not recommended for children with Down syndrome.

Nosocomial spread of RSV and other respiratory viruses can be minimized by emphasis on hand washing by care providers between contacts with patients. Without additional special precautions, an attack rate of approximately 26% has been observed.[833] Use of standard contact precautions, such as cohort nursing and the use of gowns and gloves for all contacts with RSV-infected children, can reduce the risk of nosocomial RSV infection to 9.5%.[833] Palivizumab has been reported to be a useful adjunct in the control of nursery-associated outbreaks of RSV infection.[809,834,835]

Influenza

Pregnant women are at increased risk for requiring hospitalization due to complications from influenza infection, particularly during the third trimester and if they have comorbid conditions such as diabetes and chronic pulmonary disease.[836] In the 2009 H1N1 pandemic, pregnant women accounted for 5% of the influenza-related deaths even though they were only 1% of the population.[837] Infants born to women with influenza disease during pregnancy are more likely to be premature and be admitted to the intensive care unit.[838,839] Among children who acquire influenza, the highest rates of hospitalization and mortality are in infants less than 6 months of age.[836] Oseltamivir, a neuraminidase inhibitor, is approved for infants down to 2 weeks of age and should be administered within 48 hours of symptom onset for the greatest benefit (Table 34.2).[241,840] Maternal influenza immunization during pregnancy is effective for preventing influenza disease and hospitalization in infants in the first 6 months of life.[841,842]

SARS-CoV-2

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a positive-sense RNA virus that was first identified in late 2019 and spread rapidly to cause a global pandemic. Like its close phylogenetic relatives, the SARS-CoV-1 and Middle Eastern respiratory syndrome (MERS) viruses, SARS-CoV-2 is thought to have arisen in humans via zoonotic transmission.[843,844] Infection may lead to a spectrum of disease, but the classic clinical syndrome known as coronavirus disease-19 (COVID-19) consists of severe viral pneumonia that is often associated with an exaggerated inflammatory response, organ dysfunction, and coagulopathy. While infants and children on average experience milder disease than adults, infants have a slightly higher risk of severe disease and mortality than do older children.[845]

For the developing fetus, risk is primarily related to the sequelae of severe maternal illness. Perhaps because viremia in SARS-CoV2 infection is unusual, congenital infection due to transmission in utero appears to be exceedingly rare and has not been demonstrated to cause developmental abnormalities.[846] Women who are pregnant and develop COVID-19 have higher rates of ICU admission and mortality compared to non-pregnant women.[847] Fetal loss in the setting of acute COVID-19 has been reported,[848] although cohort studies are conflicted, suggesting only a small increased risk of fetal demise, if any.[849, 849a,849b] Infection later in pregnancy is, however, associated with approximately 1.5 times higher risk of preterm birth.[850–852] There do not appear to be deleterious consequences of maternal steroids for COVID-19 on the fetus, nor are steroids to hasten fetal lung development contraindicated in acute maternal COVID-19.[853]

Intrapartum transmission is rare with appropriate isolation measures (such as maternal masking) and occurs in an estimated 2% of deliveries to SARS-CoV-2 infected mothers.[854] The mechanism of transmission in this setting may be via respiratory droplets or fecal shedding[855] as virus is very rarely found in placenta or vaginal swabs.[856] For babies born to mothers with acute COVID-19, current CDC guidelines recommend SARS-CoV-2 testing at 24 and 48 hours of life via RT-PCR from nasal, nasopharyngeal, or oropharyngeal swab.[857] Testing of placental tissue or amniotic fluid for SARS-CoV-2 by PCR does not appear to be useful for predicting infection of the newborn.[858] In 70% of infected infants, transmission appears to occur after hospital discharge and primarily via respiratory secretions and droplets, as in older children and adults.[859,860] There is no evidence that breast milk is a significant source of SARS-CoV-2 infection.[861,862]

While asymptomatic or mild COVID-19 is more likely in infants than adults, severe disease and mortality do occur. Age less than 1 month is associated with a threefold higher risk of critical care admission, although it is unclear if this represents more severe disease or higher caution among providers.[863] The most common symptoms in infants with symptomatic acute COVID-19 include respiratory distress (53%), fever (44%), gastrointestinal (36%), neurologic (19%), and hemodynamic (10%).[860] Laboratory abnormalities include leukocytosis, thrombocytopenia, elevated lactate (55%) and C-reactive protein (29%).[859,864] While older children are at risk of a post-COVID-19 inflammatory disease known as multisystem inflammatory syndrome in children (MIS-C), there have been only case reports of older infants[865] and no descriptions in neonates. Treatment of acute COVID-19 is largely supportive as the mainstays of therapy in older patients, including remdesivir, dexamethasone, and monoclonal antibody therapy, have not been evaluated in infants.[860]

Prevention of transmission of SARS-CoV-2 to newborns is therefore critical. The AAP has established interim guidelines for the care of infants born to mothers with acute COVID-19.[866] These allow for rooming-in between infected mothers and their infants with maternal mask use and hand hygiene prior to contact. Early data indicate that vaccination using mRNA vaccines against SARS-CoV-2 is safe and effective in pregnant women, with protective antibodies transferred to the fetus.[867,868] Infants born to mothers who developed COVID-19 more than 14 days before delivery have lower infection rates compared to those born to mothers who developed COVID-19 closer to delivery, arguing for a protective role of maternal antibodies.[850] Vaccination of pregnant mothers is an important tool for limiting COVID-19 in infants.

Adenoviruses

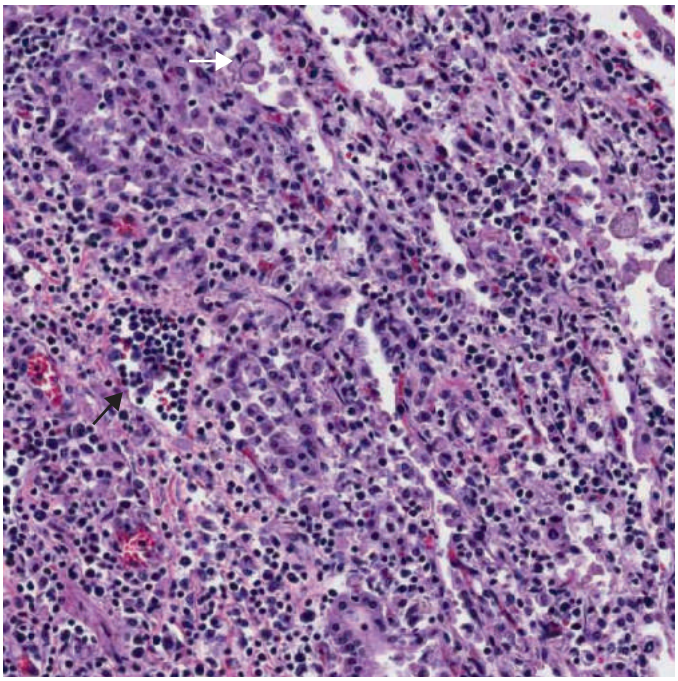
Epidemiology

Adenoviruses are medium-sized, nonenveloped viruses composed of a nucleocapsid and a double-stranded linear DNA genome. There are seven species (A to G) of adenovirus described in humans, comprising over 80 serotypes, and individual serotypes are commonly associated with specific human clinical syndromes as well as outbreaks. The most common clinical syndromes (and associated serotypes) include respiratory tract disease (Ad 1, 2, and 3), gastroenteritis (Ad 40 and 41), and keratoconjunctivitis (Ad 8).[869] Adenoviruses cause 5% to 10% of upper respiratory tract infections in febrile children and many infections in adults as well.[870] Whereas in most infants they cause primary infection, adenoviruses achieve latency and reactivation is a common problem in immunocompromised patients—particularly hematopoietic stem cell and solid organ transplant recipients. Adenovirus infections occur year-round, but they are slightly more frequent in late winter and spring.

Although a few case reports document adenovirus detection in the placenta or fetal tissue after intrauterine demise, the paucity of such cases argues against a significant role in congenital anomalies or fetal disease.[871–873] In contrast, neonatal disease is a well-described phenomenon and is often quite severe, carrying a mortality of nearly 50%.[874,875] Antenatal steroids and the presence of bronchopulmonary dysplasia increased risk of adenoviral pneumonia in one study of NICU babies.[876] Another study identified prolonged rupture of membranes, history of maternal illness, vaginal mode of delivery, and onset of illness within the first 10 days of life as risk factors for severe disease.[877] Maternal antibody appears to confer significant protection against serotype-specific infection for up to 6 to 12 months.[878,879]

Clinical Presentation

Adenovirus infection in newborns most commonly involves pulmonary (Fig. 34.9) or gastrointestinal symptoms and disseminated disease is common.[877,880] Clinical findings in various case reports and case series have included lethargy, fever or hypothermia, anorexia, apnea, hepatomegaly, bleeding, and hemophagocytic lymphohistiocytosis.[881–886] Laboratory abnormalities include thrombocytopenia, coagulopathy, and hepatitis. Acquisition of infection from the mother via vaginal delivery is the presumed mode of transmission in most cases, although transplacental spread has also been implicated. Adenovirus has been identified as a potential cause of chorioamnionitis and premature birth.[887,888] Congenital adenovirus infection (species C) has been associated with childhood leukemia in some studies,[889–891] but other studies have failed to confirm this association.[892] Adenoviruses can cause outbreaks particularly in the nursery setting, with well-documented outbreaks of keratoconjunctivitis transmitted during ophthalmologic exams.[893,894] In older infants, intussusception has a significant association with adenoviral infection of the GI tract.[895]



• **Fig. 34.9** Postmortem histologic analysis from a newborn who died of disseminated adenovirus infection at 2 weeks of age. Hematoxylin and eosin stain of lung demonstrating inflammatory infiltrates *(black arrow)* and intranuclear inclusions *(white arrow)*. This infant had a viral sepsis syndrome characterized by hepatic failure, disseminated intravascular coagulation, and pneumonitis from adenovirus infection presumed to have been acquired intrapartum.

Management

There are no FDA-approved medications for the treatment of adenovirus infection in infants. Cidofovir has become the standard treatment for severe and disseminated adenovirus infections in adults and is becoming widespread as an off-label treatment in pediatrics.[896–898] However, experience with this medication in newborns remains very limited. Intravenous ribavirin use has been described in a few cases,[899,900] but in vitro data argue against significant efficacy for adenovirus infections.[901]

Prevention

Adenoviruses are hardy and resistant to inactivation by physical and chemical methods that kill most viruses, adding to the challenge of hospital infection control during outbreaks. Nosocomial outbreaks have highlighted the importance of careful isolation measures to prevent spread, including sterilization of instruments used in retinopathy of prematurity examinations.[893,902] Adenovirus vaccines are available for use in military personnel but are not offered to the general public.[903]

Gastroenteritis Viruses

Rotavirus

Rotaviruses are historically the most important of the viruses that cause diarrhea from the perspective of the neonatal nursery. This group, with at least four serotypes, is responsible for a large proportion of diarrheal illness in infants aged 6 to 24 months.[904–907] Although the overall burden of rotavirus disease has been reduced substantially by the licensure of several rotavirus vaccines for use in the routine childhood immunization series,[908,909] rotavirus continues to produce disease in infants, particularly in parts of the world where vaccine is not available. While nursery-acquired outbreaks do occur, epidemiologic studies show that rotavirus causes mild or asymptomatic infection in infants younger than 2 months, perhaps due to less virulent strains.[910] Two studies performed in nurseries in Sydney, Australia, and London before widespread vaccination found that 30% to 50% of 5-day-old babies shed the virus, although 90% of the infants were asymptomatic.[911,912] Treatment is supportive and systemic illness is extremely rare. Many NICUs delay rotavirus vaccination for preterm infants until they are discharged due to fears of nosocomial spread of vaccine-strain virus.[913] However, available evidence argues that immunization of preterm infants with live rotavirus vaccines while in the NICU is safe and effective.[914,915]

Norovirus

Noroviruses are a genetically diverse group of single-stranded, positive-sense RNA, non-enveloped viruses. Noroviruses are the most common cause of gastrointestinal disease worldwide.[916,917] Similar to rotavirus, norovirus appears to cause less severe disease in infants less than 6 months of age, perhaps due to protective maternal antibodies.[918,919] Outbreaks of norovirus infection have been described in the NICU setting.[920,921] IUGR infants have been observed to be more likely to shed norovirus and other enteric viruses than infants who are appropriate for gestational age.[922] In addition, infection may have a variety of presentations including apnea and a sepsis-like picture.[921] Therapy is supportive.

Human Herpesviruses 6, 7, and 8

Human Herpesvirus 6 and 7

HHV-6 and HHV-7 are closely related β-herpesviruses that are ubiquitous in humans and typically cause infection in the first 2 years of life. HHV-6 was isolated in tissue culture in 1986 from peripheral blood leukocytes of patients with both lymphoproliferative disorders and HIV infection.[923,924] For several years after its discovery, its role in disease was unclear, but it is now known to be the major etiologic agent of roseola infantum (exanthem subitum) and has been associated with febrile seizures. HHV-6 is sub classified as either variant A or variant B, with HHV-6B typically associated with roseola infantum.[925] A study in Ugandan infants demonstrated that more than 75% of infants acquire HHV-6 infection in the first year of life.[926]

HHV-7 is highly related to HHV-6 and, like HHV-6, is responsible for roseola infantum.[927] As with HHV-6, infection with HHV-7 appears to be ubiquitous, although infection appears to be acquired somewhat later in life than HHV-6 infection.[928,929] Approximately 40% to 45% of children have antibodies to HHV-7 by 2 years of age, and 70% of children are seropositive by 6 years of age.

Saliva is assumed to be the main vehicle for transmission among children and adults.[930] Intrauterine infection can be the result of primary HHV-6 acquisition or maternal reactivation of latent virus. In addition, the HHV-6 genome is capable of integrating into human chromosomes, resulting in a high amount of detectable viral DNA by PCR that may not in fact represent replicating virus. Congenital HHV-6 transmission was first reported in a study of 5638 umbilical cord blood samples in which 57 samples (1%) had HHV-6 DNA by PCR, but none had HHV-7.[931] Another study including 305 umbilical cord blood samples identified HHV-6 DNA by PCR in 1.6% of samples.[932] Of note, all newborns were asymptomatic, and it is not clear if vertically transmitted DNA reactivates to produce bona fide virus particles. Indeed, vertical transmission of HHV-6 most often (90% of cases) occurs not from actively replicating virus but because of the germline passage of maternal HHV-6 through chromosomally integrated viral DNA.[933,934] The clinical consequences of such transmission and the differences between germline transmission and the more common postnatal acquisition of HHV-6 in early childhood remain unknown. Recent evidence suggests that there may be long-term neurodevelopmental concerns in infants who have germline transmission of viral DNA.[935] The HHV-6 genome appears to integrate into the telomere—a chromosomal component important in cellular aging and in cancer—suggesting there may be long-term consequences associated with vertical germline transmission.[936,937]

Peripartum and postnatal vertical transmission of HHV-6 and HHV-7 have been postulated[938] but not confirmed. In addition to saliva, both viruses can be found by PCR in cervical secretions[939–941] and breast milk,[942,943] but serological analysis in these studies suggest these are rare sources of peripartum transmission. With the advent of rapid molecular diagnostic platforms, it is increasingly common to detect HHV-6 nucleic acid in samples—particularly CSF—and identifying pathologic infection can be difficult. As many as 10% of infants with germline transmission of chromosomally integrated HHV-6 show early reactivation without symptoms.[931] Infants who acquire HHV-6 (as compared to older children) appear to have a higher incidence of symptomatic infection, including seizures and persistent viremia.[944,945] There are no approved antiviral medications to treat HHV-6. Although ganciclovir, foscarnet, and cidofovir have showed in vitro efficacy, small trials have not shown clinical benefit.[946]

Human Herpesvirus 8

In 1994 a novel herpesvirus, Kaposi sarcoma–associated herpesvirus (KSHV, also known as HHV-8) was identified in patients with AIDS-associated Kaposi sarcoma (KS).[947] This virus was assigned to the γ-herpesvirus subfamily of the *Herpesviridae*, due to its molecular and sequence similarity to the other prototypical γ-herpesvirus, EBV. Subsequent studies have linked KSHV to both AIDS-associated KS and the endemic forms of KS that are prevalent in elderly Mediterranean men. Cross-sectional studies of the seroprevalence of KSHV in children and adolescents in the United States reveal prevalence estimates ranging from 1% to 26%.[948,949] In sub-Saharan Africa, prevalence in children is even higher, approaching 60% in some studies[950] and are associated, in some cases, with single-gene inborn errors of immunity.[951] Postnatal transmission is thought to be primarily

via saliva.[952,953] There are reports of infections in infants that suggest possible vertical transmission, but congenital infection has not been confirmed by PCR techniques.[954] KSHV can also be transmitted by blood transfusion;[955] this observation suggests that transplacental transmission is at least theoretically feasible. In a study of 89 KSHV-seropositive women, KSHV DNA was detected in the peripheral blood mononuclear cells of 13 mothers (14.6%), and in the peripheral blood mononuclear cells of 2 of 89 samples drawn at birth from neonates born to these mothers. KSHV has also been demonstrated to infect placental cells, suggesting that a transplacental route of infection is feasible.[956] These findings suggest that KSHV can be transmitted perinatally, but infrequently.[957] Primary KSHV infection in immunocompetent children typically consists of mild illness with fever and rash as predominant symptoms.[958] Additional information on the epidemiology and modes of transmission of this pathogen, particularly in the prenatal and intrapartum period, is needed.

Epstein-Barr Virus

Epstein-Barr virus (EBV) is the causative agent of infectious mononucleosis and is associated with nasopharyngeal carcinoma, Burkitt lymphoma, and lymphoproliferative disease in immunocompromised patients.[959] Diagnosis based on classic evolution of early and late antibodies may be unreliable in young infants, therefore PCR is optimal to detect active infection.[960,961] Primary EBV infection during pregnancy appears to be rare,[962] and it is not clear whether transplacental passage of EBV occurs. It has been postulated that high-titer antibodies cross the placenta and protect the fetus from hematogenous transmission of virus in women who reactivate EBV during pregnancy.[963] While small case series have reported evidence of EBV infection associated with intrauterine fetal demise or developmental abnormalities,[964–968] large studies have not demonstrated a significant association of either primary or reactivated EBV with these outcomes.[969,970] Late congenital transmission appears to occur in 2% to 3% of pregnancies. In a study of 67 seropositive postpartum women and their neonates, 3% (2/67) of neonates were positive for EBV DNA by nested PCP.[971] Similarly, a 2% congenital EBV transmission rate has been described in HIV-infected newborns.[972] Early postnatal acquisition is uncommon based on serologic studies. Seropositivity increases sharply around 8 months of age, although the protective effect of maternal antibodies is a subject of debate.[960,973,974] Primary infection in newborns is almost always asymptomatic, although there are case reports of severe disease in premature infants.[968] High viral load in childhood is associated with increased likelihood of development of Burkitt lymphoma.[975]

Suggested Readings

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35

Congenital Toxoplasmosis, Syphilis, Malaria, and Tuberculosis

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KEY POINTS

- Maternal transmission of *Toxoplasma gondii* in the first trimester causes the greatest damage to the fetus, while infections later in pregnancy are more readily transmissible to the fetus.
- No neonate should be discharged from a birth hospital without documentation of maternal syphilis testing. If maternal syphilis testing is positive, adequacy and timing of maternal treatment, comparison of infant and maternal serology, and infant's examination must be taken into consideration.
- Symptoms of congenital tuberculosis are protean but should be considered in any ill neonate whose mother is from a TB-endemic area.
- Symptoms of congenital malaria are nonspecific, though fever is universal. Parasitemia with *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale*, or *Plasmodium malariae* has been reported with congenital malaria and should be considered in any infant whose mother is from a malaria-endemic region.

Congenital Toxoplasmosis

Epidemiology

Toxoplasmosis in the fetus and newborn is due to maternal infection with the parasite *Toxoplasma gondii*. This obligate protozoan parasite is ubiquitous in nature. While the cat is the definitive host, *T. gondii* can infect most warm-blooded animals. Human seroprevalence varies both geographically and by socioeconomic status. In the United States, seroprevalence studies obtained from National Health and Nutrition Examination Survey (NHANES) in 2009–2010 show a continuous decrease in seroprevalence, being around 9% for women of childbearing age compared to prior surveys conducted in 1988–1994 and 1999–2004.[1] Accordingly, over 90% of women of childbearing age in the U.S. are susceptible to primary infection with *T. gondii* during pregnancy, with risk of transmission to the fetus. Seroprevalence is increased for those born outside the United States, living below the poverty level, and with a lower level of education. Global data on seroprevalence show the highest rates in Latin America, parts of Eastern/Central Europe, the Middle East, parts of Southeast Asia, and Africa.[2]

However, seroprevalence rates differ considerably from one country to another, from one region of a country to another, and even from one ethnic group to another in the same region. These widely disparate seroprevalence rates among different adult populations throughout the world have been explained by differences in eating and sanitation practices that contribute to acquisition of infection. Eating goods that are undercooked, raw, cured, dried, as well as meat, raw oysters, clams/mussels, or unwashed raw fruits and vegetables, and drinking unpasteurized goat's milk, working with meat, having three or more kittens, and even certain climactic conditions have been associated with higher risks of infection.[3] Waterborne transmission, particularly from untreated well water, has been noted.[3,4]

Older studies through immunoglobulin (Ig) M screening of newborn blood specimens collected on filter paper showed the prevalence of congenital infection in Massachusetts and New Hampshire to be 0.08 per 1000 births.[5] Higher rates are found in other countries, ranging from 1 to 20 per 10,000 births, but are difficult to compare due to different methodology. Few countries have routine screening of pregnant women.[6] In Massachusetts, a case-control study involving 14 years of newborn screening for congenital toxoplasmosis found that the mother's birth outside the United States, particularly in Cambodia and Laos, as well as the mother's educational level and higher gravidity, were strongly predictive of congenital infection.[7] With approximately 4 million live births annually in the United States, there are an estimated minimum 400 babies born each year with congenital toxoplasmosis,[8] although the true incidence is likely much higher as the majority of infected newborns are asymptomatic at birth and therefore not diagnosed with maternal newborn screening.

Pathogenesis

T. gondii exists in three forms:

1. Sporozoite contained within oocysts that are in the cat's intestinal tract and are shed in cat feces
2. A tachyzoite or endozoite that is the proliferative form and was formerly referred to as a *trophozoite*
3. A tissue cyst that has an intracystic form termed *cystozoite* or *bradyzoite*

Nonfeline mammals or birds ingest infective oocysts from contaminated soil. Tissue cysts then accumulate in the organs and skeletal muscle of these animals. The possible routes of transmission from animal to human are direct contact with infected cat feces, ingestion of undercooked meat containing infective cysts, raw shellfish, and ingestion of fruits, vegetables, or water that

have been in contaminated soil. Rarer methods of transmission can be from infected blood transfusions or organ transplantation.[9] Congenital infection results from placental infection and subsequent hematogenous spread to the fetus.

Infection of the fetus occurs because of maternal primary infection during pregnancy or, rarely, just before conception.[10] Reactivation of latent *T. gondii* infection during pregnancy does not lead to fetal infection, except among immunocompromised women such as those infected with human immunodeficiency virus (HIV) or those receiving chemotherapy or other immunosuppressive therapy as with systemic lupus erythematosis.[11–16] Even under those circumstances, the risk is low. In addition, maternal reinfection with a new, more virulent strain can result in congenital toxoplasmosis.[17] Severity of disease is related to both host and parasite factors as well as stage of pregnancy.[18,19]

Infection of the fetus occurs transplacentally during maternal parasitemia. Placental infection is an important intermediary step, and up to 16 weeks may elapse between placental infection and subsequent infection of the fetus. This time delay has been termed the *prenatal incubation period*[20] and explains the success of therapeutic intervention during pregnancy. Infections in twins show similar clinical manifestations in monozygotic twins, whereas discrepancies in clinical findings are common in dizygotic twins.[21–23]

Overall, approximately 40% of infants born to mothers who acquired toxoplasmosis during pregnancy are congenitally infected with *T. gondii*. The rate of vertical transmission varies according to the trimester in which the mother became infected, with fetal infection rates increasing as pregnancy advances.[20,24,25] Only 15% of infants are infected with maternal infection occurring in the first trimester, whereas transmission rates are 30% and 60% with maternal infection in the second and third trimesters, respectively. The severity of clinical manifestations is greatest, however, when maternal infection is acquired early in pregnancy. Maternal infection in the first trimester results in severe disease in as many as 40% of infected fetuses, and in stillbirth or perinatal death in an additional 35% of infants.[24] Conversely, maternal infection in the third trimester is rarely if ever associated with severe fetal disease or stillbirth, and approximately 90% of infants in such situations have subclinical infection.[24]

Postnatally, transmission of *T. gondii* can occur from transfusion of blood or blood products or from transplantation of organ or bone marrow from a seropositive donor with latent infection. Although the organism has been detected in human milk, transmission by breastfeeding has not been documented.

Clinical Presentation

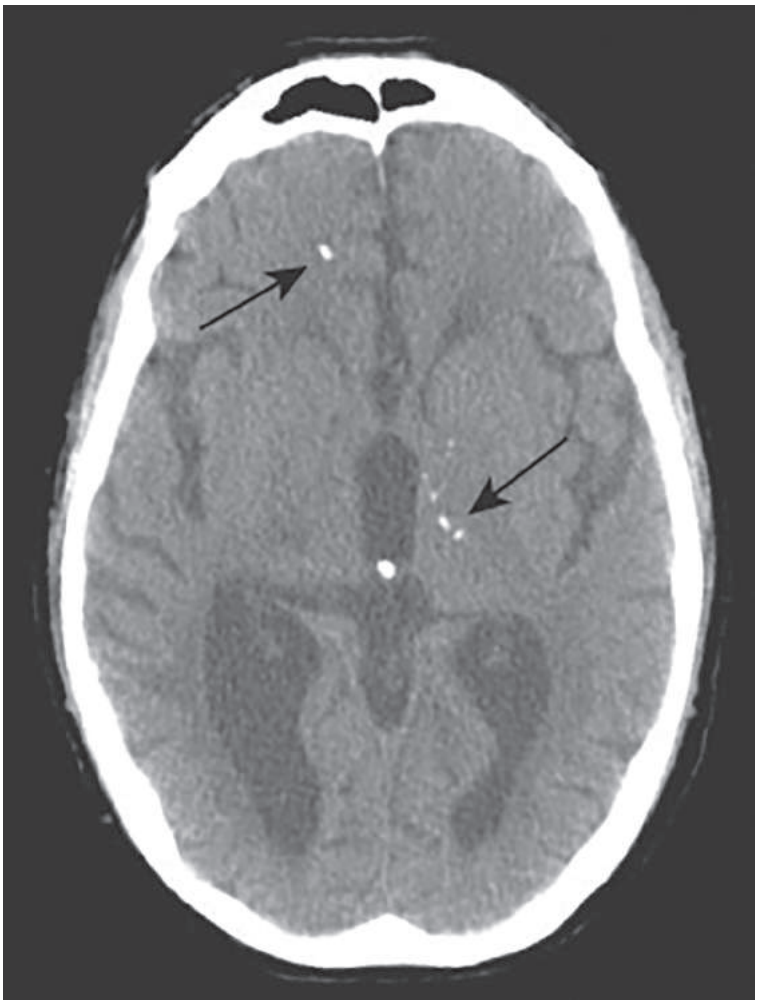
Most newborns with congenital toxoplasmosis are asymptomatic with clinically apparent disease only present in approximately 10% to 25% of infected infants,[5,26,27] although thorough evaluation may demonstrate eye or neurologic abnormalities in approximately 20% of cases. The clinical manifestations of toxoplasmosis are often indistinguishable from those seen with other congenital infections, such as cytomegalovirus or congenital syphilis. Approximately one-third of infants have a generalized form of toxoplasmosis that principally involves organs of the reticuloendothelial system. The abnormalities include temperature instability, hepatosplenomegaly, jaundice, pneumonitis, generalized lymphadenopathy, rash, chorioretinitis, anemia, thrombocytopenia, eosinophilia, and abnormal cerebrospinal fluid (CSF) indices (Table 35.1).[28] The other two-thirds of infected infants principally manifest neurologic disease.

TABLE 35.1 Clinical Findings Among Infants With Congenital Toxoplasmosis

Finding	Infants with findings (%): Neurologic Disease* (108 Cases)	Infants with findings (%): Generalized Disease† (44 Cases)
Chorioretinitis	94	66
Abnormal cerebrospinal fluid	55	84
Anemia	51	77
Convulsions	50	18
Intracranial calcification	50	4
Jaundice	29	80
Hydrocephalus	28	0
Fever	25	77
Splenomegaly	21	90
Lymphadenopathy	17	68
Hepatomegaly	17	77
Vomiting	16	48
Microcephaly	13	0
Diarrhea	6	25
Cataracts	5	0
Eosinophilia	4	18
Abnormal bleeding	3	18
Hypothermia	2	20
Glaucoma	2	0
Optic atrophy	2	0
Microphthalmia	2	0
Rash	1	25
Pneumonitis	0	41

*Infants with otherwise undiagnosed central nervous system diseases in the first year of life.
†Infants with otherwise undiagnosed nonneurologic diseases during the first 2 months of life.
Adapted from Remington JS, McLeod R, Thulliez P, Desmonts G. Toxoplasmosis. In: Remington JS, Klein JO, Wilson CV, Nizet V, eds. *Infectious Diseases of the Fetus and Newborn Infant.* 7th ed. Philadelphia: Elsevier; 2011.

Central nervous system involvement is the hallmark of congenital *T. gondii* infection.[20,29,30] Chorioretinitis, intracranial calcifications (Fig. 35.1), and hydrocephalus are the most characteristic findings, occurring in approximately 86%, 37%, and 20% of symptomatic infants, respectively.[20,28] This constellation of findings has been referred to as the *classic triad of congenital toxoplasmosis;* its presence should alert the clinician to the diagnosis. Intracranial calcifications may be single or multiple, but typically are generalized and located in the caudate nucleus, choroid plexus, meninges, and subependyma;[31] they also may occur periventricularly, as in cytomegalovirus infection. Calcifications are visualized best by computed tomography (CT) but are often detected on ultrasonography as well. Intracranial calcifications may resolve



• **Fig. 35.1** Scattered intracranial calcifications in the right frontal cortex and left thalamic area with ventriculomegaly observed in congenital toxoplasmosis.

with appropriate antimicrobial therapy.[29] Hydrocephalus may be the only manifestation of disease; it results from the extensive periaqueductal and periventricular vasculitis with necrosis that causes obstruction of the ventricular system. Ventriculoperitoneal shunting is often required.[29,32] Abnormalities of the CSF are common; characteristically, they consist of lymphocytic pleocytosis and a markedly elevated protein content. Microcephaly, when present, indicates severe brain injury. Hypothermia and hyperthermia may occur secondary to hypothalamic involvement. *T. gondii* has been detected in the inner ear and mastoid, with the associated inflammation resulting in deafness. An ascending flaccid paralysis with myelitis has also been reported.[33]

Chorioretinitis secondary to congenital toxoplasmosis can manifest at any age, and is often delayed into the second and third decades of life. It usually manifests as strabismus in infants. Defects in visual acuity are more common in older children who had never received treatment. Typically, the eye lesion consists of a focal necrotizing retinitis that is often bilateral with involvement of the macula and even the optic nerve. Complications include blindness, iridocyclitis, and cataracts.[34,35]

Other less common manifestations of congenital toxoplasmosis are nonimmune hydrops fetalis, myocarditis, nephrotic syndrome, and immunoglobulin abnormalities with both hypergammaglobulinemia and hypogammaglobulinemia described. Bony abnormalities consisting of metaphyseal lucencies similar to those seen in congenital syphilis have also been reported.[36] A variety of endocrine abnormalities may occur, including hypothyroidism, diabetes insipidus,[37,38] precocious puberty, and growth hormone deficiency.

Evaluation

Attempts to diagnose congenital toxoplasmosis occur either during pregnancy as part of a surveillance protocol, due to maternal disease or abnormal fetal findings, or, after birth, due to concern for infection. The testing methods vary depending upon the scenario and can be complicated. While commercial laboratories are able to perform some assays, reference laboratories are needed for more intensive evaluation and can be very useful with the availability of their consultative services such as the Palo Alto Medical Foundation *Toxoplasma* Serology Laboratory (PAMF-TSL; Email: toxolab@pamf.org, telephone: 650-853-4828, http:www.pamf.org/serology/).

Serologic assays for measurement of antibodies to *T. gondii* in serum and body fluids are the most widely used methods of diagnosing toxoplasmosis in a pregnant woman and the fetus or newborn, but can be difficult to interpret.[24,39–46] Most commercial laboratories can test for *Toxoplasma* IgG and IgM,

although *Toxoplasma* IgM assays performed in these laboratories are fraught with low sensitivity and, importantly, false positive results. The more commonly used tests that detect *T. gondii*-specific IgG antibodies are IgG enzyme-linked immunosorbent assay (ELISA), indirect immunofluorescent antibody test, and direct agglutination. The Sabin-Feldman dye test is considered the gold standard but requires live organisms, and, accordingly, is performed at reference laboratories.[47] If a pregnant woman is known to be seronegative prior to pregnancy and followed serially, then these IgG assays are useful to document seroconversion during pregnancy and risk of the fetus. However, most countries, including the United States, do not have routine prenatal screening, in which case it is important to decipher whether a positive test represents recent infection or chronic infection without a risk to the fetus. Reference laboratories can perform a differential agglutination test to differentiate acute from chronic maternal infection. This test compares the IgG serologic titer obtained with the use of formalin-fixed tachyzoites (HS antigen) with those obtained with acetone- or methanol-fixed tachyzoites (AC antigen). The latter preparation contains stage-specific *T. gondii* antigens that are recognized by IgG antibodies only during early infection. An additional assay to assist in ruling out maternal infection acquired in the first 3 months of pregnancy is the IgG avidity test performed by the ELISA technique.[44] This test is based on the principle that, although the antibody-binding avidity or affinity for an antigen is initially low after primary antigenic stimulation, IgG antibodies that are present from previous antigenic stimulation are usually of high avidity. Therefore, a high-avidity result in the first trimester would exclude an infection acquired in the previous 12 weeks. Finally, an enzyme-linked immunofiltration assay has been developed that allows some discrimination between IgG antibodies of maternal origin and IgG synthesized by the fetus, as well as identification of antibody subtypes in infected neonates.[48]

Tests that detect *T. gondii*-specific IgM are (1) the double-sandwich IgM ELISA, which has a sensitivity of 75% to 80% and a specificity of 100%;[5] (2) the IgM immunosorbent agglutination assay (ISAGA), which is the most sensitive test but should not be performed on umbilical cord blood because even small quantities of maternal IgM antibodies contaminating the specimen will yield a false-positive result; and (3) the IgM immunofluorescent antibody test. The last test is not recommended because it has a much lower sensitivity than either the IgM ELISA or IgM and it has poor specificity secondary to rheumatoid factors and antinuclear antibodies, contributing to false-positive results. Other tests used in reference laboratories include a *T. gondii*-specific IgA ELISA and IgA immunofiltration assay; a *T. gondii*-specific IgE immunofiltration assay; and IgG, IgM, and IgA immunoblotting tests, which are generally used in comparing samples from the infant and the mother to help determine true infection. Interferon gamma release assays on infants have been used to diagnose congenital infection, but are not available commercially.[47]

Polymerase chain reaction (PCR) analysis has been used successfully to detect *T. gondii* DNA in amniotic fluid, placenta, CSF, brain, urine, and fetal and infant blood.[49–55] PCR performed on amniotic fluid obtained by amniocentesis is the preferred method of confirming in utero infection.[56] False-negative results have been reported, however, and interlaboratory variability in performance of PCR assays has been documented.[52,55] PCR performed on neonatal CSF is recommended for the evaluation of possible central nervous system involvement.

Isolation of *T. gondii* from body fluids and tissues provides definitive evidence of infection. The organism can be isolated from placenta, amniotic fluid, fetal blood obtained by cordocentesis, umbilical cord blood, infant peripheral blood, and CSF by means of intraperitoneal and subcutaneous inoculation into laboratory mice.[20,24,57] Mouse inoculation may require as long as 4 to 6 weeks for demonstration of the parasite. Although it is not a practical method, isolation of the organism should be attempted to confirm a diagnosis and is available at the PAMF-TSL reference laboratory. In addition, tissue culture has been used to isolate *T. gondii* from amniotic fluid.

Histopathologic examination of the placenta and tissues, obtained at postmortem examination or by biopsy from stillborns or infants, should be performed because the specimens may demonstrate the presence of tachyzoites. In addition, tachyzoites have been demonstrated in CSF, ventricular fluid, and aqueous humor by specialized staining techniques.

Because most adults with acquired *T. gondii* infection are asymptomatic, evaluation of the pregnant woman and fetus is usually prompted by either seroconversion or an elevated maternal *Toxoplasma* spp. IgG titer.[58–60] The latter may reflect chronic past infection; therefore, the acuity of the maternal infection is determined serologically with the HS-AC differential agglutination test where agglutination titers to formalin-fixed tachyzoites (HS antigen) are compared with titers against acetone- or methanol-fixed tachyzoites (AC antigen). In general, an acute pattern demonstrates high AC and HS titers, while a nonacute pattern demonstrates high AC titers and low HS titers. This method can differentiate an acute from a remote infection in pregnant women, whereas IgM and IgA antibodies detectable by ELISA or ISAGA are elevated for prolonged periods. If recent maternal infection is documented by an acute pattern on the HS-AC test, seroconversion, or rising IgG antibody titers, the fetus should be evaluated by ultrasonography, and amniotic fluid should be tested for specific *Toxoplasma* spp. DNA with PCR. PCR has supplanted the need for cordocentesis, and a positive result confirms fetal infection.[53] Postnatally, serologic testing of paired maternal and infant sera should be performed at a reliable laboratory that will include assays for *Toxoplasma* spp. IgG, IgM ISAGA and IgA antibodies.[61] Waiting until the newborn is 10 days of age avoids contamination with maternal blood and false positive IgM and IgA results. Subinoculation of placental tissue, amniotic fluid, and umbilical cord blood into mice should be considered. If results of these tests suggest possible infection, the newborn should be evaluated fully with complete blood cell count and platelet determination, liver function tests, CSF evaluation (including tests for IgG and IgM antibodies and PCR),[62] cranial ultrasound or CT imaging of the head, ophthalmologic examination, and hearing evaluation. The presence of neonatal IgM antibody in serum or CSF, or a positive PCR in blood or CSF, indicates congenital infection. In addition, at-risk infants should undergo serologic follow-up to detect rising serum IgG titers during the first year of age, or persistence of IgG antibody beyond 12 to 15 months of age, when most maternal IgG antibody has disappeared.[45,47,63] Uninfected infants show a continuous decline in *T. gondii* IgG titer which usually is gone by 7 months of age with no detectable IgM or IgA antibodies.

Low IgG titers and an HS-AC differential agglutination test that indicate remote maternal infection do not require further evaluation of the mother or infant unless the mother is severely immunosuppressed. Because fetal infection has occurred during chronic *T. gondii* infection in very immunosuppressed women

such as those with poorly controlled HIV infection, their infants should be evaluated serologically at birth for evidence of congenital infection. It has been suggested that HIV-infected pregnant women who have low $CD4^+$ T lymphocyte counts-and who are seropositive for *T. gondii* antibody-receive prophylaxis to prevent fetal infection.[24,64] However, insufficient data currently are available to recommend that such therapy be given routinely for this indication. Nevertheless, if such women previously have had toxoplasma encephalitis, prophylaxis with pyrimethamine, sulfadiazine, and leucovorin (folinic acid) should be considered.[65]

Management

Fetuses and infants younger than 1 year who are infected with *T. gondii* should receive specific therapy effective against this congenital pathogen, even if they have no clinical signs of disease.[24,29,49,58,59,66–75] Compared with untreated historical controls, the outcome is improved substantially by neonatal treatment. The effectiveness of maternal and fetal treatment is less clear. Spiramycin has been used in pregnant women with acute toxoplasmosis to reduce transplacental transmission of *T. gondii*. If fetal infection is confirmed after the 17th week of pregnancy, however, treatment with pyrimethamine, sulfadiazine, and folinic acid is recommended. Prenatal treatment of congenital toxoplasmosis is believed to reduce the clinical severity of infection in the newborn while shifting the disease to a more subclinical form. This may ameliorate the long-term neurologic complications that are commonly seen among infants who have clinical manifestations in the neonatal period. A meta analysis of the effectiveness of prenatal treatment of toxoplasmosis infection found no evidence that such treatment significantly decreased clinical manifestations of disease in infected infants.[76] However, other studies in Brazil, Germany, and France suggest efficacy.[77,78]

Neonatal treatment has resulted in reductions in sensorineural hearing loss, as well as neurodevelopmental and visual handicaps. Table 35.2 shows the recommended guidelines for the treatment of congenital toxoplasmosis. In infants with congenital toxoplasmosis, the treatment consists of pyrimethamine, sulfadiazine, and folinic acid (leucovorin).[20,29,79] The optimal duration of therapy is not known, although prolonged courses of at least 1 year are preferred. Currently most experts recommend combined treatment until the patient is 1 year old.[20,74,80] Accessing pyrimethamine in the United States has recently become difficult due to extreme cost escalation. Assistance with access is available through http://www.daraprimdirect.com (phone number: 1-877-258-2033).

Complete blood cell and platelet counts must be monitored closely while the patient is receiving therapy because granulocytopenia, thrombocytopenia, and megaloblastic anemia can occur. These parameters usually improve once a higher dose of folinic acid (leucovorin) is administered or pyrimethamine and sulfadiazine are discontinued temporarily. The indications for adjunctive therapy with corticosteroids such as prednisone (0.5 mg/kg twice per day) are CSF protein concentration 1 g/dL or higher and chorioretinitis that threatens vision; corticosteroid

TABLE 35.2 Treatment Guidelines for Toxoplasmosis

Condition	Therapy	Dose (Oral Unless Specified)	Duration
Pregnant woman with acute toxoplasmosis	Spiramycin for first 21 weeks of gestation or until term if fetus not infected	3 g/day, divided twice a day without food	Until fetal infection documented or excluded at 21 weeks; if fetal infection documented, replaced with pyrimethamine, leucovorin, and sulfadiazine (see below)
Pregnant women-fetal infection confirmed (amniotic fluid PCR+)	Pyrimethamine (if fetal infection confirmed after 18th week of gestation or if infection acquired in last few weeks of gestation) *and*	Loading dose: 100 mg/day in two divided doses for 2 days followed by 50 mg/day	Until delivery
	Sulfadiazine *and*	3 g/day divided in	Until delivery
	Folinic acid	5–20 mg/day	Until delivery
Congenital *Toxoplasma gondii* infection in infant	Pyrimethamine *and*	Loading dose: 2 mg/kg/day for 2 days; then 1 mg/kg/day for 6 months; then 1 mg/kg/day on Monday, Wednesday, and Friday each week	1 year
	Sulfadiazine *and*	100 mg/kg/day in 2 daily divided doses	1 year
	Leucovorin (folinic acid)†	5–10 mg 3 times weekly	1 year
	Corticosteroids (prednisone)‡	1 mg/kg/daily in 2 daily divided doses	Until resolution of elevated (≥1 g/dL) CSF protein or active chorioretinitis that threatens vision

†Monitor blood and platelet counts weekly; adjust dosage for megaloblastic anemia, granulocytopenia, or thrombocytopenia.
‡When signs of inflammation or active chorioretinitis have subsided, dose can be tapered and eventually discontinued; use only in conjunction with pyrimethamine, sulfadiazine, and leucovorin.
Data from Boyer KM and Nadipuram SM. Toxoplasmosis. In: Cherry JD, Harrison GJ, Kaplan SL, Steinbach WJ, Hotez PJ, eds. *Feigin and Cherry's Textbook of Pediatric Infectious Diseases*. 8th ed. Philadelphia: Elsevier; 2019.

treatment is continued until either condition resolves. Current therapies are not effective against encysted bradyzoites and, therefore, may not prevent reactivation of chorioretinitis and neurologic disease.

Outcomes

Maternal toxoplasmosis acquired during the first and second trimesters has been associated with stillbirth and perinatal death secondary to severe fetal infection in approximately 35% and 7% of cases, respectively. Among infants born with congenital toxoplasmosis, the mortality rate has been reported to be as high as 12%. In addition, infants with congenital toxoplasmosis are at high risk for ophthalmologic, neurodevelopmental, and audiologic impairments, including mental retardation (87%), seizures (82%), spasticity and palsies (71%), and deafness (15%).[28,29,68,69] Of neonates with subclinical infection, long-term follow-up reveals eye or neurologic disease in as many as 80% to 90% by the time they reach adulthood.[81–85] While data from the United States National Collaborative Treatment Trial show that severity is influenced by host and parasite factors, treatment of neonates with congenital toxoplasmosis early and for 1 year resulted in more favorable outcomes than were reported for untreated infants or infants who were treated for only 1 month.[86]

Prevention

Pregnant women whose serologic status for *T. gondii* is negative or unknown, as well as women who are attempting to conceive, should be educated on the prevention of congenital toxoplasmosis through avoidance of at-risk behaviors that may expose them to cat feces or encysted bradyzoites in raw meat.[87–90] Instructions to wear gloves when changing cat litter boxes or gardening and to wash hands after such activities should be given. Daily changing of cat litter will also decrease the chance of infection, because oocysts are not infective during the first 1 to 2 days after passage. In addition, keeping domestic cats inside, and feeding them commercially prepared foods rather than undercooked meats or wild rodents, reduces the likelihood of their becoming infected and capable of transmitting the infection to a pregnant woman. Oral ingestion of *T. gondii* can be prevented by either cooking meat to well done, smoking it, or curing it in brine, and by washing kitchen surfaces and utensils that come into contact with raw meat. Vegetables and fruits should be washed, and hands and kitchen surfaces should be cleaned after handling fruits, vegetables, and raw meat. Flies and cockroaches may serve as transport hosts for *T. gondii*, so their access to food must be prevented.

Routine serologic screening of women during pregnancy has been an effective means of prevention in such countries as France and Austria, where the incidence of congenital toxoplasmosis is high. No such screening is currently in existence in the United States.[91] However, high-risk women, including those who are immunocompromised, should be screened early in pregnancy. Neonatal screening for IgM antibody has also been advocated so that asymptomatic infants can be detected and treated before neurologic symptoms develop.[92] This strategy, however, has been hampered by the lack of readily available and reliable IgM test kits. Moreover, such screening will not detect the approximately 25% of infected neonates who lack anti-*Toxoplasma* spp. IgM antibody. Further study involving cost analyses is needed to define the best preventive strategy for congenital toxoplasmosis in specific populations, regions, and countries.

Congenital Syphilis

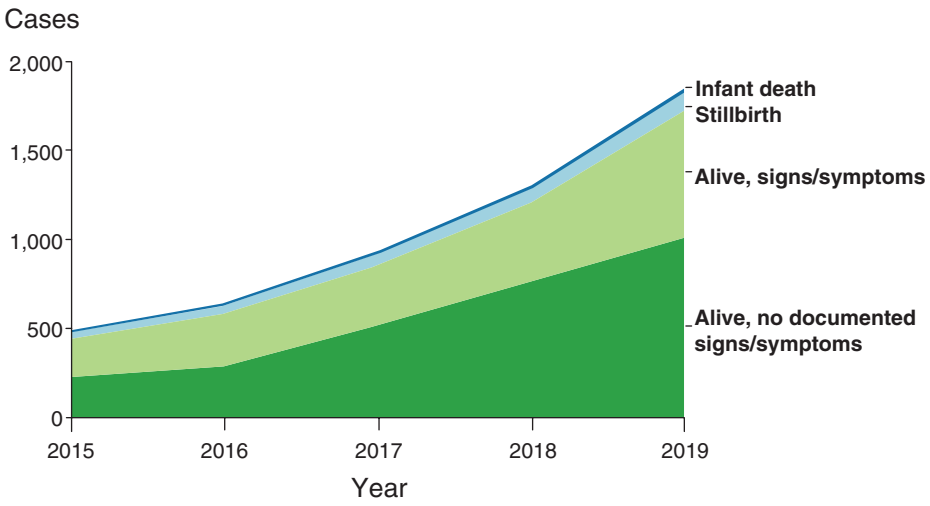
Epidemiology

Congenital syphilis, a result of fetal infection with the spirochete *Treponema pallidum*, remains a major public health problem worldwide. While adults acquire the infection sexually, infants are infected mostly in utero by a transplacental route or possibly during delivery by contact with a genital lesion of an infected mother. When maternal infection is detected during pregnancy, congenital syphilis is both preventable and treatable. However, if infected infants are not identified in a timely fashion, they may experience lifetime consequences. Accordingly, the Centers for Disease Control and Prevention (CDC) recommend that no mother or newborn be discharged from the hospital without the maternal serologic status for syphilis having been documented at least once during the pregnancy, and preferably again at delivery if the mother is at increased risk or lives in a community with high prevalence of syphilis infection.

The incidence of congenital syphilis mirrors the rates of primary and secondary syphilis in women. Overall, congenital syphilis disproportionately affects infants of black women whose prenatal care was inadequate or lacking. Less prenatal care has been associated with increased risk of fetal death that most often occurs by 31 weeks' gestation. From 1999 to 2013 in the United States, neonatal mortality secondary to congenital syphilis was 12/1000 live births, with a case fatality rate of 6.5%. Of the 418 reported deaths, 82% were stillbirths and 89% of the mothers had untreated or inadequately treated syphilis.[93]

During the pre-penicillin era of the 1930s and 1940s in the congenital syphilis clinic of the Harriet Lane Home (Baltimore, MD), 60 to 80 infants and children attended each week for arsenic therapy. Many more were lost to follow-up before completing their 2- to 3-year course of treatment. It was unusual if fewer than three or four new cases were discovered in the general outpatient department each week. Subsequently, the frequency of congenital syphilis declined for several decades, only to increase dramatically in the late 1980s and early 1990s. This increase was fueled by the crack cocaine epidemic with women exchanging drugs for sex with multiple and anonymous partners. At the same time in 1988, the CDC surveillance case definition for reporting cases of congenital syphilis was broadened to include all liveborn and stillborn infants who had reactive serologic tests for syphilis, irrespective of clinical findings, and those delivered to women with untreated or inadequately treated syphilis. This change resulted in a fourfold increase in reported cases of congenital syphilis when compared to the previously used Kaufman criteria that included only symptomatic infants (reviewed in).[94] For unclear reasons, maternal syphilis subsequently declined, and the rate of congenital syphilis decreased from 1991 to 2005 but increased slightly during 2005 to 2008. From 2008 to 2012, the overall rate of congenital syphilis again decreased from 10.5 to 8.4 cases per 100,000 live births, reflecting decreasing trends in primary and secondary syphilis among women in the United States. Since 2012, rates of congenital syphilis increased significantly (8.4 cases per 100,000 live births in 2012 to 48.5 cases per 100,000 live births in 2019). In 2019, there were 1870 cases of congenital syphilis that included 94 stillbirths and 34 infant deaths (Fig. 35.2).[95] The recent increase in syphilis has been attributed to the ongoing opioid epidemic in the United States.

Worldwide, congenital syphilis remains a major cause of fetal and neonatal mortality with more newborns affected by congenital syphilis than by any other neonatal infection.[96] The World Health



• **Fig. 35.2** Reported congenital syphilis cases between 2015 and 2019 and their manifestations. Signs and symptoms include long bone changes, snuffles, condyloma lata, jaundice, hepatitis, syphilitic rash, etc. Sexually Transmitted Disease Surveillance 2019. (Data from Centers for Disease Control and Prevention. *Sexually Transmitted Disease Surveillance 2019*. Atlanta: US Department of Health and Human Services; 2021.)

Organization estimated that, globally, nearly 1 million pregnant women were actively infected with syphilis in 2016,[97] resulting in an estimated 660,000 cases of congenital syphilis, and half of them have infants with adverse outcomes such as stillbirth or prematurity, nonimmune hydrops, and death.[98] The global burden of congenital syphilis is confounded further by the high prevalence of infection with the human immunodeficiency virus (HIV), as syphilis is a known risk factor for acquisition of HIV.

Pathogenesis

The causative agent for syphilis is *T. pallidum*, a thin, corkscrew-shaped, flagellated, highly motile spirochete. *T. pallidum* is able to invade the fetal compartment at any time during gestation, although the risk of fetal infection increases as the stage of pregnancy advances. Spirochetes have been detected in fetal tissue from spontaneous abortion as early as 9 and 10 weeks' gestation and recovered from amniotic fluid at 14 weeks of pregnancy. Vertical transmission is related directly to the maternal stage of syphilis, with early syphilis resulting in significantly higher transmission rates than late latent infection. Ingraham reported in 1950 that among 251 women with syphilis of less than 4 years' duration, 41% of their infants were born alive and had congenital syphilis, 25% were stillborn, 14% died in the neonatal period, 21% had low birth weight but no evidence of syphilis, and 18% were normal full-term infants.[99] In contrast, only 2% of infants born to mothers with late latent disease had congenital syphilis. In 1952, Fiumara and colleagues reported that untreated maternal primary or secondary syphilis resulted in 50% of infants having congenital syphilis while the other 50% were stillborn, premature, or died in the neonatal period. With early and late latent infection, 40% and 10% of infants, respectively, had congenital syphilis.[100] These data are supported by a more recent study of Sheffield and colleagues in which mothers with primary, secondary, early latent, and late latent infection had transmission rates of 29%, 59%, 50%, and 13%, respectively.[101]

Because *T. pallidum* enters the fetal bloodstream directly, the primary stage of infection is completely bypassed. There is no chancre and no local lymphadenopathy. Instead, there is widespread hematogenous spread to all organs and tissues, including the liver, spleen, pancreas, intestine, kidney, skin, mucous membranes of the lips, nose and anus, bones and cartilage, and the central nervous system. Invasion of the lung results in a characteristic "pneumonia alba" that is seen more frequently in developing countries.

Microscopically, the tissue alterations consist of interstitial fibrosis and perivascular inflammation with plasma and round cell infiltration, with visualization of spirochetes by silver or fluorescent staining. Gumma formation is infrequent in neonates, while extramedullary hematopoiesis involving the liver, spleen, dermis, kidneys, and other organs is common.

The placenta of infants with congenital syphilis often is large, thick, and pale. Histopathologic features include villous enlargement, acute villitis, and erythroblastosis. Intense inflammation of the umbilical cord results in a "barber's pole" appearance where the edematous portions have a spiral striped zone of red and pale blue discoloration, interspersed with streaks of chalky white. Histologically, the umbilical cord exhibits abscess-like foci of necrosis within Wharton's jelly and umbilical vessels. Placental and umbilical cord histopathology should be performed on every case of suspected syphilis.

Clinical Presentation

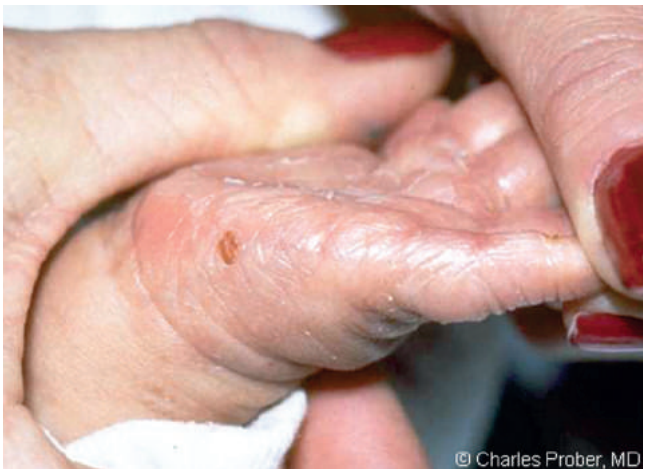
Two characteristic syndromes of congenital syphilis have been described. *Early congenital syphilis* refers to those clinical manifestations that appear in the first 2 years of age, while those features that occur after 2 years and usually at puberty are designated as *late congenital syphilis*. The clinical, laboratory, and radiographic abnormalities of early congenital syphilis are a consequence of active infection with *T. pallidum* and the resultant inflammatory response induced in various body organs and tissues. The severity of these manifestations is highly variable and can range from overwhelming involvement of multiple organs and body systems as occurs in nonimmune fetal hydrops to only laboratory or radiographic abnormalities. Most infants born to mothers with untreated syphilis appear normal and have no clinical or laboratory evidence of infection at birth but may develop manifestations of disease several months to years later if left untreated.

The signs and symptoms of early congenital syphilis are summarized in Table 35.3. Prematurity and low birth weight is seen in

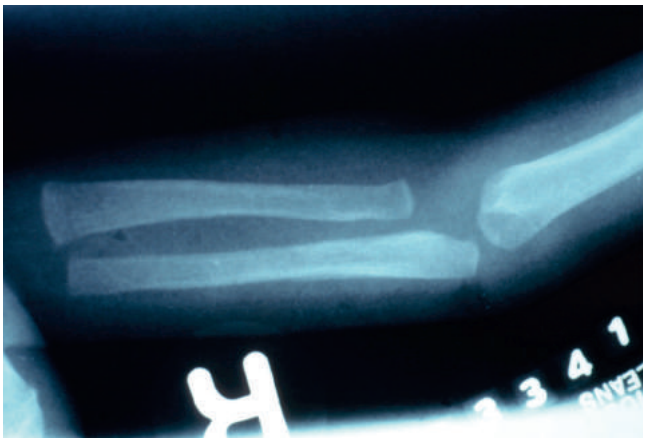
TABLE 35.3 Clinical Features of Congenital Syphilis in the Neonatal Period

Feature	Prevalence (%)
Hepatomegaly with or without splenomegaly*	60–100
Radiographic bone changes (periostitis; osteochondritis);* pseudoparalysis of Parrot	75–100 12
Lymphadenopathy	20–50
Jaundice	50–70
Skin rash*	40
Hepatitis (elevated transaminase concentrations)	40
Anemia and/or thrombocytopenia	20–50
Respiratory distress (pneumonia)	34
Fever	10†
Small for gestational age	10
Nonimmune hydrops	5
Rhinitis, mucous patch, condyloma lata, nephrotic syndrome, myocarditis, diarrhea (malabsorption), pancreatitis, chorioretinitis, cataract	Rare (<5)
Central nervous system (leptomeningitis, cranial nerve palsies, cerebral infarction, seizures, hypopituitarism)	Rare (<5)

*Prominent feature.
†More common when infant presents at >3 weeks of age.
Adapted from Kollman TR, Dobson S. Syphilis. In: Remington JS, Klein JO, Wilson CV, Nizet V, eds. *Infectious Diseases of the Fetus and Newborn Infant.* 7th ed. Philadelphia: Elsevier Saunders; 2011; and Saloojee H, Velaphi S, Goga Y, et al. The prevention and management of congenital syphilis: an overview and recommendations. *Bull World Health Organ.* 2004;82:424–430.



• **Fig. 35.3** Congenital syphilis with desquamation over the hand. (Courtesy Dr. Charles G. Prober, MD.)



• **Fig. 35.4** The radiograph displays the characteristic "celery stalking" and widening of the metaphases in long bones found in untreated congenital syphilis. (From American Academy of Pediatrics Committee on Infectious Diseases: *Syphilis: Clinical Manifestations Images. Red Book Online Visual Library*. American Academy of Pediatrics; 2015.)

10% to 40% of infants.[102] Hepatosplenomegaly is frequent, with extramedullary hematopoiesis a prominent finding in both the liver and spleen. Approximately one-third of infants have direct and indirect hyperbilirubinemia and elevated transaminase levels that may worsen transiently after initiation of penicillin therapy. Liver abnormalities may require months to resolve, but they rarely lead to cirrhosis. Generalized lymphadenopathy occurs in about 20% of infants, with characteristic enlargement of epitrochlear nodes. Anemia secondary to hemolysis or infection of the bone marrow with hematopoietic suppression may be severe.[103] Thrombocytopenia with petechiae and purpura occurs frequently and may be the sole manifestation of congenital infection. Other less common manifestations include ocular findings (chorioretinitis, cataract, glaucoma, and uveitis), pneumonitis, pneumonia alba, nephrotic syndrome, myocarditis, pancreatitis, and inflammation and fibrosis of the gastrointestinal tract leading to malabsorption and diarrhea.

Mucocutaneous lesions occur in 40% to 60% of affected infants. The rash of congenital syphilis usually is oval and maculopapular but becomes copper-colored with desquamation (Fig. 35.3) mostly in the palms and soles. A characteristic vesicular bullous eruption known as *pemphigus syphiliticus* may develop with erythema, blisters, and eventual crusting and skin wrinkling. Mucocutaneous junctions also may be involved, with the lips becoming weepy, thickened, and rough. Radial cracks may traverse the vermillion zone surrounding the margins of the lips and are the beginning of the radiating scars called rhagades that is seen with late congenital syphilis. Rarely, mucous patches of the lips, tongue, and palate, as well as white, flat, moist, raised plaques known as *condylomata lata* in the perioral and perianal areas, may occur. Rhinitis ("snuffles"), a watery nasal discharge that may become thick, purulent, and blood-tinged, occurred in almost two-thirds of patients in the early literature but is now less prevalent.[104] Both the nasal discharge and vesicular fluid contain large concentrations of spirochetes, and are highly infectious.

Bone radiographs demonstrate characteristic osteochondritis and periostitis in 60% to 80% of infants with clinical signs of congenital syphilis and 20% of well-appearing, congenitally infected infants (Fig. 35.4). These abnormalities tend to involve the long bones (tibia, humerus, femur), ribs, and cranium, and are usually symmetric, with the lower extremities involved more often than the upper extremities. Rarely, the bone lesions may be painful and result in subepiphyseal fracture and epiphyseal dislocation with

pseudoparalysis of the affected limb (pseudoparalysis of Parrot). Osteochondritis involves the metaphysis and is visualized on the long bone radiographs approximately 5 weeks after fetal infection. There is metaphyseal demineralization with a radiolucent band representing a zone of osteoporosis below a radiodense band below the epiphyseal plate that is a widened and enhanced zone of provisional calcification. Roentgenographically, this results in the classic transverse saw-toothed appearance of the metaphysis whose margins become serrated, jagged, and irregular. Bilateral demineralization and osseous destruction of the proximal medial tibial metaphysis is referred to as *Wimberger sign*. Periostitis requires 16 weeks for roentgenographic demonstration and consists of multiple layers of periosteal new bone formation in response to diaphyseal inflammation. After several months, complete healing of the affected bones occurs, even without antibiotic therapy.

Central nervous system invasion by *T. pallidum* occurs in about 50% of infants with clinical, laboratory, or radiographic signs of congenital syphilis. Clinical signs of central nervous system involvement, however, are rare in the neonatal period but can occur later if infected infants are not identified and treated.[103] Such manifestations include bulging fontanelle, seizures, leptomeningitis, cranial nerve palsies, hydrocephalus, cerebral infarction, and pituitary gland dysfunction with hypoglycemia and diabetes insipidus.

The clinical manifestations or stigmata of late congenital syphilis result from persistent inflammation or scarring following early congenital syphilis infection and is prevented by treatment during gestation or within the first 3 months of age. Infants with late congenital syphilis are not infectious. Late manifestations include dental stigmata such as Hutchinson's teeth, where the permanent upper central incisors are small, widely spaced, barrel shaped, and notched, and mulberry molars where the first lower molar has many small cusps instead of the usual four. Osteochondritis affecting the otic capsule may lead to cochlear degeneration and fibrous adhesions resulting in eighth nerve deafness, for which corticosteroid treatment may be beneficial. Late ocular manifestations include uveitis and interstitial keratitis. The constellation of interstitial keratitis, eight cranial nerve deafness, and Hutchinson's teeth is known as Hutchinson's triad.

The sequela of periostitis of the skull is frontal bossing, of the tibia is saber shins, and of the clavicle is Higouménakis sign with sternoclavicular thickening. Clutton joints, or painless synovitis and hydrarthrosis, are rare. The sequelae of syphilitic rhinitis include rhagades and short maxilla with a high palatal arch. If the inflammation of the nasal mucosa extends to the underlying cartilage and bone, perforation of the palate and nasal septum occurs, resulting in a "saddle nose" deformity. Sequelae of central nervous system infection include mental retardation, hydrocephalus, seizure disorder, cranial nerve palsies, paralysis, and optic nerve atrophy.[105,106]

Evaluation

The diagnosis of congenital syphilis is established by the observation of spirochetes in body fluids or tissue and suggested by serologic test results. *T. pallidum* may be identified by dark field microscopy, polymerase chain reaction (PCR) testing, and fluorescent antibody or silver staining of mucocutaneous lesions, nasal discharge, vesicular fluid, amniotic fluid, placenta, umbilical cord, or tissue obtained at autopsy. Its diagnosis, however, remains challenging since *T. pallidum* cannot be cultivated in artificial media and many infected infants lack clinical, laboratory, and radiographic signs of disease. In addition, the diagnosis often can only be inferred since maternal nontreponemal and treponemal IgG antibodies are transferred transplacentally to the fetus, complicating the interpretation of reactive serologic tests for syphilis in neonates. A diagnosis of congenital syphilis is supported by an infant's serum quantitative nontreponemal antibody titer that is at least fourfold higher than the mother's titer. The absence of such a finding, however, does not exclude a diagnosis of congenital syphilis.

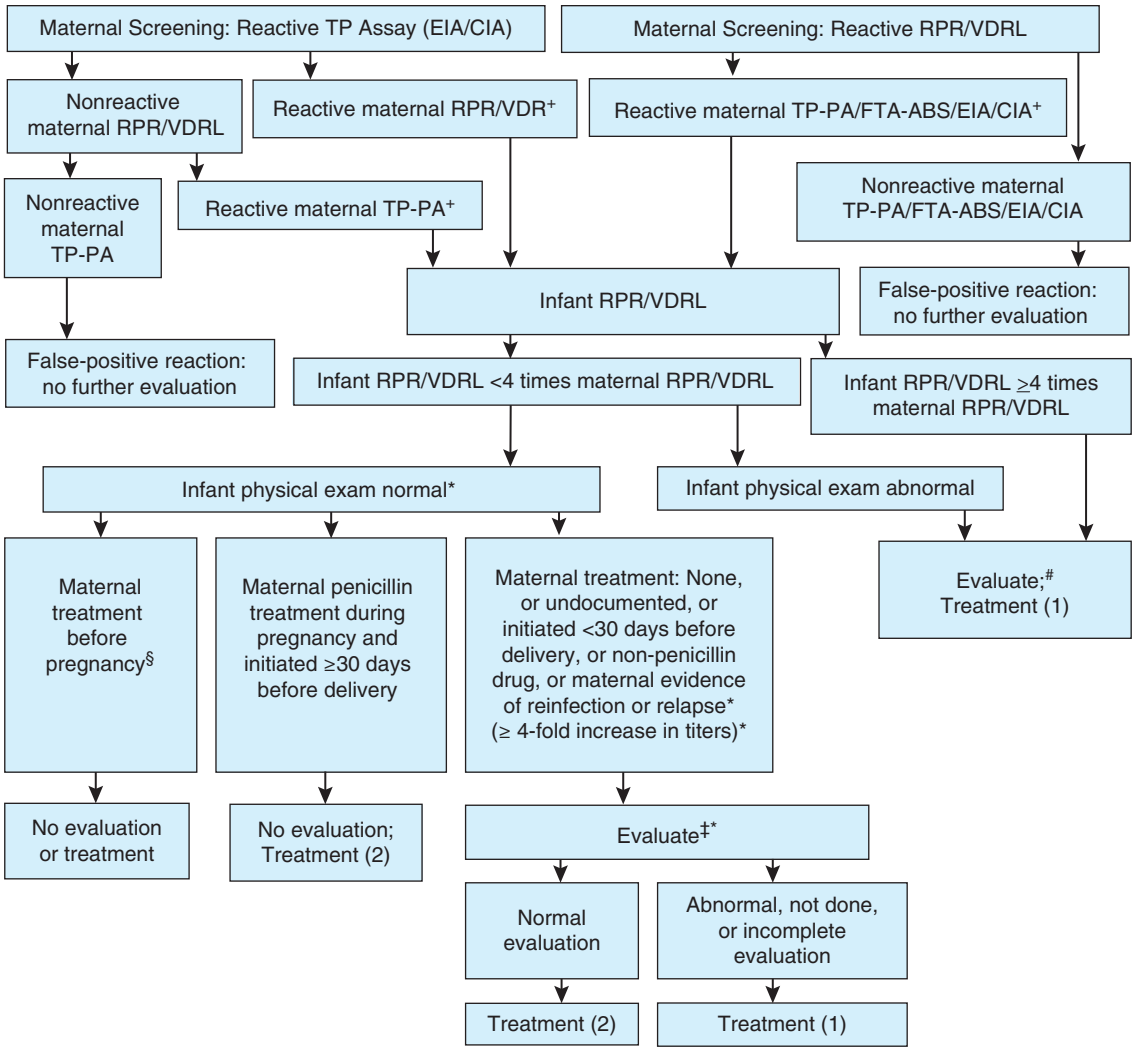
Serologic tests for syphilis are classified into nontreponemal and treponemal tests. Nontreponemal tests include the Venereal Disease Research Laboratory (VDRL) test and the rapid plasma reagin (RPR) test. The same nontreponemal test should be performed on the mother and infant so that accurate comparisons can be made. Serologic testing of the infant should be performed on serum and not umbilical cord blood since false positive test results have been reported secondary to contamination of the specimen with maternal blood or Wharton's jelly. False-negative test results also may occur when the maternal nontreponemal titer is of low dilution.[107] Measurement of total cord IgM levels, as well as use of treponemal IgM enzyme-linked immunosorbent assays (ELISA) and the fluorescent treponemal antibody absorption (FTA-ABS)-IgM test, have not proved useful in the diagnosis of congenital syphilis, and are not recommended.[108]

Treponemal tests include the *T. pallidum* particle agglutination (TP-PA) test, the fluorescent treponemal antibody-absorption (FTA-ABS) test, and treponemal immunoassay (i.e., enzyme immunoassay (EIA), chemiluminescence immunoassay (CIA), and multiplex flow immunoassay). These treponemal tests are used to confirm the diagnosis of syphilis.

The diagnosis of congenital neurosyphilis is difficult to establish, with treponemal infection of the central nervous system only inferred from abnormalities of the cerebrospinal fluid (CSF) such as a reactive VDRL test, pleocytosis (greater than 18 to 25 white blood cells per microliter), and elevated protein content (>150 mg/dL; >170 mg/dL if infant is premature). However, a reactive CSF VDRL test in neonates may be caused by passive transfer of nontreponemal IgG antibodies from serum into the CSF. By inoculation of CSF into rabbit testes with resultant syphilitic infection of the rabbit, Michelow and coworkers found that invasion of the central nervous system with *T. pallidum* occurs in 41% of infants who have clinical, laboratory, or radiographic abnormalities of congenital syphilis and in 60% of those who have an abnormal physical examination consistent with a diagnosis of congenital syphilis.[109] The sensitivity and specificity of a reactive CSF VDRL test, pleocytosis, and elevated protein content were 53% and 90%, 38% and 88%, and 56% and 78%, respectively.[110] Therefore, if clinical, laboratory or radiographic evaluation supports a diagnosis of congenital syphilis, then therapy effective against central nervous system disease is warranted.

A practical approach to the evaluation and treatment of infants born to mothers with reactive serologic tests for syphilis is presented in Fig. 35.5.[104,111]

All pregnant women and their sexual partner(s) who have syphilis should be tested for coinfection with HIV, although infants born to mothers coinfected with syphilis and HIV do not require different evaluation, therapy, or follow-up. Infants born to mothers with reactive serologic test results for syphilis should have a serum quantitative nontreponemal test (VDRL or RPR) performed and be carefully examined for physical signs



\+ Test for HIV-antibody. Infants of HIV-infected mothers do not require different evaluation or treatment.

* If the infant's RPR/VDRL is nonreactive AND the mother has had no treatment, undocumented treatment, treatment initiation <30 days before delivery, or evidence of reinfection or relapse (≥ 4-fold increase in titers), THEN treat infant with a single IM injection of benzathine penicillin (50,000 U/kg). No additional evaluation needed.

§ Women who maintain a VDRL titer ≤1:2 (RPR ≤1:4) beyond 1 year following successful treatment are considered serofast.

\# Evaluation consists of CBC, platelet count; CSF examination for cell count, protein, and quantitative VDRL. Other tests as clinically indicated: long-bone x-rays, neuroimaging, auditory brainstem response, eye exam, chest x-ray, liver function tests.

‡ CBC, platelet count; CSF examination for cell count, protein, and quantitative VDRL; long-bone x-rays

TREATMENT:

(1) Aqueous penicillin G 50,000 U/kg IV q 12 hr (≤1 wk of age), q 8 hr (>1 wk), or procaine penicillin G 50,000 U/kg IM single daily dose, x 10 days

(2) Benzathine penicillin G 50,000 U/kg IM x 1 dose

• **Fig. 35.5** Algorithm for evaluation and treatment of infants born to mothers with reactive serologic tests for syphilis.

of congenital syphilis. Neonates who have an abnormal physical examination that is consistent with congenital syphilis, a serum quantitative nontreponemal serologic titer that is fourfold or greater than the mother's titer, or a positive dark-field or fluorescent antibody test or PCR result of lesions or body fluid(s) should have a complete blood cell count (CBC) and platelet count performed as well as CSF examination for cell count, protein content, and CSF VDRL test. Other tests, such as bone and chest radiographs, liver function tests, cranial ultrasound, ophthalmologic examination, and auditory brainstem response, should be performed as clinically indicated. These infants are considered to have proven or highly probable disease. Since spirochetemia with invasion of the central nervous system is likely, it is beneficial for follow-up purposes to establish central nervous system abnormalities at presentation by performance of a lumbar puncture for CSF analysis.

For well-appearing neonates who have a normal physical examination and a serum quantitative nontreponemal serologic titer that is equal to or less than fourfold the maternal titer, further evaluation and treatment depends on the maternal treatment history (see Fig. 35.5). If the mother has untreated syphilis or the treatment is undocumented or inadequate (syphilis treatment initiated <4 weeks before delivery or with any non-penicillin G regimen), a complete evaluation consisting of CSF analysis, long bone radiographs, and complete blood cell (CBC) and platelet counts should be performed to guide optimal therapy. The evaluation must be completely normal if the infant is to be treated with a single intramuscular dose of benzathine penicillin G. Almost none of these infants will have central nervous system invasion by *T. pallidum* if their complete evaluation is normal. Alternatively, a complete evaluation is not necessary if 10 days of parenteral penicillin therapy is provided. A complete evaluation also is not indicated in newborns with normal physical examination and nonreactive nontreponemal test results as they are unlikely to have abnormalities detected on conventional laboratory and radiographic testing.[112]

However, treatment of the infant for possible incubating or asymptomatic syphilis is dependent on maternal syphilis treatment history.

Management

Penicillin G is the only known effective antimicrobial agent for prevention of vertical transmission of syphilis and treatment of fetal infection and congenital syphilis. Pregnant women with syphilis should receive the penicillin regimen appropriate for the stage of infection, and if any dose of therapy is missed for latent syphilis, the full course of therapy must be repeated (Table 35.4). Pregnant women who have a history of penicillin allergy should be desensitized and treated with penicillin.[95]

The decision to treat an infant for congenital syphilis is based on the clinical presentation, previous serologic test results and treatment of the mother, and the results of serologic testing of the infant and mother at the time of delivery (Table 35.5; see Fig. 35.5). Infants with proven or highly probable disease, or who have a normal physical examination but their evaluation is abnormal or incomplete, should be treated with either aqueous crystalline penicillin G (50,000 U/kg intravenously every 12 hours for the first week of age, followed by every 8 hours beyond 7 days of age) or aqueous procaine penicillin G (50,000 U/kg intramuscularly once daily) for 10 days. If more than 1 day of therapy is missed, the entire course should be restarted. Data are insufficient regarding the use of other antimicrobial agents (e.g., ampicillin). When possible, a full 10-day course of penicillin is preferred, even if ampicillin was initially provided for possible sepsis.

Infants who have a normal physical examination, CSF studies, CBC and platelet counts, and long bone radiographs can be treated with a single intramuscular injection of benzathine penicillin G at a dose of 50,000 U/kg. If the risk of infection in these infants is substantial and adequate follow-up cannot be ensured, the 10-day course of aqueous or procaine penicillin is recommended by the CDC and American Academy of Pediatrics, regardless of results of the CSF and laboratory examination. Failure of a single injection of benzathine penicillin in the treatment of congenital syphilis has been reported among high-risk seropositive neonates who did not have a complete evaluation. Treatment failures also have been attributed to the inability of penicillin to adequately penetrate and achieve treponemicidal concentrations in certain sites such as the aqueous humor and central nervous system.

TABLE 35.4 Recommended Treatment for Syphilis during Pregnancy

Stage of Syphilis*	Drug (Penicillin)†	Route	Dose (Units)
Primary and secondary	Benzathine penicillin G‡	IM	2.4 million units in a single dose
Early latent (≤1 year duration)	Benzathine penicillin G‡	IM	2.4 million units in a single dose
Late latent (>1 year duration) or latent syphilis of unknown duration	Benzathine penicillin G	IM	2.4 million units weekly × 3
Neurosyphilis and ocular syphilis	Aqueous penicillin G or Procaine penicillin G PLUS Probenecid	IV IM	3–4 million units every 4 h or continuous infusion for 10–14 days§ 2.4 million units IM once daily PLUS 500 mg orally four times a day, both for 10–14 days§

*Persons with HIV infection who have syphilis should be treated as those without HIV infection.
†Pregnant women who have a history of penicillin allergy should be desensitized and treated with penicillin.
‡For women who have primary, secondary, or early latent syphilis, a second dose of benzathine penicillin 2.4 million units IM can be administered 1 week after the initial dose.
§Benzathine penicillin, 2.4 million units IM once per week for up to 3 weeks, can be considered after completion of 10–14 days of intravenous treatment
From Workowski KA, Bachmann LH, Chan PA, et al. Sexually Transmitted Infections Treatment Guidelines, 2021. *MMWR Recomm Rep.* 2021;70(4):1–187. doi:10.15585/mmwr.rr7004a1

Normal neonates born to mothers adequately treated during pregnancy and initiated treatment greater than 4 weeks before delivery should be considered as a "close contact" and receive a single intramuscular injection of benzathine penicillin G (50,000 U/kg), although no evaluation is required or recommended. Similarly, normal infants who have a nonreactive serum nontreponemal test result but are born to mothers with untreated or inadequately treated syphilis can receive a single dose of intramuscular benzathine penicillin G without evaluation—an increasingly common scenario with the use of treponemal tests such as EIAs or CIAs for syphilis screening ("reverse sequence" screening).

During times of penicillin shortage when preparations of penicillin are unavailable, a 10-day course of ceftriaxone can be considered with careful clinical and serologic follow-up, including repeat CSF evaluation.[113] Research efforts are needed to evaluate whether other antibiotics such as ampicillin can effectively treat central nervous system disease.

Within 24 hours of initiation of penicillin therapy, a small percentage of infants who are treated for congenital syphilis may develop a Jarisch-Herxheimer reaction, an acute inflammatory

TABLE 35.5 Recommended Treatment of the Neonate (<30 Days Old) with Syphilis

Neonate	Maternal Stage/Treatment	Evaluation	Antimicrobial Regimen
Confirmed or highly probable disease: (a) Abnormal physical examination consistent with congenital syphilis (b) Abnormal evaluation* (c) Serum nontreponemal titer ≥4 times maternal titer (d) Visualization of spirochetes or detection of *T. pallidum* DNA by PCR of placenta, cord, lesions, or body fluid	Any or none	(1) CSF analysis: VDRL, cell count, and protein (2) CBC and platelet count (3) Other tests as clinically indicated (e.g., long bone radiographs, liver function tests, ophthalmologic examination, hearing evaluation, neuroimaging)	Aqueous crystalline penicillin G 100,000-150,000 units/kg/day IV every 12 h during first 7 days of age and every 8 h when >7 days of age for total 10 days *or* Procaine penicillin G 50,000 units/kg/dose once daily IM × 10 days
Possible congenital syphilis: (1) Normal physical examination; and (2) Serum nontreponemal titer ≤4 times the maternal titer	Any stage of infection *and* Mother was (a) not treated, inadequately treated, or has no documented treatment; (b) treated nonpenicillin regimen; or (c) received appropriate treatment but initiation of treatment <30 days before delivery	(1) CSF analysis for VDRL, cell count, and protein; *and* (2) CBC and platelet count; *and* (3) Long bone radiographs	If complete evaluation normal:† (a) Benzathine penicillin G 50,000 units/kg IM × 1 *or* (b) Aqueous crystalline penicillin G 100,000–150,000 units/kg/day IV every 12 h during first 7 days of age and every 8 h when >7 days of age for total 10 days *or* (c) Procaine penicillin G 50,000 units/kg/dose IM × 10 days
Congenital syphilis less likely: (1) Normal physical exam; and (2) Serum nontreponemal titer ≤4 times maternal titer at delivery	Mother treated appropriately for infection stage and initiation of treatment ≥30 days before delivery; and mother has no evidence of reinfection or relapse.	None	Benzathine penicillin G 50,000 units/kg once daily IM × 1
Congenital syphilis unlikely: (1) Normal physical examination; and (2) Serum nontreponemal titer ≤4 times the maternal titer; and (3) No evidence of re-infection	Mother was adequately treated before pregnancy, and mother's non-treponemal serologic titer remains low and stable before and during pregnancy and delivery (e.g., RPR ≤1:4)	None	None, but benzathine penicillin G 50,000 units/kg IM × 1 may be considered if neonate has reactive nontreponemal test and follow-up is uncertain

*CBC, platelet count, CSF examination, bone radiographs.
†If complete evaluation not done, infant must receive 10 days of penicillin therapy unless infant's RPR/VDRL is nonreactive in which case no evaluation needed but infant should receive benzathine penicillin G (50,000 U/kg IM).
CSF, Cerebrospinal fluid; *VDRL*, Venereal Disease Research Laboratory; *CBC*, complete blood cell count, *RPR*, rapid plasma regain.
From Workowski KA, Bachmann LH, Chan PA, et al. Sexually Transmitted Infections Treatment Guidelines, 2021. *MMWR Recomm Rep.* 2021;70(4):1–187. doi:10.15585/mmwr.rr7004a1

response likely due to the rapid killing of spirochetes. It is characterized by fever, tachypnea, tachycardia, hypotension, accentuation of cutaneous lesions, or even death due to cardiovascular collapse. Treatment is supportive care.

Infants with reactive serologic test results should have serial quantitative nontreponemal tests performed every 2 to 3 months until the test becomes nonreactive. In infants with congenital syphilis, nontreponemal serologic tests should decline fourfold and become nonreactive within 6 to 12 months after appropriate treatment. Uninfected infants usually become seronegative by 6 months of age. Infants with persistently low, stable titers of nontreponemal tests beyond 1 year of age may require retreatment. A reactive treponemal test beyond 18 months of age when the infant has lost all maternal IgG antibodies confirms the diagnosis of congenital syphilis. Infants with abnormal CSF findings at diagnosis no longer need a repeat lumbar puncture performed 6 months after therapy if they are clinically normal and serologic titers have decreased appropriately.[95]

If a lumbar puncture is performed, a reactive CSF VDRL test result or an abnormal protein content or cell count at that time is an indication for retreatment.

Prevention

Congenital syphilis is effectively prevented by prenatal serologic screening of mothers and penicillin treatment of infected women, their sexual partners, and their newborn infants.[114] All pregnant women should have a serologic test for syphilis performed at the first prenatal visit in the first trimester, with the test being repeated at 28 to 32 weeks' gestation and at delivery in areas with a high incidence of syphilis. Serologic screening tests should be performed on mothers and not on infants, because the infant may

have a nonreactive serologic test result, but the mother's test is reactive at a low titer.

Although nontreponemal antibody testing has been recommended for antepartum syphilis screening, treponemal antibody testing (i.e., EIA or CIA) is being used increasingly by many laboratories as a cost-cutting measure for screening pregnant women ("reverse sequence syphilis screening").[115] If a treponemal EIA or CIA test is used for antepartum syphilis screening and is positive, a quantitative nontreponemal test (RPR or VDRL) should then be performed. If the nontreponemal test is reactive, then a diagnosis of past or present syphilis is made and the management of the mother and infant should be handled as discussed previously. However, if the nontreponemal test is negative, then the results are considered discrepant and a second treponemal test (TP-PA preferred) should be performed, preferably on the same specimen. If the second treponemal test is reactive, then current or past syphilis infection is confirmed. For women with a history of adequately treated syphilis, no further treatment is necessary. Women without a history of treatment should be staged and treated accordingly with a recommended penicillin regimen. If the second treponemal test is nonreactive, then the majority of these mothers (65%) are likely to have a false-positive EIA/CIA test result.[116] The need for reflexive testing with a TP-PA is imperative to diagnose syphilis and inform newborn management (see Fig. 35.5). Infants with suspected or proven congenital syphilis can be managed with standard precautions only. If there is cutaneous lesions or mucous membrane involvement, then gloves should be worn as well until 24 hours of treatment has been completed.

All cases of syphilis must be reported to the local public health department, which performs contact investigation and identifies core environments and populations. The public health impact of syphilis in pregnancy and infancy remains substantial, and only through optimal prenatal healthcare services will elimination of maternal-to-child transmission of syphilis become a reality.

Congenital Malaria

Epidemiology

Malaria is a parasitic disease of epidemic proportion. An estimated 214 million new cases of malaria with 409,000 deaths were reported in 2019 alone.[117] The greatest burden of disease occurs in the African regions (94%) and Southeast Asia (3%). In areas of high transmission, mortality is concentrated largely among young children and pregnant women. Children below 5 years of age account for 67% of all malarial deaths[117] with a disproportionate amount occurring in African regions. Malaria is caused by four *Plasmodium* species: *P. falciparum, P. vivax, P. ovale*, and *P. malariae*. Of these, *P. falciparum* is the major cause of morbidity and mortality. Humans typically acquire infection through the bite of the *Anopheles* spp. mosquito. Transmission may also occur through blood transfusion or vertically from the mother to fetus, resulting in congenital malaria.

Congenital malaria occurs with all *Plasmodium* species. Among the 107 cases of congenital malaria reported in 1950 (mostly from Africa), 64% were infected with *P. falciparum*, 32% by *P. vivax*, and 2% by *P. malariae*.[118] Although *P. falciparum* remains the predominant pathogen in sub-Saharan Africa, *P. vivax* may account for a larger proportion of cases in Asia. Among 27 cases reported in Thailand between 1981 and 2005, 82% were caused by *P. vivax*.[119] *P. malariae* is less frequently a causative agent, with fewer than 10 cases reported worldwide since 1950.[120] In China, *P. vivax* accounted for the majority (92.5%) of the 107 cases of congenital malaria cases reported.[121] Concurrent infection with *P. malariae* and *P. vivax* has been documented.[122] In the United States, the predominant *Plasmodium* species causing congenital malaria reflects the countries of origin of the mothers. In Hulbert's (1992) review of 49 cases from 1950 to 1991, 82% of infections were caused by *P. vivax*. In the updated review in the U.S. reflecting 1966–2005, the predominant infecting species remained *P. vivax* (81%), although all four species were represented.[123]

The true rates of congenital malaria reported in the literature vary significantly based on the time span reported, method of reporting, and clinical definition. Congenital malaria is commonly defined as the presence of *Plasmodium* spp. parasites in the peripheral blood during the first 7 days of life where the transmission occurs from the mother via placental transfer.[118,124–127] In contrast, neonatal malaria is defined as the presence of *Plasmodium* spp. parasites in the peripheral blood from 8 to 30 days of life where mosquito transmission is the most likely etiology. These definitions are most applicable in areas of high malaria transmission where, among older infants, it would be difficult to distinguish congenitally acquired from mosquito-acquired disease. This has been suggested by findings from Malawi, where approximately 50% of newborns with cord blood parasitemia were infected with parasites of a different genotype than their mothers at the time of delivery.[128] Outside endemic areas, where postnatal transmission can be reasonably excluded, clinical onset of disease often does not occur until after the first week of life, and age-specific criteria are not useful for the diagnosis of congenital malaria. It is likely that because of the delay in clinical presentation, many cases of congenital malaria in endemic areas were misclassified as being acquired from mosquitoes. Covell found the prevalence of congenital malaria among nonimmune populations (i.e., Europeans residing in or visiting endemic areas) to be approximately 7%.[118] In other estimates prior to the 1970s, the prevalence of congenital malaria—defined as parasitemia detected in the first 7 days of life—was estimated to be 0.3% (16 of 5324 births) among immune mothers. Subsequent reports supported the observed low frequency of congenital malaria—defined as umbilical cord parasitemia or parasitemia in the first 24 hours of life—among indigenous populations.[129–132] These observations have been cited repeatedly in the literature to support the notion that congenital malaria is an uncommon occurrence in endemic areas despite the high prevalence of maternal and placental malaria.

Over the last several decades, however, more in-depth studies of congenital malaria have suggested that it is far more common than initially reported, as rates from endemic and nonendemic areas ranged from 0.2% to 47%.[124,133–135] Many have suggested that low prevalence previously reported for congenital malaria was likely due to inadequate recognition and underreporting.[124,134–140] In Zambia, during a season of heavy malaria transmission, incidence rates for congenital malaria ranged from 4% to 15%.[141] Congenital malaria, defined as neonatal parasitemia, was detected in 15.3% and 17.4% of neonates born in two sites in Nigeria.[142,143] The apparent increase in the frequency of congenital malaria has been attributed to increasing resistance of *P. falciparum* to antimalarial drugs, resulting in increased maternal parasitemia, increased virulence of the parasite, and reduced transmission of antibody from mother to newborn because of malaria chemoprophylaxis administered to pregnant women. Meanwhile a multicentered trial in Nigeria showed an overall prevalence rate of 5% (range of 1.1% to 11.5%).[137] In a recent meta analysis from 2000 to 2019, the overall prevalence rate was estimated at 6.9% and was

significantly higher in unstable malaria transmission areas compared to stable areas.[144] The inconsistency may also represent true environmental differences with differences in levels of maternal immunity. It is clear that congenital malaria can exist in asymptomatic infants which can make reporting in the literature even more variable.[126,127,142]

Congenital malaria in nonendemic areas is rare. As of 1995, only 300 cases of congenital malaria had been reported in the literature,[145] largely from outside of malaria endemic areas. In the United States, the occurrence of congenital malaria is well documented because the country has been free of indigenous disease since the 1950s. From 1950 to 1991, 49 cases of congenital malaria were reported in the literature,[146] and additional cases were reported during the next 15 years.[145,147–151] From 1966 to 2005, 81 cases of congenital malaria were reported to the National Malaria Surveillance system of the CDC.[123] Almost all the cases were among infants whose mothers were foreign born, suggesting that congenital malaria is primarily a health problem of recent immigrants rather than of U.S.-born travelers to malaria-endemic countries. Forty-four women (54%) had emigrated from Asia, 27 (33%) from South or Central America, and 7 (9%) from Africa. Until 1979, one to two cases were reported annually.[152] An abrupt rise to 16 cases around 1981 correlated with an increase in the total number cases of malaria that occurred as a result of a large influx of refugees and immigrants from Southeast Asia, with 15 of the 16 infants being born to mothers from that region.[153] In 2017, the CDC reported two cases of congenital malaria among the 2161 reported cases in the United States.[154,155] The lack of familiarity with this disease in the United States renders it a diagnostic and therapeutic challenge for clinicians, with delays in diagnosis potentially leading to significant morbidity and mortality.[156]

Pathogenesis

Two key features play a critical role in the pathogenesis and natural history of congenital malaria:

1. Maternal and placental parasitemia; and
2. A correlation between umbilical cord parasitemia and neonatal parasitemia.

Pregnancy itself increases the risk of severe malaria. Malaria also increases the risk for adverse pregnancy outcomes, including prematurity, abortion, and stillbirth. Despite major global health efforts, there were 12 million pregnant women at risk for malaria in 2019 in the African region.[157,158] It is well established that both the frequency of disease and density of parasitemia are higher in pregnant women compared with nonpregnant women.[133,159,160] Among 20 studies conducted between 1985 and 2000, the median prevalence of maternal malaria infection (defined as peripheral or placental infection) in all was 28%.[161] More recent studies of endemic areas within the last decade have reported significant geographic variation ranging between 8.1% and 52% prevalence of malaria among pregnant women.[162–164] For this reason, it is widely contended that one in four pregnant women in areas of stable transmission in Africa have evidence of malaria infection at the time of delivery.

The clinical features of *P. falciparum* malaria in a pregnant woman depend to a large degree on her immune status, which in turn is determined by her prior exposure to malaria. Pregnancy itself suppresses both the humoral and cell-mediated portions of the immune system, increasing the susceptibility to severe malaria. Impaired T cell responses reduce systemic control of the infection and the ability to control liver stage infections, resulting in relapse among some forms of *Plasmodium* spp.[165] Primigravidae women have a twofold to fourfold increased risk of placental malaria compared with multigravidae women.[132,133,166] This may be due to levels of antigen specific *Plasmodium* spp. antibodies that appear to be boosted with successive pregnancies.[165] In pregnant women with little or no preexisting malaria immunity, such as women from nonendemic countries or travelers to malaria-endemic areas, infection is associated with high risks of severe disease with significant maternal and perinatal mortality. In contrast, women residing in areas of stable malaria transmission usually have a high level of immunity to malaria. Infection may be frequently asymptomatic and therefore unsuspected or undetected, but it is associated with placental parasitization, with consequent effects on maternal and fetal outcomes.

The most significant consequence of pregnancy-associated malaria is maternal anemia. It is estimated that in sub-Saharan Africa between 200,000 and 500,000 pregnant women develop anemia as a result of malaria, and that up to 10,000 maternal anemia-related deaths are a consequence of *P. falciparum* parasitemia.[166] Malaria in pregnancy also has potentially devastating effects on the fetus and newborn, including spontaneous abortion, still birth, premature delivery, congenital infection, and neonatal death.[128,159] In areas of high transmission in Africa, the risk of LBW approximately doubles if women have placental malaria, with the greatest effect in primigravidae.[133] Pregnancy-associated malaria is believed to be responsible for 30% to 35% of LBW infants and for 75,000 to 200,000 infant deaths each year.[159] In 2019, an estimated 822,000 low birthweight infants were attributed to malaria infection during pregnancy alone.[117]

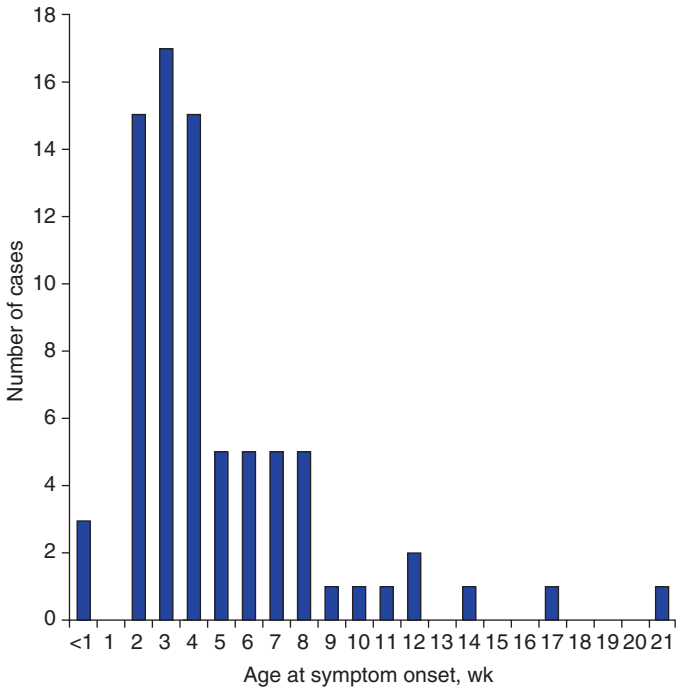
The timing and mechanism of transmission of *Plasmodium* spp. parasites from the mother to the fetus is not well understood. Postulated mechanisms include maternal transfusion into fetal circulation either during pregnancy or at delivery, or direct penetration of parasitized red blood cells through the chorionic villi or through premature separation of the placenta. Plasmodium infected erythrocytes have the unique ability to sequester in the placenta which may facilitate transmission to the fetus.[160,167] In utero transmission is supported by the finding of malarial parasites in fetal tissues at autopsy,[168] by umbilical cord blood parasitemia[132,138,169] and the onset of clinical signs of malaria within hours of birth.[118,149,170] Vertical transmission of malaria probably does not occur as a result of transplacental passage of exoerythrocytic parasites. More likely, transmission occurs by transfusion of parasitized maternal erythrocytes through a breach in the placental barrier that may occur either prematurely during pregnancy or during labor. Transmission of malaria by breastfeeding is not known to occur.

The fate of the *Plasmodium* spp. parasite is unclear after it is transmitted to the fetus. Reinhardt found parasites in thick smears of umbilical cord blood in 22% of 19 infants born to women in the Ivory Coast, but the peripheral blood smears were negative for all of the infants.[171] Similarly, 4% of 1009 infants born to Tanzanian women had parasites in cord blood, but parasitemia was detected on peripheral smear in only 2 of 11 infants.[132] Parasitemia detected shortly after birth may also resolve without evolving into clinically symptomatic disease. The correlation between maternal, placental, umbilical cord, and peripheral parasitemia was evaluated in 1875 mother-baby pairs in Nigeria. Thick and thin blood smears were obtained within 4 hours of birth, and smear-positive (cord or peripheral) neonates were retested on days

2, 3, and 7 of life. Treatment was provided for those with symptoms or persistent parasitemia. The overall prevalence of congenital malaria was 5.1%, with parasitemia detected in 19% of infants born to mothers with peripheral parasitemia, 21% of those born to mothers with placental malaria, and 45% of those with positive cord smears. Spontaneous clearance of parasitemia occurred in 62% of infants before day 2, whereas 33% were symptomatic within 3 days of birth.[137] Depending on the region, spontaneous clearance of peripheral parasitemia has been documented in 87% to 100% of neonates.[123,142] Larkin and Thuma[169] found peripheral parasitemia within 24 hours of age in 19 of 51 newborns (65%), but only 7 had clinical signs of disease. Because all 19 newborns received antimalarial therapy, it is unknown how many would have manifested disease if untreated. The spontaneous clearance of *Plasmodium* spp. has been attributed to the protective effects of passive maternal antibody and weakened adherence to fetal hemoglobin.[172]

Clinical Presentation

Infants with congenital malaria may be asymptomatic or can develop symptoms several weeks after birth. The clinical picture of overt congenital malaria is detailed in cases reported outside of endemic areas.[123,146,173,174] The manifestation of disease, although occasionally noted within hours of birth,[149,170] is typically delayed until the infant is several weeks old. In the classic review of 49 infants with congenital malaria reported in the United States between 1950 and 1992, the mean age at onset of symptoms was 5.5 weeks, with 96% of infants presenting between 2 and 8 weeks of age.[146] Among cases reported to the CDC from 1966 to 2005,[123] the median age of symptom onset for 81 infants was 21.5 days for all species combined (Fig. 35.6). Infants infected with *P. malariae* were significantly older at symptom onset (mean, 53 days) compared with those infected with *P. vivax* or *P. falciparum*.



• **Fig. 35.6** Age in weeks at symptom onset of infants with reported congenital malaria, United States, 1966–2005. (From Lesko CR, Arguin PM, Newman RD. Congenital malaria in the United States: a review of cases from 1966 to 2005. *Arch Pediatr Adolesc Med*. 2007;161:1062–1067.)

The prolonged interval between birth and onset of clinical manifestations may be explained by transmission late in pregnancy or at delivery, such that multiple erythrocytic life cycles are required to produce clinically evident disease. Alternatively, the delay may be attributed to the presence of transplacentally acquired maternal antimalarial antibodies. When such antibodies are present in sufficient concentrations, as in infants born to immune mothers, parasitic replication can be prevented or attenuated, and clinical signs can be mild, delayed, or even absent. The presence of a high concentration of fetal hemoglobin in newborns may also promote resistance to a multiplication of parasites. Among infants born to mothers with low or nonexistent immunity, parasitic replication is more likely uninhibited, and clinical signs of malaria may supervene. Preterm infants, who do not benefit from passive immunity, can manifest clinical signs earlier than full-term infants. In a review of premature neonates with congenital malaria, four of five infants received a diagnosis in the first week of life,[175] although the prompt medical evaluation afforded these infants may have facilitated earlier detection.

The clinical features of congenital malaria are nonspecific and often resemble those of bacterial or viral sepsis and other congenital infections. Fever is almost uniformly present, although without the classic paroxysmal pattern described for malaria beyond the neonatal period. Hulbert[146] noted fever in all 44 infants for whom clinical information was available. In the cases reported from 1966 to 2005, fever was reported in 70 of 81 cases (86%).[123] Hepatomegaly and splenomegaly suggestive of a transplacentally acquired infection are found in a substantial portion of infants (Table 35.6). Anemia (often hemolytic), thrombocytopenia, and hyperbilirubinemia are the most commonly reported laboratory findings. Additional signs, symptoms, and laboratory findings are listed in Table 35-6.

In endemic areas, the traditional belief has been that congenital malaria is rare and that when it occurs the infant is typically asymptomatic and develops no clinical features. The lack of symptoms has been attributed to transplacentally acquired antibodies from the mother as well as the protective effects of high levels of fetal hemoglobin. Falade et al.[137] noted spontaneous clearance of parasitemia in 62% of 95 neonates before day 2 of life. Of the remaining infants, 34% were symptomatic within 3 days of birth, with fever and refusal to eat being the most common signs of disease. When active surveillance for malaria was conducted in newborns being evaluated for possible bacterial sepsis in Nigeria, 16 of 203 (8%) neonates had parasitemia, and 10 (5%) met the definition of congenital malaria.[139] Predominant features of disease included fever, respiratory distress, anemia, and hepatomegaly. In another area in Nigeria, of 202 neonates younger than 1 week who were admitted for evaluation of sepsis, 71 (35%) were diagnosed with congenital malaria.[176] Fever was the most common symptom and was present in 93% of infants. Refusal to feed and jaundice were reported in approximately 33%. These observations suggest that, as in infants diagnosed outside endemic areas, the clinical presentation of congenital malaria in endemic areas does not differ significantly from bacterial sepsis. Because the clinical symptoms of congenital malaria may be indistinguishable from that of neonatal sepsis, it is suggested that screening for malaria be included as part of routine investigation of newborns with fever in areas of high malaria transmission.[143,176]

TABLE 35.6 Frequency of Symptoms, Signs, and Laboratory Findings Among 81 Infants With a Diagnosis of Congenital Malaria (United States, 1966–2005)

Symptoms, Signs, and Laboratory Findings	Infants, No. (%)*
Fever	70 (86)
Anemia	28 (36)
Splenomegaly	25 (31)
Hepatomegaly	16 (20)
Thrombocytopenia	12 (15)
Jaundice	11 (14)
Irritability	8 (10)
Anorexia	8 (10)
Vomiting	8 (10)
Cough	6 (7)
Diarrhea	3 (4)
Lethargy	3 (4)
Hemolysis	3 (4)
Pallor	3 (4)
Hyperbilirubinemia	2 (3)
Failure to thrive	2 (3)
Seizures	2 (3)
Dyspnea	1 (1)
Purpura	1 (1)
Tachycardia	1 (1)
Monocytosis	1 (1)

*Percentages do not total 100% because each case can have more than one symptom, sign, or laboratory finding.
From Lesko CR, Arguin PM, Newman RD. Congenital malaria in the United States: a review of cases from 1966 to 2005. *Arch Pediatr Adolesc Med.* 2007;161:1062–1067.

Evaluation

Diagnostic tests for malaria include blood smears, rapid antigen detection tests (RDTs) and PCR. Definitive diagnosis of congenital malaria is based on the microscopic demonstration of parasites on stained thick and thin blood films. Thick blood smears test for the presence of parasites by concentration of red blood cells, whereas thin blood smears allow species identification and quantification of parasitemia. In cases of suggested congenital malaria, specimens for smears should be obtained from both the infant and the mother. If test results from the initial set of smears are negative, additional sets should be obtained every 12 to 24 hours; three sets are generally considered sufficient for diagnostic evaluation. Response to therapy may also be measured by clearance of parasitemia on blood films.

RDTs are based on the immunochromatographic detection of parasite-specific antigens circulating in the bloodstream. Many RDTs are commercially available outside the United States, and, depending on the antigens targeted, the tests may detect only *P. falciparum* or all *Plasmodium* species. RDTs are simple to use, do not require specialized training or facilities, and offer a useful alternative to microscopy in situations where reliable microscopic diagnostics are not readily available. However, the tests have demonstrated mixed results in multiple trials, and sensitivity remains a problem, especially at low parasite densities. To assure standard high-quality control among RDTs used worldwide, the World Health Organization has provided a laboratory-based evaluation to compare the performance of numerous RDT used amongst malaria programs. (The minimum performance criteria is >90% sensitive, detection of at least 75% of low-density samples, false positive rate of <10% and invalid rate of <5%.) In the most recent round of testing in 2014–2015, all products tested could detect high levels of parasitemia; however, low parasite density (200 parasites/μL) detection varied significantly.[177] Information regarding the sensitivity of these tests is limited for neonatal or congenital malaria. Currently the World Health Organization recommends microscopy or malaria RDT in all patients with suspected malaria before treatment is initiated.

The major advantage of PCR for the diagnosis of malaria is its ability to detect low level parasitemia with species specification. Currently, PCR is used mainly to confirm positive blood smears, particularly when the results of the smear are not definitive or there is a mixed species infection. PCR may detect DNA from circulating nonviable parasites after treatment, resulting in difficulty differentiating an active infection from a recently cleared infection. Currently, PCR is only used for epidemiologic research or survey mapping in endemic areas. Although PCR is a highly sensitive alternative to microscopy, the infrastructure and expertise required preclude its use in malaria-endemic areas and in many health care settings in the United States.[178]

As with malaria in general, the diagnosis of congenital malaria outside of endemic areas is often delayed because of nonspecific features and lack of clinical suspicion. Among the 81 cases reviewed by Lesko et al.[123] a median length of delay of 8.5 days was noted for 15% of the infants. Occasionally, the diagnosis is made incidentally. In all four cases of congenital malaria reported by Quinn et al.[153] *Plasmodium* spp. parasites were noted by hematology technicians on routine smears performed for blood cell counts. Maternal history of recent travel to or emigration from an endemic area may suggest the diagnosis but is often obscured by the lack of clinical or laboratory findings in the mother. Lesko et al.[123] found that, of the mothers for whom a history was available, 67% reported having fever during pregnancy, and 26% reported a diagnosis of malaria during pregnancy. Maternal blood films were performed after either symptomatic illness or malaria diagnosis in the infant. Overall, only 42% of women had parasitemia detected, although it is not clear whether an adequate number of smears was conducted for each patient. As a result, lack of peripheral parasitemia in the mother of an infant with suspected congenital malaria does not exclude the diagnosis.

Further confounding the early recognition of disease in the infant is the potentially prolonged lapse between malaria exposure in the mother and transmission of infection to the infant. *P. vivax* and *P. ovale* may remain dormant in the liver, especially if the infected individual did not receive therapy for the exoerythrocytic stage, which can cause a delayed relapse of malaria in travelers or immigrants. *P. malariae* can persist for 20 to 40 years before clinical symptoms or demonstrable parasitemia appear.[148] Congenital malaria has been reported in an infant whose mother lived in the United States for 5 years before delivery and had

no signs or symptoms or malaria for more than 20 years.[174] In North Carolina, congenital *P. malariae* infection was reported in a 10-week-old infant who was born to a mother who had emigrated from the Democratic Republic of Congo 4 years before delivery.[148] In a review by Lesko et al.[123] the median duration from the mother's last exposure to delivery was 9.5 months. The time elapsed since exposure was longest for those with *P. malariae* infection, ranging from 2 to 12 years.

Recognizing that congenital malaria is an exceptional occurrence in the United States, it is still important to include malaria in the differential diagnosis of fever in infants born to mothers who have been exposed to malaria, even if the exposure is remote and even if the woman is asymptomatic. Of 11 infants with congenital malaria in the United States born to women known to have parasitemia at or shortly after delivery, only five underwent testing by blood smears, and all five had negative test results at the time of delivery.[123] Data are insufficient to determine the overall risk of an infant developing congenital malaria when born to a woman at risk for parasitemia or identified with parasitemia at birth. Consequently, the evaluation of infants born outside endemic areas to women with epidemiologic risk factors for parasitemia should be individualized. In malaria endemic areas, and as a public health measure, it has been recommended that blood smears should be checked as part of the evaluation of neonates with fever born to mothers who have had fever within a few weeks of delivery.[127]

Management

The management of malaria consists of supportive care and antimalarial therapy. Information regarding treatment of congenital malaria is limited, and recommended chemotherapy is similar to that of noncongenital infections. The treatment regimen is based on the infecting species, the possibility of drug resistance, and the severity of disease. For mild infections caused by *P. vivax, P. ovale*, and *P. malariae* or chloroquine-sensitive *P. falciparum*, chloroquine orally (10 mg base/kg initially followed by 5 mg base/kg 6, 24, and 48 hours later) is recommended. Treatment with primaquine is not necessary for congenitally acquired *P. vivax* or *P. ovale* infection because, like transfusion-associated malaria, congenital infection does not involve the exoerythrocytic phase.[179]

The treatment of congenital malaria due to chloroquine-resistant *P. falciparum* is poorly defined. In older children, the three treatment options currently recommended are: (1) oral quinine plus either tetracycline, doxycycline, or clindamycin; (2) atovaquone-proguanil; or (3) mefloquine. For the treatment of congenital malaria, oral quinine sulfate and trimethoprim-sulfamethoxazole for 5 days was recommended by Quinn et al. (1982), who used the regimen to treat a 1-month-old infant.[153] Ahmed et al.[175] used a similar regimen for the treatment of an infant born at 28 weeks' gestation to a mother from Zaire. Quinine and clindamycin have been used for *P. falciparum* congenital malaria.[180] Artemisinin-based combination treatments, such as dihydroartemisinin-piperaquine, can also be used in infants for drug resistant *Plasmodium* spp.[134,181] Other regimens used successfully in neonates include oral quinine sulfate and pyrimethamine-sulfadoxine[149] and intravenous quinine hydrochloride followed by oral quinine.[182] Intravenous quinine is no longer available in the United States. Because of the rarity of congenital malaria in the United States, the changing pattern of resistance, and the potential toxicity associated with drugs used for therapy, current treatment recommendations should be sought from the Malaria Branch of the CDC (www.cdc.gov/malaria). For health care professionals, assistance with management of malaria is also available 24 hours a day through the CDC Malaria Hotline (855-856-4713).

Severe malaria occurs most commonly with *P. falciparum* infection and is characterized by one or more of the following: (1) parasitemia greater than 5% of red blood cells, (2) central nervous system or other end-organ involvement, (3) shock, (4) acidosis, (5) severe anemia, or (6) hypoglycemia. Management of severe malaria involves parenteral treatment in an intensive care setting. Until recently, the only parenteral therapy available in the United States was quinidine gluconate. Quinidine is more cardiotoxic than quinine and should be administered with continuous cardiac monitoring. Artesunates can be given either intravenously or intramuscularly for 24 hours until oral medication can be tolerated, at which time combination therapy should be instituted.[181] Exchange transfusion may be warranted when parasitemia exceeds 10% or if there are complications at lower parasite densities.

The efficacy of treatment should be monitored by examining blood smears (i.e., malaria smears) every 12 hours until negative for malaria parasites. Response to therapy with chloroquine for non-*P. falciparum* malaria is usually favorable.[170,173,183] It has been suggested that infants born to mothers with parasitemia at delivery should be treated presumptively for congenital malaria.[123] Data are insufficient to determine the risk of an infant developing congenital malaria when born to a mother with parasitemia. Although there is evidence from endemic areas that parasitemia detected at or shortly after delivery may clear spontaneously, the clinical relevance of this observation in nonendemic areas is unclear. It is recommended that physicians judge each case individually, considering factors such as access to medical care and reliability of follow up in deciding whether to treat infants presumptively.

Outcomes

Malaria during pregnancy is likely an underappreciated risk factor for increased infant morbidity and mortality in endemic areas. In a review of studies published between 1985 and 2000, a 3% to 8% infant mortality rate was calculated based on population-attributable risks for maternal malaria.[161] It was estimated that 75,000 to 200,000 infant deaths annually are associated with malaria during pregnancy, although what proportion of these are related to congenital malaria is unknown. Outside endemic areas, the short-term outcome of congenital malaria has been favorable. Most infants respond rapidly to therapy, with clearance of parasitemia. There were no reports of death or adverse outcomes in the 49 cases reported from 1950 to 1992 or in the 81 cases reported to the CDC from 1966 to 2005.[123,146] It is unclear, however, whether the outcomes are due to an overall favorable prognosis or to a reporting bias.

Prevention

The prevention of congenital malaria is based on a pregnant woman's avoidance of exposure and use of chemoprophylaxis. The burden of malaria among pregnant women in endemic areas is well recognized, and prevention and control strategies for areas of high *P. falciparum* transmission are aimed at reducing maternal and infant mortality. The World Health Organization has proposed a three-pronged approach: (1) long-lasting insecticidal nets, (2) intermittent preventive treatment with an effective antimalarial agent in pregnancy, and (3) prompt diagnosis and

effective treatment of malaria infection. In areas of moderate to high transmission of *P. falciparum*, this three-pronged intervention is strongly recommended along with intermittent preventive treatment with sulfadoxine/pyrimethamine. This drug regimen is administered during antenatal care starting in the second trimester, at least 1 month apart, until the time of delivery.[184] A meta-analysis of more recent intervention trials suggests that successful prevention of these infections reduces the risk of severe maternal anemia by 38%, LBW by 43%, and perinatal mortality by 27%, among women in their first and second pregnancies.[133] Unfortunately, the full implementation of antenatal malaria prevention efforts is burdened by the challenges associated with health care delivery in the developing world. In 2015, 15 million of the 28 million pregnant women at risk for malaria did not receive a dose of preventive medication.[185] About 80% of pregnant mothers in the African countries at highest risk used antenatal services, of which only 62% received intermittent preventive treatment and only 50% received a second dose.[117]

Pregnant women originally from areas where malaria is endemic, but who are now living in nonendemic areas, may be only partially immune. When traveling to their countries of origin, they should be considered nonimmune and, thus, should receive the same recommendations as nonimmune women. For women in the United States who are pregnant or likely to become pregnant, the CDC advises they avoid travel to areas with malaria transmission. If such travel is unavoidable, consultation with an infectious disease or malaria expert is advised. The use of mosquito netting, mesh screens on windows, insecticides, and mosquito repellents can decrease potential exposure to malaria parasites. For pregnant women traveling to areas where there is no chloroquine-resistant *P. falciparum* malaria, prophylaxis with chloroquine is recommended. The safety of chloroquine for the fetus when used at the recommended doses for malaria prophylaxis is well established.[122] For travel to areas where chloroquine resistance has been reported, mefloquine can be used prophylaxis during pregnancy. Use of atovaquone-proguanil during pregnancy was not associated with increased risk of birth defects[186] and can be used for treatment of malaria in the second and third trimester.[187] Doxycycline is contraindicated because of potential adverse effects on the fetus caused by a related drug, tetracycline, which include enamel hypoplasia and discoloration of teeth and inhibition of bone growth. Health care professionals caring for women who cannot take the recommended antimalarial agent should contact the CDC Malaria Hotline (855-856-4713).

Congenital Tuberculosis

Epidemiology

Tuberculosis (TB) remains one of the leading causes of infectious disease deaths worldwide[188,189] and it remains one of the deadliest communicable diseases. One quarter of the world's population is infected with *Mycobacterium tuberculosis*. In 2019, 10 million new TB cases were identified, and 1.2 million people died from the disease.[188] The greatest burden of disease is in developing countries, where TB remains a major public health threat. While case rates have decreased in the United States and Europe, the corresponding numbers have increased dramatically in the former Soviet Union, where public health efforts are impeded by political unrest, and in sub-Saharan Africa, where TB has been fueled by the epidemic of human immunodeficiency virus (HIV).

In the United States, the incidence of TB declined steadily from 1953 through 1984, reaching a nadir of 9.4 cases per 100,000 people. The resurgence of disease was attributed to multiple factors, including the HIV epidemic, increased immigration, and a decline in public health funding for tuberculosis control. With the availability of antiretroviral therapy for HIV and fortification of public health measures, the epidemiologic trend was reversed. The overall incidence of TB in the United States has decreased dramatically since 1992 from 26,673 per 100,000 to only 9421 per 100,000 in 2014.[190] Despite the decrease in the total burden of disease, tuberculosis continues to disproportionately affect the foreign-born and racial and ethnic minorities. Among the 47,718 people with reported TB in the United States, 67% occurred in foreign-born persons between 2012 and 2016.[191]

The current epidemiology of tuberculosis in pregnancy is not well defined. Historical studies prior to the 1900s indicated that the severity of disease was greater during pregnancy though later reports appeared to contradict these early reports.[192] With the resurgence of tuberculosis in the 1980s, the largest increase in the incidence of disease occurred in the 25- to 44-year age group, and the number of cases among women of childbearing age rose by 40%.[193] In 2014, nearly 40% of TB cases among women were in those of child bearing age between 15 to 45 years of age.[194] Thus women of childbearing age, especially those who are foreign born, and their newborns are at continued risk. An estimated 1.04 million children were treated for TB in 2018 and 2019 worldwide.[188]

The prevalence of congenital TB is likely rare as fewer than 400 case reports have been published in the English-language literature. Most published cases were those in the pre-chemotherapy era.[195] Hageman et al.[196] reported two cases of congenital TB and reviewed another 24 reported in the English-language literature since the introduction of isoniazid (INH) in 1952. In the subsequent 30 years, more than 34 additional cases of neonates with congenital tuberculosis have been described (Table 35.7).[193,195,197–209] The majority of reports describe infants born in low-burden countries to mothers who have emigrated from high-burden countries. Although some reports originate from countries where TB is endemic, it is likely that congenital tuberculosis is underrecognized and underreported in these areas, largely due to the nonspecific clinical features of disease and the limited diagnostic capability. In a recent review of Chinese medical literature, 92 cases of congenital TB were reviewed that included 21 cases between 2011 and 2018.[210] With increased global mobility and the epidemiologic trends of tuberculosis, it is likely that tuberculosis and congenital tuberculosis will continue to be observed in developed countries. The nonspecific features of congenital tuberculosis and the mortality associated with untreated disease underscore the importance of maintaining a high index of suspicion for tuberculosis in pregnant women and young infants.

Pathogenesis

Transmission of *M. tuberculosis* from the mother to the neonate can occur in utero, intrapartum, or postpartum. Although congenital infection is classically considered the result of in utero infection of the fetus, the term *congenital tuberculosis* has historically referred to infection acquired either in utero or intrapartum. The infection can be transmitted by direct spread to the fetus from the placenta via the umbilical vein or by aspiration/ingestion of infected amniotic fluid, either in utero or intrapartum.

TABLE 35.7 Reviews of Cases of Congenital Tuberculosis Cases Reported in the English-Language Literature in the Era of Chemotherapy

Reference	Years Cases Reported	No. of Cases	Age at Clinical Presentation (d)	No. of Infants With Reactive TST	Common Symptoms	Mortality (%) (With Treatment)
Hageman et al. (1980)	1952–1980	26	NR	2 of 14	Respiratory distress, fever, hepatomegaly	46 (12)
Cantwell et al. (1994)	1980–1994	31	Median 24 (range 1–84)	0 of 9	Hepatosplenomegaly, respiratory distress, fever	38 (22)
Abughali et al. (1994)	1952–1994	58	NR	1 of 19	Respiratory distress, hepatomegaly, fever	45 (14)
Laartz et al. (2002)	1994–2002	16	Mean 17.4 (range, 1–60)	1 of 4	Respiratory distress, hepatomegaly, fever	20

TST, Tuberculin skin test.

Historically, congenital tuberculosis criteria were described by Beitzke in 1935 to distinguish congenital tuberculosis from postnatally acquired tuberculosis. The criteria required that the infant have proven tuberculosis lesions and one of the following: (1) a primary hepatic complex as evidence of dissemination of the tubercle bacilli via the umbilical vein or (2) in the absence of a primary complex, the presence of tuberculous lesions in the first few days of life or the exclusion of postnatal infection by separation of the infant at birth from the mother and other potential sources of infection. These criteria were developed before the introduction of chemotherapy, when infant mortality with congenital tuberculosis was high and diagnosis was largely based on autopsy findings. The demonstration of a primary hepatic complex with liver and regional node involvement requires an open surgical procedure; a percutaneous liver biopsy may demonstrate caseating granulomas, but the primary complex will seldom be identified. Given the impractical nature of such criteria, Cantwell et al.[193] proposed a revised set of diagnostic criteria that became more applicable to current practice with improved diagnostic sensitivity. The infant must have proven tuberculous lesions and at least one of the following: (1) lesions in the first week of age, (2) a primary hepatic complex or caseating hepatic granulomas, (3) tuberculous infection of the placenta or maternal genital tract, or (4) exclusion or postnatal transmission by thorough investigation of contacts.

The risk of congenital tuberculosis in infants born to women with tuberculosis is unknown but likely low. Blackall[211] reported only three cases among infants born to 100 mothers with tuberculosis. Ratner et al.[212] identified no cases among infants born to 260 mothers with the disease. In a study of 1369 infants separated at birth from their tuberculous mothers and placed in foster care, only 12 became tuberculin-positive during 4 years of observation, and in all 12 cases there was a source of infection in the postnatal environment.[193] The low incidence of congenital TB is in part attributable to the high likelihood of infertility in women who have endometrial TB.[213] However, in areas with high rates of tuberculosis transmission, neonates may be undiagnosed or underreported, and the incidence of congenital infection or vertical transmission remains unknown.

Congenital TB is transmitted in one of three ways: (1) hematogenous spread from the infected placenta via the umbilical vein, (2) in utero aspiration or ingestion of amniotic fluid infected from the placenta or endometrium, or (3) ingestion of infected amniotic fluid or secretions from maternal genital lesions during delivery. In pregnant women, tuberculous bacillemia can result in dissemination of infection to the placenta, the endometrium, or the genital tract. Hematogenous seeding and in utero aspiration likely account for many congenital TB cases though actual documented sources are not well documented.[209,214–216] Several anecdotal cases of congenital TB have been reported in the literature associated with in vitro fertilization, for which, in retrospect, TB salpingitis was the likely cause of sterility.[217–219] While such events are likely to be associated with a very low frequency of congenital infection, they occur in more medically sophisticated areas that are likely to report them in the medical literature.

Tuberculous bacilli have been demonstrated in the decidua, amnion, and chorionic villi of the placenta.[214] It is unlikely that the fetus can be infected directly from the mother without the presence of a caseous lesion in the placenta, although massive involvement of the placenta does not always result in congenital tuberculosis. When a tubercle ruptures into the fetal circulation, bacilli in the umbilical vein can infect the liver, forming a primary focus with involvement of periportal lymph nodes. The bacilli also may pass through the liver and right ventricle and into the lungs, or they can enter the left ventricle via the foramen ovale and pass into the systemic circulation. The organisms in the lung remain dormant until after birth, when oxygenation and circulation result in their multiplication and the subsequent development of a primary pulmonary focus. Alternatively, if the caseous lesion in the placenta ruptures directly into the uterine cavity and infects the amniotic fluid, the fetus can inhale or ingest the bacilli, leading to primary foci in the lung, intestine, or middle ear. Pathologic examination of tuberculosis in the fetus and newborn usually demonstrates disseminated disease, with the liver and lungs being principally involved. Among 38 postmortem cases,[220] the lungs were involved in 97%, the liver in 82%, and the spleen in 76% of the infants. Other sites described are the gastrointestinal tract, kidneys, adrenal glands, and skin.[196,221,222] It is not always possible to determine whether sites represent multiple primary foci or are secondary to primary lesions in the lung or liver. The only lesion in the neonate that is unquestionably associated with congenital infection is a primary complex in the liver; all others may be acquired congenitally or postnatally.

M. tuberculosis infection acquired in utero or perinatally may be indistinguishable from postpartum infection. Postnatal acquisition of *M. tuberculosis* acquired by airborne inoculation, either from the mother or another contagious adult in the infant's environment, is the most common route of infection of the neonate. In addition, postnatal infection can occur from ingestion of infected breast milk from a mother with a tuberculous breast abscess. In the absence of a breast abscess, transmission of tuberculosis via breast milk has not been documented.[223] The distinction between congenital tuberculosis and postnatally acquired disease may be important for academic or epidemiologic purposes, but the management, treatment, and prognosis of the disease processes are the same.

Clinical Presentation

The clinical manifestations of congenital tuberculosis are protean. Complications from tuberculosis during pregnancy include stillbirth, recurrent abortion, and infertility.[224] A retrospective cohort study from Mexico of infants born to 35 mothers whose pregnancies were complicated by TB demonstrated an approximately two fold risk of prematurity compared with newborns of mothers without tuberculosis.[225] Manifestations of disease resemble those of neonatal sepsis or other congenital infections. The affected infant is commonly born prematurely.[226–231] Clinical signs may be evident shortly after birth, but typically do not appear until 2 to 4 weeks of age (see Table 35.8). Among the 29 cases reviewed by Cantwell et al.[193] the median age of presentation was 24 days. In an updated review of 16 cases reported since 1994, the mean age at presentation was slightly younger, at 17.4 days.[195]

Before the availability of INH, congenital tuberculosis was almost uniformly fatal. Notable signs included failure to thrive, jaundice, and central nervous system involvement. In the post-INH era, the most commonly described features of disease are respiratory distress, hepatomegaly with or without splenomegaly, and fever.[193,196,197,232] Additional findings are listed in Table 35.8. Although it is important to evaluate for meningitis in an infant with suspected congenital tuberculosis, central nervous involvement occurs in fewer than 50% of cases.[196,233] Otitis media with aural discharge has been described as the presenting sign of congenital tuberculosis,[234,235] accompanied by regional lymphadenopathy[201,236] or facial palsy.[205] It is presumed that the infection is due to the accumulation of infected amniotic fluid in the eustachian tube, either in utero or at birth. Cutaneous manifestations of congenital tuberculosis include papular, pustular, or vesicular lesions often surrounded by erythema.[222,237,238] Biopsy of the lesions is often confirmatory, demonstrating granulomatous inflammation and the presence of acid-fast bacilli (AFB) on tissue stain.[196,237] A unique case of congenital tuberculosis involving the spine was recently reported in India.[200]

Laboratory abnormalities among infants with congenital TB are also nonspecific, but include leukocytosis (63.8%), thrombocytopenia (80%), elevated inflammatory markers such as CRP (94.7%) or erythrocyte sedimentation rate (60.8%), and elevated liver function tests (76.4%).[232]

Evaluation

The timely diagnosis of congenital tuberculosis requires a high index of suspicion. Clinical signs of disease in the neonate are nonspecific, and disease in the mother may be unsuspected (as many cases are not diagnosed until the infant has been diagnosed), contributing to further delay in diagnosis. The diagnosis of congenital tuberculosis should be considered in any neonate with suspected infection who is unresponsive to conventional antimicrobial therapy, especially when the mother is from TB endemic regions. Evaluation for suspected disease should include a tuberculin skin test (TST), chest radiography, lumbar puncture, and mycobacterial culture of appropriate specimens. Biopsy specimens of affected tissue, either from the infant or the mother, and the placenta have been confirmatory in several case reports.[193,195–197,237,239]

The TST is the most commonly used diagnostic test for tuberculosis. The test uses five tuberculin units of purified protein derivative injected intradermally on the volar surface of the forearm. The reaction is measured 48 to 72 hours later as millimeters of induration. An estimated 10% to 40% of immune competent children with culture-proven tuberculosis do not initially react to a TST. Host factors such as young age and immunocompromised state can also decrease the sensitivity of the TST. Thus, the TST cannot be used to exclude the diagnosis and must be interpreted in the context of each patient. Specificity of the TST may be compromised by cross-reactivity with Bacille-Calmette-Guérin (BCG) vaccine or with environmental nontuberculous mycobacteria. The TST result is usually negative in neonates with congenital or perinatal tuberculosis, either secondary to immature cell-mediated immunity or because of overwhelming disease. Hageman et al.[196] found that only 2 of 14 infants who underwent skin testing had positive test results; on repeated testing, seven infants subsequently demonstrated positive tuberculin skin tests, the earliest being at 6 weeks of age, almost 4 weeks after presentation with clinical signs. Similarly, results of TSTs performed in 9 of 29 patients described by Cantwell et al.[193] were all negative, with results of subsequent testing being positive in two of the nine infants. Among the 16 infants with congenital tuberculosis

TABLE 35.8 Clinical Signs and Symptoms of Congenital Tuberculosis in 170 Infants

Sign	No. of Patients	% of Patients
Fever	107	64.4
Respiratory distress	106	63.8
Hepatomegaly with or without splenomegaly	108	65.6
Lethargy or irritability	66	39.7
Poor feeding	65	39.1
Failure to thrive	42	25.3
Cough	59	35.5
Cyanosis	39	23.4
Lymphadenopathy	34	20.4
Jaundice	23	13.8
Abdominal distention	37	22.2
Ear discharge	9	15
Skin lesions	17	10.2
Vomiting	14	8.4
Seizure	6	3.6

Adapted from Peng W, Yang J, Liu E. Analysis of 170 cases of congenital TB reported in the literature between 1946 and 2009. *Pediatr Pulmonol.* 2011;46:1215–1224.

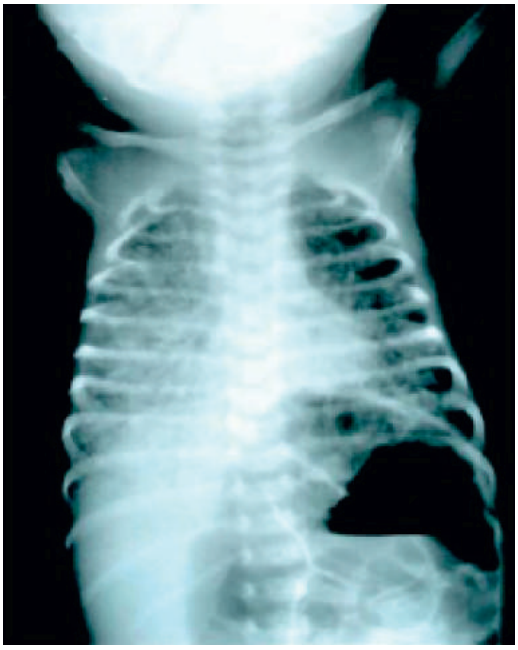
recently reviewed by Laartz et al.[195] three of four infants tested had nonreactive TST results.

Recent advances in diagnostic tools for tuberculosis include whole-blood interferon γ release assays (IGRAs), which are immunologically based tests that measure interferon γ production from lymphocytes in response to antigens that are specific to *M. tuberculosis*. The two types of assays currently available include the Quantiferon Gold (Cellestis, Valencia, California) and the enzyme-linked immunosorbent spot assay commercially known as the T-Spot.TB (Oxford Immunotec, Abington, Oxfordshire). Advantages of these tests include lack of cross-reactivity with the BCG vaccine and most nontuberculous mycobacteria. However, the correlation between IGRAs and TSTs is variable, and negative results do not definitively exclude tuberculosis. Moreover, published experience with the use of IGRAs in children is limited, and the negative predictive value of these tests in this population is unclear. Although IGRAs are endorsed by the CDC for use in circumstances in which a TST is indicated,[240] the tests are not FDA-approved for use in children younger than 5 years of age.[241] However, many experts in the pediatric infectious disease field have used the IGRAs in children between 2 and 4 years of age.[241] The sensitivity of IGRAs in children less than a year of age is reduced compared to adults,[242] and data on the use of IGRAs in newborns is limited to case reports.[219,243,244] For children 2 years of age or older, TST or IGRA can be used unless they have been BCG vaccinated, in which case the IGRA is preferred.[245]

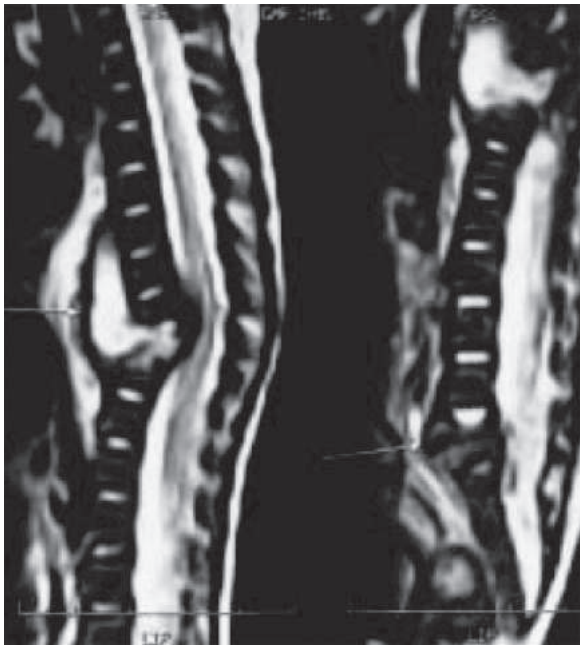
Given the frequency of respiratory distress in infants with congenital TB, it is not surprising that chest radiograph findings are frequently abnormal at first examination. In a meta-analysis of 170 infants with congenital tuberculosis between 1946 and 2009, 93.1% of all infants had an abnormality on chest radiograph. Among the 29 cases reviewed by Cantwell et al.[193] 23 infants (79%) had chest radiographic abnormalities, the majority being nonspecific infiltrates. Miliary disease was the most common specific radiographic characteristic (46.8%) (Fig. 35.7) followed by multiple pulmonary nodules (11.1%), lobar pneumonia (11.8%), bronchopneumonia (9.7%), and interstitial pneumonia (9%). Cavitation secondary to progressive pulmonary involvement has been reported.[246] Mediastinal adenopathy was also reported in 9.7% of infants. CT imaging of the chest may demonstrate adenopathy suggestive of TB or confirm miliary disease.[247,248] An ultrasound or CT image of the abdomen may reveal enlargement of the liver, spleen, or both, possibly with areas of abscesses,[200,226,235,249] necrotic retroperitoneal of intra-abdominal lymphadenopathy, or ascites.[232] Spinal disease associated with congenital tuberculosis identified by radiograph and confirmed by magnetic resonance imaging has been reported (Fig. 35.8).[200]

Microbiologic confirmation of disease in the neonate should be sought using specimens from multiple sites. For infants and children unable to expectorate sputum, gastric aspirates are considered the specimens of choice. Additional sources for culture include endotracheal aspirate, bronchial washing, middle-ear discharge, bone marrow, lymph node tissue, peritoneal fluid, or other suspected sites of disease. CSF should be analyzed and cultured, although isolation of *M. tuberculosis* from CSF is uncommon.[196,197] Traditionally, the detection of mycobacterial organisms by smear or culture has been considered difficult because children have paucibacillary disease relative to adults. With three morning gastric aspirates collected appropriately in hospitalized children with a clinical diagnosis of tuberculosis, only 40% of children had positive cultures.[250] Only 5% to 12% of samples are actually acid-fast smear positive[251] though fluorescent staining methods are more sensitive than AFB smear.[252] In comparison, cultures of aspirates from infants (younger than the childhood studies discussed before) evaluated at the same institution had a 75% yield.[250,253]

The improved diagnostic yield in infants likely reflects more widely disseminated and progressive disease, with higher bacillary



• **Fig. 35.7** Miliary tuberculosis in a neonate with congenital tuberculosis. (From Singh M, Kothur K, Dayal D, et al: Perinatal tuberculosis a case series, *J Trop Pediatr* 53:135-138, 2006.)



• **Fig. 35.8** Sagittal MRI on the *left* demonstrates destruction of T9 to T11 vertebral bodies with the collapse of T10, leading to a kyphotic deformity causing cord compression. A large prevertebral collection is also seen. The image on the *right* demonstrates destruction with collapse of L5 and S1 vertebral bodies. (From Grover SB, Pati NK, Mehta R, et al. Congenital spine tuberculosis: early diagnosis by imaging studies. *Am J Perinatol*. 2003;20:150.)

loads. Hageman et al. found positive cultures of *M. tuberculosis* in 10 of 12 gastric aspirates, 3 of 3 liver biopsy specimens, 3 of 3 lymph node specimens, and 2 of 4 bone marrow biopsy specimens.[196] Among the 31 cases reviewed by Cantwell et al.[193] noninvasive procedures and biopsy were useful for the diagnosis of congenital tuberculosis in the majority infants (Table 35.9). More recent reports confirm the high yield of cultures from a variety of specimens in neonates.[203,230,231,239,249] Histologic examination of tissue may suggest the diagnosis before culture results are available. For example, histopathological evidence of granulomas or acid fast bacilli on stained tissue samples of skin lesions, lymph nodes, and the liver have suggested the diagnosis before culture results were available.[196,227,249]

Whereas *M. tuberculosis* can require 7 to 42 days for growth by standard culture technique, results from techniques such as PCR may be available within 48 hours. A comparison of PCR, AFB smear, and culture with clinical diagnosis in children found a sensitivity of 60% and a specificity of 97%.[254] Although PCR has been useful for diagnosing congenital tuberculosis in a few case reports, it is not sensitive enough to preclude obtaining specimens for culture. Moreover, isolation of *M. tuberculosis* by culture is still important to determine susceptibilities and to optimize treatment.

The mother of a newborn in whom congenital tuberculosis is suspected is often asymptomatic or has subclinical disease. In the series of congenitally infected infants reported by Hageman et al. the majority of mothers (16 of 26) did not have a diagnosis until after the disease became apparent in their infants.[196] Cantwell et al. found that 50% of the mothers of infected infants were not ill at the time their newborns exhibited clinical signs of disease.[193] Evaluation of the mother should include a TST, a chest radiograph, and, if the radiograph is consistent with TB disease, collection of sputum for microbiological confirmation. Extrapulmonary disease such as meningitis or peritonitis occurs with some regularity among mothers with children born with congenital tuberculosis,[195,255] and evaluation may need to be extended to identify such sites if pulmonary disease is not discovered. In mothers with no clinical evidence of disease, endometritis should be considered.[256] Pathologic examination and culture of the placenta (if available) or endometrial biopsy can confirm the diagnosis of genital transmission.[193,213,257–260] In several case reports, the diagnosis of maternal tuberculosis was ultimately made by endometrial biopsy and culture.[205] Culture of amniotic fluid should be performed when genital involvement is suspected. All mothers with tuberculosis should be tested for HIV infection and, if the mother is seropositive, the infant should be evaluated for perinatally acquired HIV infection.

TABLE 35.9 Results of Diagnostic Procedures Performed on 29 Infants With Congenital Tuberculosis Reported from 1980 to 1994

Type of Specimen	Acid-Fast Smear*	Mycobacterial Culture	Smear or Culture
Gastric aspirate	8/9	8/9	9/11
Endotracheal aspirate	7/7	7/7	7/7
Ear discharge	2/2	1/1	2/2
Cerebrospinal fluid	1/2	1/2	1/2
Urine	0/2	0/2	0/2
Peritoneal fluid	1/1	1/1	1/1
Bronchoscopic specimen	1/1	1/1	1/1
Biopsy specimen	14/19	11/12	16/21
Lymph node	7/8	6/6	7/8†
Liver	4/6	1/2	4/6†
Skin	1/3	1/1	1/3
Lung	1/1	1/1	2/2
Bone marrow	—	1/1	1/1
Ear	1/1	1/1	1/1

*Results expressed as number of positive results per number of patients tested.
†All biopsy specimens of lymph node and liver that tested negative on smear and culture showed histopathologic changes consistent with tuberculosis (i.e., giant cell transformation of granulomas, with or without caseation).
Adapted from Cantwell MF, Shehab ZM, Costello AM, et al. Brief report: congenital tuberculosis. *N Engl J Med.* 1994;330:1051.

Management

The successful management of congenital tuberculosis depends on early recognition and treatment of disease. In suspected cases, treatment should not be delayed while awaiting culture results or other diagnostic tests. Multiple drug therapy for an extended duration has long been recognized as the standard of care for tuberculosis. Because of the rarity of the condition, clinical trials have not been conducted to establish the optimal treatment regimen for congenital tuberculosis. It is assumed that the regimens used for older infants and children are safe and effective for the treatment of neonates with congenital tuberculosis. Consultation with a pediatric infectious disease specialist or tuberculosis expert is advised.

Until susceptibility results are known, infants with proven or suspected tuberculosis should be treated with a four-drug regimen consisting of INH, rifampin (RIF), pyrazinamide (PZA), and ethambutol or an aminoglycoside (Table 35.10). Some experts would recommend administration of three drugs (INH, RIF, PZA) if antimicrobial resistance is not suspected in the mother (e.g., known susceptible strain in either mother or the source case or mother has no risk factors for resistant *M. tuberculosis*). Supplementation with pyridoxine, although not routinely recommended for otherwise healthy older children, should be provided to breastfeeding infants receiving INH. If the *M. tuberculosis* isolate is determined to be susceptible, the regimen can be narrowed to three drugs (INH, RIF, PZA) for the first 2 months of initial treatment, and subsequently to two drugs (INH, RIF) to complete the continuation phase of treatment. The adjunctive use of corticosteroids is recommended for the treatment of tuberculosis meningitis based on decreased mortality and morbidity demonstrated in adults and children[261] and has been used (though controversial) in cases of endobronchial obstruction, pericardial/pleural disease. Once the infant is discharged to home, directly observed therapy is recommended to ensure adherence and to prevent relapse.

The optimal duration of treatment for infants with congenital tuberculosis is unknown. The typical duration of treatment for susceptible *M. tuberculosis* is 6 months for pulmonary disease, pulmonary disease with hilar adenopathy, or hilar adenopathy

TABLE 35.10 Commonly Used Drugs for Treatment of Tuberculosis in Infants, Children, and Adolescents

Drugs	Dose Forms	Daily Dose (mg/kg)	Twice per week Dose (mg/kg)	Maximum Dose	Adverse Reactions
Ethambutol	Tablets (100, 400 mg)	20 (15–25)	50	2.5 g twice a week, 1 g daily	Optic neuritis (usually reversible), decreased red-green color discrimination, gastrointestinal tract disturbances, hypersensitivity
Isoniazid	Scored tablets (100, 300 mg) Syrup 10 mg/mL	10 (10–15)*	20–30	300 mg daily	Mild hepatic enzyme elevation, hepatitis,* peripheral neuritis, hypersensitivity Diarrhea, and gastric irritation caused by vehicle in the syrup
Pyrazinamide	Scored tablets (500 mg)	30–40	50	2 g	Hepatotoxic effects, hyperuricemia, arthralgia, pruritus, rash, gastrointestinal tract upset
Rifampin	Capsules (150, 300 mg) Syrup formulated capsules	15–20	15–20	600 mg	Orange discoloration of secretions or urine, staining of contact lenses, vomiting, hepatitis, influenza-like reaction, thrombocytopenia, pruritus; oral contraceptives may be ineffective

*When isoniazid in a dose exceeding 10 mg/kg/day is used in combination with rifampin, the incidence of hepatotoxic effects may be increased.
From American Academy of Pediatrics Committee on Infectious Diseases. Section 3: Summaries of infectious diseases, tuberculosis. In: Kimberlin DW, Barnett E, Lynfield R, Sawyer MH, eds. *Red Book: Report of the Committee on Infectious Diseases*. 30th ed. Elk Grove Village, IL: American Academy of Pediatrics; 2021.

alone.[245,262] Except for meningitis, extrapulmonary disease can be treated for the same duration as pulmonary disease. For meningitis, the duration is extended to 9 to 12 months. The duration of treatment for drug-resistant disease can be prolonged up to 24 months or longer depending on the clinical course.[262] Most experts would treat infants with congenital tuberculosis for 9 to 12 months because of the decreased immunocompetence of neonates.[233]

Although there is a substantial amount of data to support the safety of INH, data on the safety and pharmacokinetics of other agents are limited. While INH toxicity is rare in otherwise healthy infants and children for which monitoring is not required, routine determination of serum transaminases and careful monitoring of hepatitis symptoms is indicated in children with severe tuberculosis (e.g., miliary or meningitis), those with concurrent liver or biliary disease, or for those receiving other potentially hepatotoxic drugs. Routine monitoring of liver function should also be considered for neonates with congenital tuberculosis given the paucity of data on adverse effects of antituberculosis agents in this age group.[263] The risks of optic neuritis with ethambutol should be considered when this agent is used, and vision should be monitored periodically.

Outcomes

The prognosis for congenital tuberculosis was dismal in the pre-chemotherapy era, the diagnosis often being only made at autopsy. Although the survival rate subsequently improved, mortality remained approximately 50% due to delayed diagnosis. In a review of 26 cases reported between 1952 and 1980, 12 (46%) patients died, 9 of whom were untreated but with a diagnosis made at autopsy. Similarly, in a review of 92 cases from China between 1976 and 2018, the mortality rate was 44%.[210] The subsequent reviews demonstrate a decrease in case fatality (see Table 35.8), with earlier diagnosis and treatment. Timely diagnosis and initiation of antituberculosis therapy are critical for a favorable outcome.

Prevention

Early diagnosis and treatment of *M. tuberculosis* infection in women of childbearing age is the optimal method of preventing congenital tuberculosis. Risk factors for acquiring tuberculosis infection or progressing to disease should be assessed at prenatal visits, and women with high risk for *M. tuberculosis* infection should undergo tuberculin skin testing or IGRA as recommended by the CDC.[264] More recent studies indicate that IGRA performance is not altered during pregnancy with better compliance in test completion rates compared to TST.[265,266] Previous studies have shown the greatest discordance with IGRA and TST, especially among those with prior BCG vacation.[267] Women who test positive for *M. tuberculosis* infection either by TST or IGRA should undergo evaluation for active disease. The treatment of active tuberculosis during pregnancy is considered standard, and early treatment has been shown to improve maternal and neonatal outcome.[268] The benefit of treating tuberculosis during pregnancy far outweighs the potential risk to the pregnant woman and her fetus. The treatment of latent Mtb infection (LTBI) during pregnancy is somewhat more controversial. In a multicenter, double-blind, placebo-controlled, non-inferiority trial, HIV-infected pregnant women living in TB endemic areas were randomized to either receive INH during pregnancy or 3 months post partum. While the earlier INH treatment was noninferior to postpartum treatment with respect to the incidence of TB and maternal adverse events, there were more adverse pregnancy outcomes (e.g., stillbirth, low birth weight) among the early treatment group.[269] For this reason, the CDC recommends that LTBI treatment in pregnancy be deferred until 2 to 3 months postpartum unless there is significant increased risk of progression to active TB, such as recent infection. This recommendation contrasts with the WHO in which the results of several other studies showed no evidence of adverse outcomes. Accordingly, the WHO recommends that treatment for LTBI should be given to pregnant women at risk for TB regardless of degree of immune

suppression and LTBI testing.[270] In short, benefits and risk of treatment should be considered for each pregnant patient as the postpartum period may be associated with an increased risk of more severe forms of active disease.[245,271]

The management of infants born to mothers with LTBI or tuberculosis disease are based on the categorization of infection in the mother and the potential risk of transmission of tuberculosis to the infant.[245,262] Infants born to mothers with potentially contagious tuberculosis should be evaluated for congenital tuberculosis that includes a TST and IGRA (though its sensitivity is unknown), chest radiograph, lumbar puncture, and appropriate cultures (e.g., gastric aspirate, CSF).[245] Separation of the infant and mother is necessary only in cases in which the mother is considered to be infectious at the time of delivery, nonadherent to medication, has drug-resistant disease, or who has not been on at least 2 weeks of treatment.[224] A mother with latent tuberculosis infection is not contagious. To prevent reactivation disease in the mother and subsequent exposure of the infant, the mother should receive treatment with INH for LTBI if she was not treated during pregnancy. In addition, latent tuberculosis infection in the mother may be a marker for contagious tuberculosis within the household, and it is recommended that all household members and close contacts of the mother be evaluated for tuberculosis.

Breastfeeding is not contraindicated in women with LTBI. The breast milk of a woman with tuberculosis does not contain tubercle bacilli. For women with tuberculosis who are potentially infectious and separated from the newborn, breast milk may be manually expressed and fed to the infant. Once the mother is noninfectious or the infant is receiving therapy, breastfeeding can be resumed. The exception, however, is the mother with an active tuberculous breast lesion. In this situation, the breast milk may be pumped and discarded until resolution of the lesion.[272]

With regard to isolations practices, airborne precautions are recommended for the following pediatric patients: (1) children with and adolescents with adult-type cavitary disease, (2) extensive pulmonary involvement, (3) laryngeal involvement, (4) those with smears positive for AFB, and (5) congenitally infected neonates undergoing oropharyngeal procedures (e.g., endotracheal intubation).[262] In general, children below the age of 10 years are not considered contagious given the lack of tussive force they are able to generate and the paucibacillary load. However, the adult contacts of that child may be the source case and potentially contagious. Thus, visitation of the hospitalized pediatric patient should be restricted to adults in whom contagious tuberculosis has been excluded. Hospitalized children with negative sputum AFB smears (if obtained) require standard precautions, assuming that contagious tuberculosis has been excluded in the visitors.

Compared with older children, neonates likely have a higher concentration of bacilli in their sputum. As noted previously, AFB smears on tracheal aspirates and other specimens are frequently positive in this population compared with older children. Transmission of tuberculosis from congenitally infected neonates to health care workers and other hospitalized infants has been reported and is likely related to aerosolization of bacilli during respiratory manipulation.[195,273–275] Neonates suspected of having congenital tuberculosis should be cared for with airborne infection isolation precautions if intubated or if undergoing any procedure with the potential for aerosolization of infected sputum. Exposed infants, visitors, and health care workers should undergo evaluation for tuberculosis infection or disease.

Suggested Readings

Congenital Toxoplasmosis

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36

Fungal Infections in the Neonatal Intensive Care Unit

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KEY POINTS

- Fungal infections account for approximately 12% of neonatal late-onset sepsis, with a mortality rate of approximately 32%, and the most important risk factor remains the gestational age at birth.
- When a lumbar puncture is performed, 10% to 50% of candidemic infants have associated meningitis and a significant percentage of extremely low birth weight (ELBW, BW<1000 g) infants with *Candida* meningitis have negative blood cultures.
- *Candida* species isolated from a blood culture should be considered as an infection, not a contaminant.
- Rapid institution of parenteral amphotericin B deoxycholate is the therapy of choice for systemic fungal infection; if there is also meningitis, 5-flucytosine or fluconazole should be added.
- Central venous catheters should be removed within 24 hours after the identification of yeasts in the blood culture, if possible.
- The finding that prophylactic fluconazole reduces the incidence of invasive neonatal fungal infections should be interpreted with caution; nevertheless, recent guidelines recommend fluconazole prophylaxis in ELBW infants in nurseries with high rates (>10%) of invasive candidiasis.

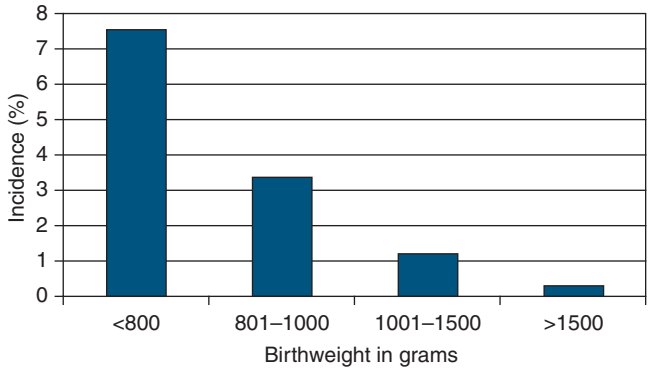
Epidemiology

Invasive fungal infection accounts for approximately 10% of healthcare associated infections in very low birth weight infants[1] and occurs in approximately 1% to 2% of all infants admitted to US neonatal intensive care units (NICUs).[2] The incidence rises dramatically with decreasing gestational age and birth weight. Fungal infections are associated with high mortality and morbidity, including poor neurodevelopmental outcomes in extremely low and very low birth weight infants.[3] Among the fungi, *Candida* species (spp.) are the dominant pathogens. There are over 200 identified species of *Candida*,[4] but fewer than 12 appear to cause disease in neonates. These infections are evenly distributed among *C. albicans* and non–*C. albicans* spp. (*C. parapsilosis, C. orthopsilosis/C. metapsilosis, C. glabrata, C. guilliermondii, C. krusei*, and *C. lusitaniae*). *Candida* spp. are the third most common pathogen in neonatal nosocomial bloodstream infections and carry a fatality rate more than sevenfold greater than that of *Staphylococcus epidermidis*, the most common pathogen found in the NICU.[5,6] In late-onset sepsis, fungi account for approximately 12% of cases, with a mortality rate of 32%.[7] Other fungi encountered include *Aspergillus (fumigatus* and *flavus)* and *Malassezia (furfur* and *pachydermatis)*. The yeast *Cryptococcus neoformans* and the fungi *Histoplasma capsulatum, Blastomyces dermatitidis*, and *Coccidioides immitis* are rarely seen in the NICU.

Pathophysiology

The most important risk factor for fungal infection is gestational age. In a survey of 2847 infants from six different nurseries, the incidence of candidemia in infants weighing less than 800 g (7.55%) was 25 times that of the infants weighing more than 1500 g (Fig. 36.1).[8] This latter group acquires bloodborne candidal infection in association with congenital anomalies, especially those of the gastrointestinal tract.[9] Candidemia carries a mortality rate exceeding 25% in most studies.[10,11]

Colonization with ubiquitous fungal spp. occurs in at least 25% of very low birth weight (VLBW) infants,[12] and both the amount of *Candida* in the gastrointestinal tract[13] and colonization at sites such as endotracheal tubes[14] have been correlated with increased risk of invasive disease caused by *Candida* spp. Prospective studies correlating colonization by other fungal genera (e.g., *Aspergillus, Malassezia*) with risk of invasive disease have not been done.



• **Fig. 36.1** Incidence of Candidemia related to birthweight in grams. (Data from Saiman L, Ludington E, Pfaller M, et al. Risk factors for candidemia in neonatal intensive care unit patients. The National Epidemiology of Mycosis Survey study group. *Pediatr Infect Dis J*. 2000;19:319–324.)

Apart from colonization and gestational age, other host factors that contribute to the susceptibility of the NICU neonate to fungal infection include a 5-minute Apgar score of less than five and an age-dependent immunocompromised state ascribable to reduced numbers of T cells, impaired phagocyte number and function, and reduced levels of complement.[15–18] Other factors thought to increase the risk of fungal infections include length of NICU stay greater than 1 week, indwelling central venous catheters (CVCs), abdominal surgery, parenteral nutrition, intralipids, H_2 blockers, endotracheal intubation, and prolonged use of broad-spectrum antimicrobials, especially third-generation cephalosporins.[6,8,19] Some studies have identified associations with systemic steroids and catecholamine infusions and with topical petrolatum.[5,20,21]

A number of variables appear to not be associated with candidal colonization, including use of antibiotics in the mother, premature rupture of the membranes, the infant's gender, use of antimicrobial agents other than third-generation cephalosporins in the infant, surgical procedures, or frequency of intubation.[6] Although approximately 5% of NICU staff carry *C. albicans* on their hands and 19% carry *C. parapsilosis*, there is no correlation with site-specific rates of neonate colonization.[6]

This chapter will place its major emphasis on infections caused by *Candida* spp. and will also discuss infections caused by other fungi, as well as the approach to diagnosis, treatment, and management of infants with fungal infection.

Infections Caused by *Candida* Species

Congenital Candidiasis

Congenital candidiasis typically presents within the first 24 hours of life in both full-term and premature neonates. The infection manifests as a deeply erythematous skin rash in the setting of pronounced neutrophilia, with white blood cell counts often rising to 50,000 mm^3 or more. *Candida* funisitis (discussed later in the chapter) can be an infrequent accompaniment. In the full-term neonate, there are usually no invasive consequences, and desquamation typically ensues within 2 to 3 days. In contrast, the condition is life-threatening in the premature neonate[22,23] and is distinguished by a pustular rash, hazy infiltrates reminiscent of respiratory distress syndrome on chest radiograph, and frequently positive blood cultures. The premature neonate is thought to acquire the organism from inhalation of infected amniotic fluid.

The diagnosis of congenital candidiasis in both premature and full-term neonates requires visualization of the organism on Gram stain from a bullous lesion or an opened pustule. On rare occasions, placental culture can yield the diagnosis. Treatment for the full-term neonate requires only the full-body application of topical antifungal creams containing either nystatin or azoles such as miconazole or clotrimazole. In the premature neonate, the initiation of parenteral amphotericin B deoxycholate at a dose of 1 to 1.5 mg/kg is mandatory (this dose may be reduced to 1.0 mg/kg), but respiratory involvement typically heralds death despite antifungal therapy. (Except where noted, all dosages come from *Nelson's Pediatric Antimicrobial Therapy*, 2017 edition, AAP Press.)

Local Infections With *Candida* Species

Diaper Dermatitis

This entity presents as an erythematous, erosive dermatitis of the perineal region, typically with pustular "satellite lesions" beyond the borders of the rash. Predisposing factors include systemic antibiotics, glucosuria, and wet diapers. Care must be taken to differentiate this tractable condition from invasive fungal dermatitis (discussed later in the chapter). Candida diaper dermatitis responds well to topical antifungal ointments.

Funisitis

Infection of the umbilical cord with *Candida* spp., while rare, is an indicator of chorioamnionitis and carries a poor prognosis, especially in the premature neonate.[24]

Urinary Tract Infection

Isolation of *Candida* spp. from a catheterized specimen or via suprapubic bladder aspiration, as opposed to a bagged sample, is a reliable indicator of infection. However, asymptomatic colonization of urinary catheters, stents, or nephrostomy tubes can be difficult to distinguish from true infection.[25]

The presence of candiduria in the NICU neonate is associated with renal candidiasis—the latter manifested by cortical abscesses or fungal mycelia in the collecting system ("fungus balls")—nearly half the time and may be a cause of frank obstruction.[26] Thus, in contrast to older children or adults, the finding of candiduria in a neonate should prompt blood cultures and renal imaging, at the very least. If blood cultures are positive, a full evaluation for disseminated candidiasis should be undertaken (discussed later in the chapter).

Because of the high prevalence of associated upper urinary tract disease, imaging of the kidneys by ultrasonography should be performed upon isolation of *Candida* spp. from a catheterized urine specimen or from a suprapubic bladder aspiration. Approximately half of patients who eventually develop upper tract manifestations will display them on the first ultrasound.[26] Therefore, follow-up imaging is recommended both to ensure the clearance of fungal mycelia, if present, and to monitor for later development of this complication. Unfortunately, no standard interval for monitoring has been proposed. However, in the infant who remains persistently funguric or candiduric, a single negative ultrasound should not be considered definitive.

Removal of a colonized urinary catheter may suffice for treatment in a patient without pyuria or systemic symptoms. Disease confined to the lower tract is best addressed with azoles (e.g., fluconazole, 4 to 6 mg/kg/day). Upper tract disease requires parenteral amphotericin B in systemic doses (1 mg/kg/day). Liposomal amphotericin B may not be an acceptable alternative. The particles in at least one liposomal preparation (Abelcet) appear to be too large to penetrate the adult kidney, and treatment failures with Abelcet in premature newborn with persistent candiduria have been reported.[27] Therefore, amphotericin B deoxycholate appears to be the best alternative.[27]

Peritonitis

Candida peritonitis typically develops as a consequence of bowel perforation or, rarely, as a complication of peritoneal dialysis. In the former situation, multiple organisms such as gram-negative rods and enterococci may also be involved, and the neonate is at risk for sepsis with any one of them.[28] Peritonitis associated with a peritoneal dialysis catheter usually occurs as an isolated process, and the outcome is much better.

Spontaneous intestinal perforation associated with *Candida* peritonitis with or without sepsis has been described within 7 to 10 days of birth in neonates weighing less than 1000 g, typically in the absence of necrotizing enterocolitis.[29–32] Hallmarks include

bluish discoloration of the abdomen and a gasless pattern on abdominal film. A substantial proportion of these neonates will have systemic candidiasis, although *S. epidermidis* can also be seen. In a small study of seven patients,[32] deficiency of the muscularis propria was found in six.

Diagnosis of Candida peritonitis requires visualization of the organism on a Gram stain of sterilely obtained peritoneal fluid or culture of the organism from the same source. Isolation of *Candida* spp. from the peritoneal fluid should always prompt a search for bowel perforation, either by radiology or by surgical exploration, depending upon the clinical circumstances.

Treatment of *Candida* peritonitis caused by necrotizing enterocolitis or bowel perforation requires surgical evaluation, supportive therapy, and directed treatment of all contaminating microorganisms in the peritoneal fluid and the bloodstream. The typical regimen should address enterococci, gram-negative rods, and anaerobes together with systemic antifungal therapy, with the most likely choice being amphotericin B. *Candida* spp. isolated from peritoneal dialysate can be treated with removal of the catheter and a short course (7 to 10 days) of amphotericin B therapy in a dose of 0.3 to 0.5 mg/kg/day. In cases of *Candida* peritonitis associated with a peritoneal dialysis catheter, the catheter can typically be reinserted within 24 to 48 hours, once the Gram stain is free of yeast cells.

Systemic Infection

Candidemia Associated With Central Venous Catheters

The association between prematurity and bloodborne candidal infections has been recognized for 35 years.[33,34] Over this same period of time, the incidence of candidemia has escalated from 25 per 10,000 NICU admissions to 123 cases per 10,000 NICU admissions.[8,35] The median time of onset is approximately 30 days of age.[33] In a large multicenter study, colonization of the gastrointestinal tract preceded candidemia in 43% of cases.[8]

A variety of nonspecific clinical findings may be associated with this presentation of candidal disease, including respiratory decompensation, feeding intolerance, temperature instability, or mild thrombocytopenia. It is unclear whether the latter manifestation relates more to the use of heparin in intravenous (IV) and peripheral catheters or to the presence of *Candida* spp. in the bloodstream.

Any *Candida* spp. isolated from a blood culture should be regarded as an infection and should prompt an immediate search for evidence of dissemination, which occurs in approximately 10% of premature newborns with candidemia.[36,37] A thorough evaluation includes ophthalmologic examination and ultrasonography of the heart, venous system, and abdomen. When lumbar puncture is performed, 10% to 50% of candidemic neonates may have associated meningitis;[3,38] in one prospective study, nearly 50% of ELBW infants with *Candida* meningitis (13/27) had negative blood cultures.[3]

Numerous studies have shown that CVCs should be removed within 24 hours of identification of yeasts in the blood culture;[39] in particular, removal of the CVCs within 3 days is associated with a significantly shorter median duration of candidemia (3 days vs. 6 days) and a reduced mortality rate (0% vs. 39%). In at least one study of candidemia, delayed removal of CVCs was associated with neurodevelopmental impairment at 18 to 22 months.[3] Many experts recommend routine echocardiograms for patients with catheter-associated candidemia, to look for thrombi before removal of the catheter. However, even with the prompt removal of the catheter and institution of appropriate antifungal therapy, a substantial proportion of infants may exhibit prolonged candidemia lasting 1 to 3 weeks.[10]

Disseminated Candidiasis

Disseminated candidiasis is a dreaded complication of candidemia, and mortality rates from disseminated candidiasis approach 30%. As in other forms of candidiasis, *C. albicans* is the leading pathogen. Organ involvement is most common in the vascular tree at catheter sites (15%), followed by the kidneys (8%).[36,37] Eye involvement occurs in approximately 6% of infants. Thrombi within the vascular bed may be particularly difficult to eradicate with antifungal therapy; infants with right atrial thrombi may benefit from atriotomy.[40] Other sites less frequently involved include the liver, spleen, and skeletal system. In infection of the bones and joints in premature newborns, *Candida* spp. are typically the second most likely pathogen, preceded by *S. aureus*.[41]

Antifungal Therapy for Systemic Infection

As is true of most medications used in the NICU, dosing recommendations for antifungal therapies have not undergone rigorous testing in this patient population. With that caveat in mind, practice guidelines for this difficult clinical problem are suggested (Table 36.1). There are four widely used classes of drugs in the treatment of invasive fungal infections in children and infants, including the polyenes, pyrimidine analogues, azoles, and echinocandins.[42] Fluconazole and Amphotericin B are the most commonly used antifungals in the neonatal period.[43] Amphotericin B is the "gold standard" antifungal agent for treatment of systemic neonatal fungal infection. amphotericin B binds to ergosterol in the membrane of fungi, facilitating membrane leakage. Rapid institution of parenteral amphotericin B deoxycholate in doses of 1 mg/kg/day, given by IV infusion over 2 to 6 hours, is the therapy of choice for systemic infection, including catheter-associated candidemia and disseminated candidiasis. Liposomal amphotericin B in a single dose of 5 mg/kg/day as an IV infusion over 2 hours[44,45] is an equivalent choice for initial therapy, but clinicians should be aware that some lipid formulations (e.g., Abelcet) have decreased renal penetration and are therefore not appropriate choices in disseminated candidiasis with renal infection.

Azoles are fungistatic agents that interfere with ergosterol synthesis by inhibiting C-14 alpha demethylase, a cytochrome P450 enzyme. Some experts recommend this class of antifungals as initial therapy for *C. albicans* infection because of their fungistatic effects; others use them primarily to complete a course of antifungal therapy after clearance of the infecting organisms. Fluconazole, the most frequently used antifungal in neonates, requires an IV loading dose of 25 mg/kg on day 1, with subsequent IV doses of 12 mg/kg/day for the remainder of therapy. Azoles such as itraconazole and posaconazole are preferable to fluconazole for *Aspergillus* and zygomycetes. However, *Aspergillus* and zygomycetes are extremely rare in the NICU; therefore, no studies have been done to recommend neonatal dosing guidelines.

In the 2016 Infectious Diseases Society of America candidiasis guidelines, echinocandins are the primary drugs of choice for invasive candidiasis.[46] Caspofungin, an echinocandin, has also been used in dosages of 25 mg/m^2/day to treat invasive candidal disease in the newborn in several case reports. Caspofungin and other echinocandins such as micafungin (10 mg/kg/day) or anidulafungin

TABLE 36.1 Summary of Antifungals in Infants

Drug	Neonatal	FDA Approved in Children	FDA Indication
Polyenes			
Amphotericin B deoxycholate	1 to 1.5 IV mg/kg/day	No	Potentially life-threatening fungal infections: aspergillosis, cryptococcosis (torulosis), North American blastomycosis, systemic candidiasis, coccidioidomycosis, histoplasmosis, zygomycosis including mucormycosis due to susceptible species of the genera *Absidia, Mucor*, and *Rhizopus*, and infections due to related susceptible species of *Conidiobolus* and *Basidiobolus*, and sporotrichosis
Liposomal amphotericin B	3 to 7 IV mg/kg/day	1 mo to 16 yr	Empirical therapy for presumed fungal infection in febrile, neutropenic patients; treatment of *Cryptococcus* meningitis in HIV-infected patients; treatment of patients with *Aspergillus* species, *Candida* species, and/or *Cryptococcus* species infections refractory to amphotericin B deoxycholate, or in patients where renal impairment or unacceptable toxicity precludes the use of amphotericin B deoxycholate; treatment of visceral leishmaniasis
Amphotericin B lipid complex	5 mg/kg/day	<16 yr	Invasive fungal infections in patients who are refractory to or intolerant of conventional amphotericin B therapy
Pyrimidine analogues			
Flucytosine	General dosing: 75 mg/kg/day PO divided q6h to q8h Candidal meningitis: 75 to 100 mg/kg/day PO divided q6h to q8h in combination with amphotericin B	No	Serious infections caused by susceptible strains of *Candida* and/or *Cryptococcus* Use with extreme caution in renally impaired patients and monitor closely for hematologic, renal and hepatic toxicity
Azoles			
Fluconazole	Treatment of systemic infection Postnatal age < 8 days Loading dose: 12 to 25 mg/kg IV Maintenance: 12 mg/kg IV/PO q48h Postnatal age > 8 days Loading dose: 12 to 25 mg/kg IV Maintenance: 12 mg/kg IV/PO q24h Candidiasis prophylaxis < 30 weeks' gestation Postnatal age < 7 days 3–6 mg/kg IV twice weekly Postnatal age > 7 to 42 days 3 mg/kg IV q24h or 6 mg/kg IV q72h Postnatal age > 42 days 6 mg/kg IV q48h Candidiasis prophylaxis 30 to 40 weeks' gestation 6 mg/kg IV q48h	>6 mo	Oropharyngeal candidiasis, esophageal candidiasis, systemic candidiasis, cryptococcal meningitis and prophylaxis of invasive candidiasis in immunocompromised children

Continued

TABLE 36.1 Summary of Antifungals in Infants—cont'd

Drug	Neonatal	FDA Approved in Children	FDA Indication
Echinocandins			
Caspofungin	Preterm neonates and infants < 3 mo 25 mg/m² IV q24h Infants >3 mo Initial: 70 mg/m² IV once Maintenance: 50 mg/m² IV q24	>3 mo	Empirical therapy for presumed fungal infections in febrile, neutropenic patients; treatment of candidemia and the following *Candida* infections: intraabdominal abscesses, peritonitis, and pleural space infections; treatment of esophageal candidiasis; treatment of invasive aspergillosis in patients who are refractory to or intolerant of other therapies
Micafungin	Neonates 10 mg/kg IV q24h Infants and children 2 to 4 mg/kg IV q24h	>4 mo	Candidemia, acute disseminated candidiasis, Candida peritonitis and abscesses; treatment of patients with esophageal candidiasis; prophylaxis for patients undergoing hematopoietic stem cell transplantation
Anidulafungin	Initial: 3 mg/kg once followed by 1.5 mg/kg q24h	No	Candidemia and other forms of *Candida* infections (in intraabdominal abscess and peritonitis), esophageal candidiasis

(1.5 mg/kg/day) interrupt biosynthesis of β-(1,3)-D-glucan, an integral part of the fungal cell wall. However, identification of infecting spp. has important implications for choice of therapy. Although amphotericin B formulations, the azoles, and the echinocandins are all appropriate for infections caused by *C. albicans*, non–*C. albicans* spp. such as *C. glabrata, C. guilliermondii*, and *C. krusei* may have decreased susceptibility to fluconazole and can be variably sensitive to the echinocandins.[47–49] *C. lusitaniae* is usually resistant to amphotericin B formulations.

Some experts recommend institution of empiric antifungal therapy in acutely thrombocytopenic neonates of less than 25 weeks' gestation, especially if there is a recent exposure to third-generation cephalosporins.[50] Dosage adjustment for renal dysfunction is necessary only if serum creatinine increases significantly during therapy. Amphotericin B has poor cerebrospinal fluid penetration; in neonates with accompanying meningitis, fluconazole may also be added to amphotericin B. The use of 5-flucytosine in doses of approximately 25 mg/kg per dose (range 12.5 to 37.5 mg/kg) given orally every 6 hours in patients with normal renal function is no longer routinely recommended because of bone marrow suppression or hepatotoxicity when serum concentrations rise above 40 to 60 µg/mL.

With prompt removal of an offending CVC and no evidence of dissemination, the duration of therapy for catheter-associated candidemia is typically 10 to 14 days after the blood culture becomes negative.[51] Disseminated candidiasis, including *Candida* meningitis, requires at least three or more weeks of parenteral therapy; the course is typically completed when all foci have been eradicated. Most infectious disease experts will use fungicidal doses of parenteral amphotericin B or a liposomal preparation for the entire course.

Antifungal Prophylaxis

Appropriate use of antifungals in infants is important for both treatment and prevention of infection. Fluconazole prophylaxis for the prevention of invasive candidiasis is effective and safe in randomized control trials in premature infants.[52] Five randomized controlled trials comparing IV fluconazole (3 mg/kg/day) with placebo or no treatment in VLBW or ELBW infants for 4 to 6 weeks[53–57] met criteria for analysis in Cochrane reviews.[58] Some authors[53,56] reported significantly lower incidences of invasive fungal infection, while there was no difference in treated versus untreated infants in the studies of others.[54,55,57] The only study to evaluate neurologic outcomes found no difference in neurologic impairment at 16 months.[53] Fluconazole prophylaxis was not associated with a significant difference in the risk of death before discharge in any of the five studies or in a metaanalysis.[58] No study documented clinically significant adverse effects of fluconazole or the emergence of fluconazole resistance. In a recent multicenter, randomized, placebo-controlled trial of fluconazole prophylaxis, the MIC of colonizing *Candida* isolates increased in the fluconazole group, with most isolates remaining in the susceptible range.[59]

One study from a single center compared nonrandomized fluconazole prophylaxis from 2002 to 2006 with an untreated, retrospective cohort from 2000 to 2001 and reported that invasive candidiasis decreased from 0.6% to 0.3%, although the proportion of invasive disease caused by non–*C. albicans* spp. increased from 26% to 41% after the introduction of fluconazole prophylaxis.[60] Interestingly, fluconazole prophylaxis in this study was extended to several infants with birthweights greater than 1000 g, if risk factors were present (e.g., maternal human immunodeficiency virus infection, intestinal abnormalities).

The finding that prophylactic fluconazole reduces the incidence of invasive fungal infection must be interpreted "with caution"[58,61] as:

1. The incidence of invasive fungal infection in the placebo groups in these[53,56,57] studies was significantly higher (13% to 16%) than in other large cohort studies of VLBW or ELBW infants in the United States (6% to 7%) or the United Kingdom (1% to 2%).

2. Fluconazole prophylaxis may have impaired the microbiologic isolation of some fungal spp. and led to underdiagnosis of infection in the treatment group.
3. Six years after the introduction of fluconazole prophylaxis, one study reported that non–*C. albicans* spp. with relatively reduced susceptibility to the azoles were the most common causes of invasive fungal infection.[57] This study did not detect a significant effect of fluconazole prophylaxis in reducing invasive candidal disease.

Current guidelines from the Infectious Diseases Society of America[46] recommend IV or oral fluconazole, 3 to 6 mg/kg twice weekly for 6 weeks in ELBW infants in nurseries with high rates (>10%) of invasive candidiasis. Oral nystatin, 100,000 units three times daily for 6 weeks, is an alternative in neonates less than 1500 g where issues with availability or resistance to fluconazole militate against its use.

Infections Ascribable to Other Fungi

Invasive Fungal Dermatitis

Invasive fungal dermatitis typically presents in the infant weighing less than 1000 g who displays macerated or bruised lesions that are contaminated with fungal species. In one report, three of seven confirmed cases of invasive fungal dermatitis had *C. albicans*. *C. parapsilosis, C. tropicalis, Trichosporon beigelii, Curvularia*, or *Aspergillus niger*, and *fumigatus* were cultured from the remainder.[62] Among cases considered "probable," seven of eight had *C. albicans*. Systemic complications, including fungemia, meningitis, or infection of the urinary tract, occurred in four of seven confirmed cases and seven of eight probable cases. More cases than controls had postnatal steroids and prolonged hyperglycemia. Disseminated infection occurred in 69%, all ascribable to *Candida* spp.

The diagnosis of invasive fungal dermatitis requires a skin biopsy demonstrating fungal invasion beyond the stratum corneum or a positive potassium hydroxide preparation of skin scrapings; growth of the identical organism from an otherwise sterile site is confirmatory (e.g., blood, cerebrospinal fluid, or urine obtained via sterile technique). Treatment requires systemic doses of amphotericin B in the range of 0.7 to 1.0 mg/kg/day; in those infants who do not develop systemic infection, oral therapy with fluconazole or topical antifungal creams may suffice. Oral therapy is not advisable for pathogens like *Aspergillus*, and repeated skin biopsies may be necessary to define duration of therapy.

Line Infections Caused by Lipophilic Organisms

The spp. *M. furfur* and *M. pachydermatis* are lipophilic organisms commonly carried on the skin, even in patients without tinea versicolor.[63] Cutaneous colonization can infect hyperalimentation fluids or parenteral lipid formulations. Infants with lipophilic organisms infections typically present with mild but nonspecific signs: respiratory decompensation, glucose intolerance, or thrombocytopenia.[64,65] Diagnosis requires isolation of the organism from blood by growth on fungal medium overlaid with olive oil, since *Malassezia* spp. will not grow in the absence of lipids and may take up to 2 weeks to grow.[66] Removal of the intravascular catheter usually suffices for therapy, although some experts recommend the addition of amphotericin B in doses of 0.5 mg/kg/day for 7 days.

Miscellaneous Fungal Infections

Aspergillus Species

Although rarely seen in neonates, systemic infection with *Aspergillus* suggests severe immunocompromise, such as in DiGeorge syndrome or myeloperoxidase deficiency.[67,68] Invasive aspergillosis most commonly presents as pulmonary or disseminated disease in neonates, but can also present in the CNS and GI tract.[69,70] Disseminated *Aspergillus* disease has occurred in premature newborns without additional immunologic abnormalities.[71] The diagnosis of invasive aspergillosis requires isolation of the fungus from a normally sterile tissue site or visualization by Gomori-methenamine silver stain on biopsy of infected tissue. Of note, a commercially available enzyme-linked immunosorbent assay for diagnosis of aspergillosis on serum specimens had an 83% rate of false positives in premature newborns.[72] PCR assays for *Aspergillus* spp. have been developed and may aid in the diagnosis, but have low sensitivity and specificity (80% for both) and are not available at all centers.[73] Treatment requires systemic amphotericin B in doses of 1 mg/kg/day. Fungistatic therapies such as the triazoles are not recommended for aspergillosis.

Trichosporon beigelii

In a cluster of five neonatal cases of infection caused by *T. beigelii*, a yeast found ubiquitously in soil, no common source was identified.[74] Two of three premature neonates infected with this organism died. Resistance to achievable concentrations of amphotericin B complicates therapy.

Suggested Readings

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37

Healthcare-Associated Infections

LAKSHMI SRINIVASAN

KEY POINTS

- Healthcare-associated infections (HAIs) are important preventable causes of morbidity and death in neonates.
- Central line–associated bloodstream infections cause the bulk of HAIs in the neonatal intensive care unit; other HAIs include ventilator-associated pneumonias, urinary tract infections, surgical site infections, and nosocomial viral infections.
- Several modifiable risk factors have been identified for these conditions. Preventive strategies must focus on basic hand hygiene and infection control measures, along with specific measures for the infection.
- Quality improvement collaboratives have achieved great strides in infection reduction by developing improved surveillance and benchmarking strategies and implementation of bundles of preventive measures.
- However, some high-risk infants continue to experience infections; additional customized strategies may be needed to enhance infection prevention in these high-risk patients.

Healthcare-associated infections (HAIs) are infections acquired by patients during hospitalization. For decades, HAIs were considered an unavoidable problem in the neonatal intensive care unit (NICU). Several factors converge to create a high risk of infection in the NICU patient: immature innate and adaptive immune defenses, the need for life-sustaining invasive interventions, broad-spectrum antibiotic exposure, colonization with potentially pathogenic microorganisms, and prolonged hospital stays.[1–3] The improved survival of very low birthweight (VLBW) infants and their attendant need for invasive technologies during the NICU course magnify the impact of this problem. HAIs have been shown to impact neonatal outcomes, including short-term and long-term morbidities, hospital length of stay, healthcare costs, and death significantly and independently.[4–13]

Continuous surveillance and monitoring of HAI rates and pathogens are necessary to establish reference points in each nursery and facilitate early identification of epidemics. Clinicians can prevent HAIs by consistently and reliably following best practices for infection prevention and minimizing invasive procedures to the extent possible. Quality improvement strategies, education of staff on bundles of preventive care, standardization of practice, and hospital programs such as antimicrobial stewardship are essential to preventing harm. Many centers have used these strategies to reduce the burden of HAIs with great success.[14–20] In this chapter we will review the definitions, epidemiology, and adverse outcomes related to HAIs, as well as current concepts and evidence surrounding strategies for prevention of HAIs in NICUs.

Defining Neonatal Healthcare-Associated Infection

The Centers for Disease Control and Prevention (CDC) surveillance definition states that an HAI is an illness associated with a pathogen or its toxins that is not present or incubating at the time of admission.[21] Although this definition appears straightforward, additional special considerations exist for neonates. Infections that manifest themselves early in the first week of life are typically related to perinatal risk factors and vertical transmission from the mother. HAIs occur later and are more often related to patient colonization and environmental risk factors. Although most sources define HAIs in neonates as infections occurring after 3 days of postnatal life,[2,22] there is no specific age that clearly distinguishes maternally transmitted infections from HAIs.[22] There may be substantial temporal overlap between HAIs. The CDC National Healthcare Safety Network (NHSN) currently defines HAIs in neonates as those infections initially identified on day 3 or later (or 7 days and later for group B streptococcus infections) and thus may include some infections that may have been acquired perinatally but manifest clinically beyond 3 days of life.[21] Neonatal HAI rates may be slightly overestimated during the first few weeks of life. Definitions of central line–associated bloodstream infection (CLABSI), ventilator-associated pneumonia (VAP), pediatric ventilator-associated event (pedVAE), and catheter-associated urinary tract infection (UTI) are given in Box 37.1.[23]

Diagnosing Neonatal Healthcare-Associated Infection

Diagnosis of Central Line–Associated Bloodstream Infection

Although coagulase-negative staphylococci (CoNS) are common skin commensals, they are also the most common cause of CLABSIs in the NICU. Differentiating contamination from true infections with CoNS is one of the greatest challenges surrounding neonatal CLABSI definitions. The CDC introduced a change to its definition in 2008, namely, the requirement for two blood cultures positive for skin commensals to fulfill the definition of a CLABSI.[21] However, the Vermont Oxford Network (VON), the National Institute for Child Health and Human Development (NICHD), and Children's Hospital Neonatal Consortium (CHNC) include infants with only one culture positive for CoNS

• BOX 37.1 Definitions of Healthcare-Acquired Infections for Patients Younger Than 12 Months*

Nosocomial Bloodstream Infections

Laboratory-confirmed bloodstream infection (LCBI). Must meet one of the following definitions:

- Patient has a recognized bacterial or fungal pathogen (not included on the NHSN common commensal list), identified from one or more blood specimens obtained by a culture *AND* organism(s) identified in blood is not related to an infection at another site
- Patient has at least one sign or symptom (fever >38.0°C, hypothermia <36.0°C, apnea, or bradycardia) *AND* organism(s) identified in blood is not related to an infection at another site *AND* the same NHSN common commensal is identified by a culture from two or more blood specimens collected on separate occasions. Common commensal organisms include, but are not limited to, diphtheroids (*Corynebacterium* spp. not *C. diphtheria*), *Bacillus* spp. (not *B. anthracis*), *Propionibacterium* spp., coagulase-negative staphylococci (including *S. epidermidis*), viridans group streptococci, *Aerococcus* spp., *Micrococcus* spp., and *Rhodococcus* spp.

Central Line–Associated Bloodstream Infection

- LCBI (as defined above) *AND*
- Central line or umbilical catheter in place for more than 2 days *AND*
- Central line in place on day of or day before CLABSI diagnosis

Pneumonia

- Imaging evidence: Two or more serial chest radiographs with new/progressive and persistent infiltrate, cavitation, consolidation, or pneumatoceles for patients with underlying pulmonary or cardiac disease (respiratory distress syndrome, bronchopulmonary dysplasia, pulmonary edema) *or* one chest radiograph with the aforementioned abnormalities for patients without underlying pulmonary or cardiac disease *AND*
- Worsening gas exchange *AND*
- At least three of the following: (1) temperature instability, (2) white blood cell count less than 4000/μL or greater than 15,000/μL with 10% or more bands, (3) new-onset purulent sputum, change in character of sputum, increased respiratory secretions, or increased suctioning requirements, (4) physical examination findings consistent with increased work of breathing or apnea, (5) wheezing, rales, or rhonchi, (6) cough, (7) bradycardia (<100 bpm), or tachycardia (>170 bpm)

Ventilator-Associated Pneumonia

- Pneumonia (as defined above) *AND*
- Patient on ventilator for more than 2 days *AND*
- Ventilator in place on day of or day before VAP diagnosis

Pediatric Ventilator-Associated Events

- Patient has a baseline period of stability or improvement on the ventilator, defined by ≥2 calendar days of stable or decreasing daily minimum FiO_2 or MAP values
- After a period of stability or improvement on the ventilator, the patient has at least one of the following indicators of worsening oxygenation: (1) Increase in daily minimum FiO_2 of ≥0.25 (25 points) over the daily minimum FiO_2 of the first day in the baseline period, sustained for ≥2 calendar days. (2) Increase in daily minimum MAP values of ≥4 cmH_2O over the daily minimum MAP of the first day in the baseline period, sustained for ≥2 calendar days

Urinary Tract Infection

Symptomatic Urinary Tract Infection (SUTI)

- At least one of the following symptoms: (1) fever (temperature >38.0°C), (2) hypothermia (temperature <36.0°C), (3) apnea, (4) bradycardia, (5) lethargy, (6) vomiting, or (7) suprapubic tenderness *AND*
- Urine culture with no more than two species identified, at least one of which is present at more than 10^5 CFU/mL

Asymptomatic Bacteremic Urinary Tract Infection (ABUTI)

- Urine culture with no more than two species identified, at least one of which is present at more than 10^5 CFU/mL *AND*
- Bacteria identified in blood (culture-based or nonculture-based microbiologic method) that matches at least one of the bacteria present at more than 10^5 CFU/mL in urine

Catheter–Associated Urinary Tract Infection:

- Urinary tract infection (as defined above, either SUTI or ABUTI) *AND*
- Indwelling urinary catheter for more than 2 days *AND*
- Urinary catheter in place on day of or day before urinary tract infection diagnosis

Surgical Site Infection

Superficial Incisional SSI

- Occurs within 30 days after the NHSN operative procedure *AND*
- Involves only skin and subcutaneous tissue of the incision *AND*
- At least one of: (1) purulent drainage from the superficial incision. (2) organism(s) identified from an aseptically obtained specimen from the superficial incision or subcutaneous tissue. (3) superficial incision deliberately opened and microbiologic testing of the superficial incision or subcutaneous tissue is not performed *and* at least one of: localized pain or tenderness; localized swelling; erythema; or heat. (4) diagnosis of a superficial incisional SSI by a physician or physician designee

Deep Incisional SSI

- Occurs within 30 or 90 days after the NHSN operative procedure *AND*
- Involves deep soft tissues of the incision (e.g., fascial and muscle layers) *AND*
- At least one of: (1) purulent drainage from the deep incision; (2) a deep incision that spontaneously dehisces, or is deliberately opened or aspirated *and* at least one of the following: fever (>38°C); localized pain or tenderness; (3) abscess or other evidence of infection involving the deep incision detected on gross anatomical or histopathologic exam, or imaging test.

Organ/Space SSI

- Occurs within 30 or 90 days after the NHSN operative procedure *AND*
- Involves any part of the body deeper than the fascial/muscle layers that is opened or manipulated during the operative procedure *AND*
- At least one of: (1) purulent drainage from a drain that is placed into the organ/space; (2) organism(s) identified from fluid or tissue in the organ/space by microbiologic testing; (3) abscess or other evidence of infection involving the organ/space that is detected on gross anatomic or histopathologic exam, or imaging test evidence suggestive of infection; *AND*
- Meets at least one criterion for a specific organ/space infection (e.g., osteomyelitis, myocarditis, intraabdominal infection etc. as defined by NHSN module)

*Centers for Disease Control and Prevention National Healthcare Safety Network; modified from CDC NHSN modules for LCBI, CLABSI, VAP, pedVAE SSI.

bpm, Beats per minute; *CFU*, colony-forming unit; *SSI*, surgical site infection.

in the presence of clinical signs of infection.[1] Furthermore, there is considerable clinical practice variation regarding whether single or multiple cultures (from phlebotomy and/or central line) are drawn, which could impact rates of CoNS diagnosis.[24] Refinements of CLABSI definitions (relating to the timing of infection following hospital admission, line placement, etc.) may impact the number of CLABSIs reported.[25,26]

Another area of uncertainty surrounds source attribution. For example, some CLABSIs in infants with underlying gastrointestinal (GI) conditions may result from translocation caused by

mucosal barrier injury. However, studies to date have not fully borne out this hypothesis.[27] Studies have demonstrated that International Classification of Diseases, Ninth Revision (ICD-9) coding of hospital administrative data has low concordance with NHSN definitions of CLABSI.[28,29] Although these studies were not limited to neonatal data, the findings reflect limitations of the NHSN definitions in certain situations where clinicians may diagnose secondary etiologies for the infection, but infections remain attributed to the central line per NHSN criteria.

Diagnosis of Ventilator-Associated Pneumonia

Prior to 2013, VAP definitions were used by NHSN for surveillance of ventilator-associated events (VAEs). These definitions, including the NHSN definitions, are problematic to apply in neonates because overlap of signs and symptoms and radiographic findings with underlying respiratory conditions posed significant challenges to the diagnosis of VAP.[1,30–35] Identifying a suitable source of pathogens is an additional cause of variability. Because of these challenges with defining VAP accurately in the neonatal population, in 2014 the NHSN discontinued surveillance of VAP in the NICU. However, many NICUs and collaboratives continue surveillance and internal benchmarking of this condition.

The CDC organized a working group in 2012 to consider whether the VAE surveillance approach used in adults could also be applied to neonatal and pediatric inpatient populations. Following the publication of a study on pediatric VAE, the CDC created a pedVAE definition. PedVAEs are identified by objective criteria defining deterioration in respiratory status after a period of stability or improvement on the ventilator. However, this approach has limitations because many pedVAE are not due to infectious etiologies but rather due to progression of underlying respiratory or cardiac disease.[31]

Diagnosis of Urinary Tract Infection

Clinicians often use less stringent microbiologic criteria (ranging from 100 to 10,000 colony-forming units [CFUs] of bacteria per milliliter in urine) for neonatal UTI diagnosis compared with CDC definitions.[36–38] CoNS may be isolated in urine samples, raising the question similar to bloodstream infections of whether they represent contamination or true infection.[38,39]

Healthcare-Associated Infection Surveillance and Data Sources

Sources of data on neonatal HAIs in the United States include the CDC, NHSN, VON, NICHD, NRN, and the CHNC.[1] Reporting overall incidence rates may be misleading because of wide variations in case mix and device utilization rates. In 2005, the CDC NHSN system developed monitors device-associated HAI rates by using an approach that accounts for variability in device use and length of hospital stay.[40–42] These data are also stratified by birthweight categories and expressed as incidence density per 100 or 1000 patient or device days, which facilitates risk adjustment based on birthweight and the duration of exposure to the risk factor. Another approach to surveillance is the use of the standardized infection ratio (SIR), which is a summary measure used to track HAIs at a national, state, or local level over time. The SIR is calculated by measuring observed HAIs over predicted HAIs (derived using data from a baseline period) and adjusts for facility and/or patient-level factors that contribute to HAI risk within each facility.

NHSN tracks HAIs in more than 17,000 medical facilities and shares the data with the facilities, the CDC, other partners, and quality improvement organizations to help guide efforts at infection prevention. Outside of the United States, the International Nosocomial Infection Control Consortium runs a surveillance network for HAIs, using CDC NHSN definitions, in more than 1000 hospitals around the world, and provides aggregate data on HAI from several countries.[43]

Epidemiology of Healthcare-Associated Infection

Healthcare-Associated Infection in the Newborn Nursery

Accurate rates of HAI in the "well-baby" nursery is difficult to ascertain because there is no systematic reporting or surveillance network for this location; however, the incidence appears to be low. Some researchers estimate rates of less than 1 per 100 patients discharged.[22] Typical risk factors for acquiring an HAI are uncommon in this population. Discharge from the hospital within 48 hours of birth, "baby-friendly" hospital policies, and rooming-in practices have helped to further decrease the risk of exposure in modern mother-baby units. However, early discharge may also limit surveillance efforts and documented rates of HAI, as patients may be discharged home before they become symptomatic from HAIs.[44]

HAIs in the well-baby nursery are commonly superficial, involving the skin, mouth, or eyes, and include omphalitis, pustules, abscesses, and bullous impetigo.[44] Community-acquired methicillin-resistant *Staphylococcus aureus* (MRSA) has been implicated in outbreaks of skin and soft tissue infections (SSTIs) and pneumonia in newborn nurseries.[45–49] Legionella outbreaks have been reported in term neonates, because of nosocomial contamination from water sources (water births, humidifiers).[50,51] Nursery epidemics of other bacterial enteropathogens and viral enteropathogens have been reported but occur infrequently in the United States in the current era.[44,52,53]

Healthcare-Associated Infection in the Neonatal Intensive Care Unit

Most neonatal HAIs occur in term and preterm infants hospitalized in NICUs. Comparison of surveillance data between institutions needs to be carefully performed with attention to differences in patient demographics, device utilization rates, and use of neonate-specific definitions for HAI.

Data from the NICHD NRN in 2002 demonstrated that 21% of VLBW infants who survived beyond 3 days postnatally had at least one episode of late-onset sepsis.[2] There was significant variability among participating centers, with rates ranging from 10.6% to 31.7% at individual sites.[2] In another study, 19% of VLBW infants had HAIs, with the highest rates in infants with the lowest birthweights.[54] A point prevalence survey conducted by 29 level II to level IV nurseries participating in the Pediatric Prevention Network revealed a prevalence of 11.4% for HAI.[55] Data from the NICHD NRN showed that the rates of late-onset sepsis declined from 2005 to 2012 among VLBW infants of all gestational ages: the rates for infants born at 24 weeks' gestation

declined from 54% to 40% (adjusted relative risk [RR] 0.94, 95% confidence interval [CI] 0.93 to 0.95), for infants born at 26 weeks' gestation the rates declined from 37% to 27% (adjusted RR 0.93, 95% CI 0.92 to 0.94), and for infants born at 28 weeks' gestation the rates declined from 20% to 8% (adjusted RR 0.91, 95% CI 0.90 to 0.92).[14,56]

CLABSIs constitute most HAIs in the NICU.[40,55] CLABSIs contribute to 25% to 60% of HAIs in the neonatal population.[57] The remaining cases involve the respiratory tract, eye, ear, nose, throat, GI tract, or urinary tract or may be surgical site infections (SSIs). VAPs are difficult to diagnose in the NICU but can contribute to morbidity, especially in patients with evolving lung disease.[32] Although the use of indwelling urinary catheters is low in the NICU compared with other intensive care units, UTIs in the NICU are still a problem. Surveillance data for meningitis are limited. There are widespread differences in clinical practice regarding the inclusion of a lumbar puncture with cerebrospinal fluid analysis in the evaluation for possible neonatal sepsis. Although early-onset meningitis is rare, studies suggest that late-onset meningitis may be underdiagnosed in VLBW infants.[58,59] Neonates may experience SSI, most frequently with abdominal, cardiac, or neurosurgical procedures.

Rates of device-associated infections in NICUs in the United States are presented in Table 37.1. HAI rates remained constant

TABLE 37.1 Healthcare-Associated Infection Rates in the United States and Internationally

CLABSI Rates for Level III NICUs in the United States, 2013 (CDC NHSN)

Birthweight Category (g)	No. of Locations†	No. of CLABSIs	Central Line Days	Pooled Mean CLABSI Rate
≤750	389 (345)	403	191,246	2.1
751–1000	411 (354)	210	156,909	1.3
1001–1500	429 (385)	136	173,835	0.8
1501–2500	433 (349)	91	161,626	0.6
>2500	432 (324)	134	182,144	0.7

CLABSI Rates for Level III NICUs Internationally, 2012–17 (INICC)

Birthweight Category (g)	No. of Locations†	No. of CLABSIs	Central Line Days	Pooled Mean CLABSI Rate*
≤750	70	137	7,468	18.3 (15.4–21.7)
750–1000	70	255	17,553	14.5 (12.8-16.4)
1001–1500	70	566	36,978	15.3 (14.1–16.6)
1501–2500	70	156	20,310	7.7 (6.5–9)
>2500	70	180	19,376	9.3 (8–10.8)

VAP Rates for Level III NICUs in the United States, 2013 (CDC NHSN)

Birthweight Category (g)	No. of Locations†	No. of VAP Cases	Ventilator Days	Pooled Mean VAP Rate
≤750	114 (106)	56	54,201	1
750–1000	119 (91)	29	25,356	1.1
1001–1500	123 (70)	11	16,264	0.7
1501–2500	125 (64)	8	14,719	0.5
>2500	124 (60)	3	20.906	0.1

VAP Rates for Level III NICUs Internationally, 2012–17 (INICC)

Birthweight Category (g)	No. of Locations†	No. of VAP Cases	Ventilator Days	Pooled Mean VAP Rate*
≤750	70	26	7807	3.3 (2.2–4.9)
750–1000	70	62	12582	4.9 (3.8–6.3)
1001–1500	70	298	22650	13.2 (11.7–14.7)
1501–2500	70	114	17728	6.4 (5.3–7.7)
>2500	70	112	20534	5.5 (4.5–6.6)
Pooled	70	612	81301	7.5 (6.5–8.1)

continued

TABLE 37.1 Healthcare-Associated Infection Rates in the United States and Internationally—cont'd

Comparison of HAI Rates per 1000 Device Days in the NICUs of the INICC (2012–17) and the US NHSN (2013)

Birthweight Category (g)	CLABSI		VAP	
	INICC 2012–17 Pooled Mean*	US NHSN 2013 Pooled Mean*	INICC 2012–17 Pooled Mean*	US NHSN 2013 Pooled Mean*
≤750	18.3 (15.4–21.7)	2.1 (1.9–2.3)	3.3 (2.2–4.9)	1.0 (0.8–1.3)
750–1000	14.5 (12.8–16.4)	1.3 (1.2–1.5)	4.9 (3.8–6.3)	1.1 (0.8–1.6)
1001–1500	15.3 (14.1–16.6)	0.8 (0.7–0.9)	13.2 (11.7–14.7)	0.7 (0.3–1.2)
1501–2500	7.7 (6.5–9)	0.6 (0.5–0.7)	6.4 (5.3–7.7)	0.5 (0.2–1.1)
>2500	9.3 (8–10.8)	0.7 (0.6–0.9)	5.5 (4.5–6.6)	0.1 (0.0–0.4)

*The 95% confidence interval is given in parentheses.
†The number in parentheses is the number of locations meeting minimum requirements for percentile distributions (i.e., ≥50 device days for rate distributions, ≥50 patient days for device utilization ratios) if less than the total number of locations. If this number is <20, percentile distributions are not calculated.
CDC, Centers for Disease Control and Prevention; *CLABSI*, central line–associated bloodstream infection; *HAI*, healthcare-associated infection; *INICC*, International Nosocomial Infection Control Consortium; *NHSN*, National Healthcare Safety Network; *NICU*, neonatal intensive care unit; *VAP*, ventilator-associated pneumonia.
Data from Dudeck MA, Edwards JR, Allen-Bridson K, et al. National Healthcare Safety Network report, data summary for 2013, Device-associated module. *Am J Infect Control*. 2015;43(3):206–227 and Rosenthal VD, Bat-Erdene I, Gupta D, et al. International Nosocomial Infection Control Consortium. International Nosocomial Infection Control Consortium (INICC) report, data summary of 45 countries for 2012–2017: Device-associated module. *Am J Infect Control*. 2020;48(4):423–432.

in the 1990s[40,60] but decreased in the later years of the first decade of this century, likely because of the impact of collaborative quality improvement efforts.[3,16,17,19,57,61,62] The most recent epoch has demonstrated a plateauing of CLABSI rates.[63] The impact of the COVID-19 pandemic on HAI rates in neonatal populations needs to be more thoroughly elucidated, but overall data demonstrate significant increases in the national SIRs for CLABSI, CAUTI, VAE, and MRSA bacteremia in 2020.[64]

Although catheter-associated UTIs are not very frequent in the NICU, non–catheter-associated UTIs remain a common cause of infections and need for antibiotic treatment in the NICU.[65] VAP rates also decreased from 1.6 to 0.6 per 1000 ventilator days from 2007 to 2012.[57] SSIs occur in about 5.6% of neonates who undergo surgical procedures.[66]

The International Nosocomial Infection Control Consortium published surveillance data from 2012 to 2017 from NICUs in Africa, Asia, Europe, and Latin America.[67] Rates of CLABSI and VAP were generally higher than the corresponding CDC NHSN rates in the United States (see Table 37.1).[43] Recent advances in resources, technology, and regulations in healthcare in many countries have led to reductions in infection rates, which are reflected in the reductions in NICU CLABSI and VAP rates seen in the later years of surveillance (see Table 37.1).[43]

Risk Factors for Development of Healthcare-Associated Infection

Minimizing exposure to known modifiable risk factors for infection is important to reduce the rate of HAIs in the nursery. Many HAIs previously thought to be not preventable can in fact be substantially decreased in frequency with appropriate interventions.

The risk of developing an HAI is inversely related to gestational age and birthweight.[2,40,57,68–70] Infants with birthweights less than 1000 g experience twice the rate of nosocomial bloodstream infections compared with infants with birthweights greater than 1000 g.[2,40,54,69,71] Overall, VLBW infants are more vulnerable to infection because of the immaturity of their immune response, severity of illness, and their greater need for invasive devices.[44,72]

The use of any type of invasive device increases the risk of infection. The most common invasive devices used in the nursery are intravascular catheters, mechanical ventilators, ventriculoperitoneal shunts, and urinary catheters. In general, risk rises as the duration of exposure to invasive devices lengthens. Compared with adult patients, neonates are at higher risk of CLABSI and at lower risk of VAP and UTI.[62,73] These patterns correlate with the frequency with which these invasive devices are used in the neonatal population.

Histamine-blocking agents, proton pump inhibitors, and postnatally administered corticosteroids are the medications most associated with an increased risk of HAIs among newborns.[74–76] It is hypothesized that the reduced gastric pH associated with the use of histamine-blocking agents promotes bacterial overgrowth and invasion of pathogenic bacteria.[77] With greater understanding of the adverse neurodevelopmental consequences of prolonged ventilator need and consequent bronchopulmonary dysplasia (BPD), there has been an increase in use of lower-dose steroids after the first 2 weeks postnatally, especially in infants at high risk of developing BPD.[78–83] However, steroid use has been associated with an increased risk of infection in VLBW infants,[84,85] and therefore neonatologists need to include this concern in any risk-benefit analysis of the use of steroids in their patients.

Nursery design and staffing influence the risk of infection. Overcrowding and larger workloads decrease compliance with hand washing and raise the risk of HAI.[86–91] Inadequate numbers of staff and the use of temporary or inexperienced staff members both adversely affect the rate of infection. Studies have shown a relationship between nurse-to-patient ratio and colonization of patients with MRSA and CLABSI rates.[86,91] Furthermore, strategic nursery design and improvement in nursing staffing correlate with lower rates of HAIs.[92] However, there continue to be nurse staffing challenges, as exemplified by a VON study that identified rates of understaffing and infections in NICUs across the United

States.[90] NICU design may also impact infection rates. Several NICUs are transitioning to single-patient room design to reduce infection risk among other reasons. The evidence surrounding the effectiveness of single patient rooms in reducing infection risk for neonates is mixed.[93–95] Many of these HAI risk factors hold true globally. Nosocomial infection rates are much higher in lower-income countries than middle-income and higher-income countries (see Table 37.1), likely reflecting a combination of personnel and resource limitations, overcrowding, lack of infection control regulatory and auditing mechanisms, hospital accreditation, and healthcare workers inexperienced in infection prevention standard practices.[43]

Risk Factors for Central Line–Associated Bloodstream Infection

CDC NHSN data demonstrate that VLBW infants have a twofold increased risk of developing CLABSI compared with normal-weight infants.[57] Infants with an underlying GI condition such as necrotizing enterocolitis, gastroschisis, or omphalocele may be predisposed to mucosal barrier injury and at heightened risk of CLABSI. In a multicenter cohort study in 14 NICUs, 40% of infants who developed CLABSIs had underlying GI conditions associated with loss of mucosal integrity or impaired gut motility or both.[27] However, the proportion caused by "enteric pathogens" was not different in the overall group, except for infants with intestinal failure who developed multiple CLABSIs; this latter group experienced higher rates of infections with enteric pathogens.[27] A study from the NICHD NRN also demonstrated that VLBW infants with intestinal failure were at increased risk of recurrent bloodstream infections; however, gram-positive organisms were the most frequent pathogens.[96]

Intravascular devices commonly used in the neonatal population are peripheral intravenous catheters (PIVCs), umbilical catheters, peripherally inserted central catheters (PICCs), surgically placed central venous catheters (CVCs), and percutaneous arterial catheters. Regardless of the type of device used, the rate of CLABSI appears to be related to the number of days the catheter is in place.[2,40,70,97] Table 37.2 summarizes evidence regarding several of the interventions associated with increased CLABSI risk.[98] A 2015 Cochrane review of trials comparing PICCs with PIVCs found that use of a PICC line decreases the risk of complications associated with PIVCs, without increasing the rate of infection.[99] For central lines, theoretically the risk should be lower for tunneled catheters, because the Dacron cuff proximal to the exit site of a surgically placed catheter can inhibit the migration of organisms into the catheter track.[100] Adult data suggest that tunneled catheters have lower infection rates than nontunneled catheters; however, studies of neonates with tunneled lines show rates of infection comparable with or worse than the reported rates of PICC line infections in other NICU populations.[101,102] Data regarding umbilical catheters is mixed, with some studies showing higher risk,[103] whereas others found similar or lower risk than PICC lines.[97,104–108] Studies on the relationship between catheter dwell time and CLABSI rates show conflicting findings, with some studies demonstrating no association.[97,107] However, several

TABLE 37.2 Studies Identifying Interventions With Increased Risk of Bloodstream Infections

Intervention	Adjusted Risk for Each Intervention[a]			
Mechanical ventilation	6.8 (5.9–7.8)[b]	1.7 (1.4–2.1)[g]	4.2 (1.4–12.4)[d]	
Central line	6.1 (5.0–7.4)[b]	9.3 (5.9–14.8)[h]		
Central line >7 days	6.2 (5.0–7.6)[b]	3.5 (1.3–9.2)[i]		
Central line >21 days	6.1 (4.6–8.0)[b]	80.6 (6.9–945)[j]		
Umbilical catheter >7 days	1.9 (1.7–2.1)[b]			
PICC >7 days	2.9 (2.5–3.3)[b]			
PAL >7 days	3.7 (3.0–4.6)[b]			
Parenteral nutrition > 7 days	14.2 (8.8–22.9)[c]	12.9 (9.7–17.2)[b]	7.1 (2.8–18.1)[i]	4.7 (2.2–9.9)[h]
Vancomycin	6.1 (1.9–20.1)[d]			
Steroids	1.8 (1.0–3.3)[e]	4.8 (1.7–13.2)[i]		
H_2 blocker/PPI	6.7 (3.8–12.9)[f]	3.1 (1.3–7.6)[e]	7.9 (2.8–21.1)[d]	3.1 (1.0–10.2)[i]

[a]The 95% confidence interval is given in parentheses.
[b]From Stoll et al.[2]
[c]From Holmes.[92]
[d]From Smith et al.[70]
[e]From Stoll et al.[79]
[f]From Bianconi.[93]
[g]From Makhoul et al.[94]
[h]From Perlman.[95]
[i]From Mahieu et al.[91]
[j]From Graham et al.[69]
H_2 blocker, Histamine H_2 receptor antagonist; *PAL*, peripheral arterial line; *PICC*, peripherally inserted central catheter; *PPI*, proton pump inhibitor.
Modified from Cantey JB, Milstone AM. Bloodstream infections: epidemiology and resistance. *Clin Perinatol.* 2015;42:1–16.

others have suggested that increasing risk of CLABSI is associated with increased dwell time of PICC and umbilical catheters.[103,108] A large retrospective review of more than 13,000 infants found that although dwell time did not affect CLABSI risk for PICCs, it did impact CLABSI risk with tunneled catheters, with increased CLABSI risk beyond week 7 from placement.[109]

Exposure to parenteral nutrition has been shown to be associated with increased risk of bloodstream infections, which may in part be mediated by increased use of central lines for delivery of parenteral nutrition.[110,111] A meta-analysis identified parenteral nutrition and lipid infusions as independent predictors of neonatal bloodstream infections.[76] Lipid emulsions may decrease the flow rate through the intravenous (IV) catheter, potentiate growth and proliferation of some microorganisms, and interfere with host defense mechanisms by impairing the function of neutrophils and reticuloendothelial cells.[112–114] Use of lipid emulsions was independently associated with the development of CoNS bacteremia.[114] Administration of such emulsions has also been linked to a higher risk of HAI with *Candida* and *Malassezia* spp. in neonates.[115–117]

Risk Factors for Ventilator-Associated Pneumonia

CDC NHSN data indicate that VLBW infants have a 3.5-fold increased risk of VAP compared with normal-weight infants.[57] VAPs are more common in infants with underlying cardiopulmonary disease such as BPD, as well as infants who have experienced previous thoracoabdominal surgery.

Prolonged duration of mechanical ventilation is the primary risk factor for development of hospital-acquired pneumonia. Endotracheal tubes circumvent normal airway protective and clearance mechanisms and become coated with biofilms of bacteria, which have the potential to enter the lower airways and cause infections.[30] Contamination of respiratory equipment, especially with gram-negative organisms that thrive in moist environments, such as *Acinetobacter, Pseudomonas*, and *Flavobacterium* species (spp.), frequently leads to colonization of the respiratory tract.[118] Aspiration of gastric and oropharyngeal secretions around uncuffed endotracheal tubes could be a mechanism for VAP.[30,119,120] Closed-system suctioning devices may decrease the risk of environmental contamination during suctioning; however, there is a theoretical risk that they could reintroduce pathogens suctioned from the secretions from major airways into smaller lower airways. Although there is a paucity of NICU evidence on the subject, adult studies have demonstrated higher colonization rates but lower VAP rates with closed suctioning systems.[121–126] Supine positioning and dependent position of the endotracheal tube in relation to the ventilator circuit may increase the risk of VAP by increasing the risk of aspiration.[127–129] Data on nasal continuous positive airway pressure suggest that its lack of disruption of normal airway protective mechanisms compared with endotracheal tubes may be responsible for lower rates of nosocomial pneumonia; however, the association of nasal continuous positive airway pressure with greater rates of gram-negative sepsis needs further elucidation.[74,130]

Risk Factors for Urinary Tract Infection

UTIs are more prevalent in ex-preterm infants; neonatal males are at higher risk than neonatal females, as are infants with underlying renal anomalies.[39,65]

Healthcare-Associated Infection: Distribution by Pathogen

The predominant pathogens responsible for nosocomial bloodstream infections have changed over time. Goldmann[44] proposed that these trends are explained by changes in the neonatal intensive care patient population and advancing technology. *S. aureus* was the most common nosocomial pathogen in the 1950s and 1960s. By 1970, gram-negative organisms emerged as the predominant pathogens; globally, these organisms are the most important pathogens responsible for HAIs in the nursery.[131] In the United States, CoNS was the most common nosocomial pathogen in the 1990s and early years of the first decade of this century.[2,40] Among the cohort of VLBW infants born between 2002 and 2008 with late-onset infections, the NICHD reported that in singleton infants, gram-positive organisms were responsible for 77% of cases, gram-negative organisms were responsible for 16%, and fungi were responsible for 8% of cases.[132] These findings were similar to studies from the previous decade.[2,133] A study comparing pathogens causing late-onset sepsis in extremely preterm infants from two eras—2000 to 2005 and 2006 to 2011—also demonstrated that pathogens remained similar across epochs, except for a decline in fungal infections (Table 37.3).[14] However, reports from at least one center after the introduction of standardized central line insertion and maintenance bundles and checklists noted declines in CoNS as a causative pathogen.[134]

Microbial Resistance

Antimicrobial resistance is increasing across NICUs.[135,136] Drug-resistant gram-negative pathogens are associated with the highest attributable mortality rates.[137–139] Cantey and Milstone[98] identified a marked increase in the number of NICU publications associated with MRSA, vancomycin-resistant enterococcus (VRE), extended-spectrum β-lactamase (ESBL), and carbapenemase-producing bacteria (CPB) between 1993 and 2013. NHSN data show that MRSA infection rates tripled in NICUs between 1995 and 2004 and more recently demonstrate that NICU infants continue to experience a considerable proportion of infections caused by resistant pathogens.[140,141] Resistant bacteria have been implicated in more than 15% of NICU outbreaks worldwide.[135] Colonization with resistant bacteria has been associated with approximately a 33% infection risk with the same pathogen.[75,142,143] Many NICUs have adopted periodic surveillance strategies to detect resistant bacteria such as MRSA or VRE.[144]

Gram-Positive Bacteria

Coagulase-Negative Staphylococci

CoNS (e.g., *S. epidermidis, S. capitis, S. hominis, S. warneri*, and *S. haemolyticus*) are commonly thought of as skin commensals but are also the most common endemic nosocomial pathogen in neonates.[2,54,72,132,133] Most CoNS infections are bloodstream infections, with a reported incidence of 51% to 78% among VLBW infants.[2,14,68,72,132,145] CoNS are lower-virulence pathogens, with low mortality rates.[134,146] Known risk factors for CoNS infection are low birthweight, lower gestational age, use of CVCs, prolonged parenteral nutrition, use of IV lipid emulsions, postnatal administration of corticosteroids, and prolonged hospital stay.[44,54,110,114] CoNS produce a capsular polysaccharide adhesin—poly(*N*-succinyl glucosamine)—which forms a "biofilm," enhancing its ability to

TABLE 37.3 Pathogens Associated With Late-Onset Sepsis in Extremely Preterm Infants (2000–11)

Pathogens in First Episode of Late-Onset Sepsis	Era 1 (2000–05) (*N* = 1896)	Era 2 (2006–11) (*N* = 1728)	P value
Gram-positive, *N* (%)	1504 (78%)	1386 (79%)	0.55
CoNS	973 (50%)	1007 (57%)	
Staphylococcus aureus	217 (11%)	212 (12%)	
Staphylococcus spp. (unspecified)	136 (7%)	0 (0%)	
Enterococcus spp.	61 (3%)	92 (5%)	
Streptococcus spp. (unspecified)	52 (3%)	0 (0%)	
Group B streptococcus	30 (2%)	45 (3%)	
Streptococcus pneumoniae	1 (0.1%)	1 (0.1%)	
Group A streptococcus	0 (0%)	6 (0.3%)	
Clostridia spp.	0 (0%)	1 (0.1%)	
Possible contaminants	34 (2%)	22 (1%)	
Gram-negative, *N* (%)	371 (19%)	329 (19%)	0.74
Escherichia coli	103 (5%)	117 (7%)	
Klebsiella spp.	92 (5%)	90 (5%)	
Enterobacter spp.	74 (4%)	45 (3%)	
Pseudomonas spp.	58 (3%)	30 (2%)	
Serratia spp.	30 (2%)	24 (1%)	
Citrobacter spp.	7 (0.4%)	10 (0.6%)	
Proteus spp.	4 (0.2%)	6 (0.3%)	
Acinetobacter spp.	2 (0.1%)	6 (0.3%)	
Stenotrophomonas maltophilia	0 (0%)	1 (0.1%)	
Possible contaminants	1 (0.1%)	0 (0%)	
Fungus, *N* (%)	188 (10%)	111 (6%)	<0.001
Candida spp.	182 (9%)	96 (6%)	
Other fungi	6 (0.3%)	15 (0.9%)	

CoNS, Coagulase negative staphylococci.
Data from Greenberg RG, Kandefer S, Do BT, et al. Eunice Kennedy Shriver National Institute of Child Health and Human Development Neonatal Research Network. Late-onset sepsis in extremely premature infants: 2000–2011. *Pediatr Infect Dis J.* 2017;36(8):774–779.
Data from all studies have been adjusted for gestational age.

adhere to intravascular devices.[147] Although some studies suggest that prophylactic use of vancomycin or vancomycin locks reduces the risk of CoNS catheter-related infections, this practice is not recommended because of the serious risk of encouraging antibiotic-resistant organisms, especially VRE and vancomycin-resistant *S. aureus* (VRSA).[148]

Staphylococcus aureus

S. aureus has caused epidemics of infections in well-baby nurseries and in NICUs and causes up to 10% of CLABSIs.[45–47,49,149–151] The skin, nares, and umbilicus are the most common sites of colonization. MRSA rates differ greatly between institutions but can account for 50% to 55% of staphylococcal infections.[141,149,152] MRSA colonization increases the risk of MRSA infection.[153] Consequently, when one is covering for a possible *S. aureus* infection, it is critical to select an antibiotic that is effective against methicillin-resistant strains. Attributable mortality due to *S. aureus* HAI is between 5% and 18%, with rates as high as 25% in VLBW infants, irrespective of whether the strain is methicillin resistant or not.[149,152,154] *S. aureus* HAI has been found to be associated with increased rates of adverse neurodevelopmental outcomes.[154]

Enterococcus

Enterococci *(Enterococcus faecalis, Enterococcus faecium)* are responsible for both endemic and epidemic HAIs in the NICU and are responsible for approximately 3% of NICU bloodstream infections.[2,132] Prematurity, use of CVCs, prolonged hospital stay, and prior antibiotic use are recognized risk factors for colonization with these organisms.[155] The GI tract is often the primary source of infection; however, the pathogens are typically spread via the hands of healthcare workers or through environmental contamination. The widespread use of antibiotics has led to the emergence of VRE.[156] There are published guidelines to prevent the spread of VRE, which include periodic surveillance, hand washing, isolation, barrier precautions, and cohorting of infected patients.[157,158]

Group B Streptococcus

Group B streptococcus remains an important cause of early-onset and late-onset infection in neonates, but neither has a clear role as an HAI. CDC NHSN guidelines have modified exclusion criteria such that group B streptococcus identified from blood, within the first 6 days of life, will not be reported as a CLABSI.

Gram-Negative Bacteria

Gram-negative organisms are a particularly important cause of nosocomial bloodstream infections, pneumonia, and meningitis because of the high burden and severity of disease. They are the predominant cause of infection in low- and middle-income countries.[43] *Escherichia coli* is the most common gram-negative pathogen.[132] Other gram-negative organisms responsible for HAI include *Klebsiella, Pseudomonas, Enterobacter, Acinetobacter, Serratia, Haemophilus, Citrobacter*, and *Salmonella* spp. (see Table 37.3).[132] Gram-negative infections are currently responsible for approximately 15% of infections in the NICU.[132] The GI tract is thought to serve as the reservoir for these bacteria, and prolonged antibiotic therapy may promote selection of these bacteria.[75,159] The attributable mortality is much higher for gram-negative infections than for gram-positive infections.[137–139] The occurrence of a gram-negative infection in an NICU patient has been found to be associated with a 3.5-fold higher risk of death.[2] *Pseudomonas* spp. are particularly virulent, causing death in 42% to 75% of infected neonates.[2,139,160–162]

Fungi

Fungal infections are discussed in detail in Chapter 36. Invasive fungal infection is estimated to occur in 1% to 4% of VLBW infants and up to 10% of extremely low birthweight (ELBW) infants, with the highest risk in infants weighing less than 750 g.[163–168] The rates and predominant fungal species differ considerably between centers. The smallest and most premature infants appear to be at the highest risk, particularly when they are exposed to broad-spectrum antibiotics and long courses of antibiotics. Other identified risk factors are prolonged mechanical ventilation, prolonged use of CVCs, exposure to lipid emulsions, antenatal antibiotics, and histamine H_2-receptor antagonists.[117,163,166]

Prophylactic fluconazole therapy has shown promise in reducing the rates of colonization and infection with candida, with the greatest impact seen in NICUs with high rates of candida infection.[169–176] However, a large randomized controlled trial of prophylactic fluconazole did not show reductions in mortality or the incidence of invasive fungal disease.[177] Although no study has found an increase in the incidence of fluconazole-resistant fungus, resistance remains a concern with any prophylactic strategy. Limiting prophylaxis to NICUs with high rates of invasive candidal disease and to patients at highest risk provides the greatest benefit with the least incurred risk of antifungal resistance.

Viruses

Viral organisms that commonly cause HAI in the NICU include respiratory syncytial virus (RSV), rhinovirus, metapneumovirus, influenza virus, rotavirus, and enterovirus. Isolated infections generally result from contact with infected caregivers or family members. Nursery epidemics may occur in addition to isolated individual cases. An analysis of infectious outbreaks in NICUs globally found that approximately 10% of outbreaks affecting neonates were attributed to viral causes, with the most common etiologic agents being rotavirus, RSV, enterovirus, hepatitis A virus, and adenovirus.[178] Through the COVID-19 pandemic, nosocomial transmission of SARS CoV-2 has been rarely reported in the NICU, likely due to early adoption of personal protective equipment and isolation precautions.[179] Measures to prevent and contain viral infections include standard precautions such as isolation and cohorting of affected patients, meticulous hand hygiene and use of personal protective equipment, and surveillance of patients and healthcare personnel during outbreaks. Alarmingly, mortality rates for viral outbreaks appear to be similar to those for bacterial outbreaks (7.2% vs. 6.4%).[178]

Respiratory Syncytial Virus

RSV is a fastidious organism capable of surviving on inanimate objects for prolonged periods and which can cause severe disease in neonates, particularly those who are premature or who have cardiopulmonary disease. Rapid testing to detect RSV in nasal washings facilitates efforts to cohort infected patients.[180] Guidelines from the American Academy of Pediatrics (AAP) recommend that all high-risk infants born at less than 29 weeks' gestation, or with chronic lung disease, or with hemodynamically significant congenital heart disease receive up to five doses of palivizumab (an RSV monoclonal antibody) during the RSV season but starting only at hospital discharge.[181] Although current guidelines do not list prophylactic palivizumab for prevention of nosocomial transmission of RSV, some centers choose to provide RSV prophylaxis to at-risk inpatient neonates because of sporadic occurrences of inpatient nosocomial deaths from RSV.[182–186]

Influenza

Influenza is spread primarily via airborne transmission. Hand washing and immunization of healthcare workers are the primary tools to prevent nosocomial spread.[187] Some institutions mandate yearly immunization among healthcare workers. Infection control guidelines recommend that every healthcare worker wear a mask during contact with infected patients.[187] At-risk infants should receive the influenza vaccine during the winter months once they reach the age of 6 months.[188] Parents and other close contacts of at-risk infants should also receive influenza vaccination. Oseltamivir is approved by the US Food and Drug Administration (FDA) for treatment (within the first 48 hours of symptoms) of patients older than 2 weeks infected with influenza A virus or influenza B virus; the AAP and CDC recommend its use for treatment of infected infants of any age.[188] Although safety and efficacy of oseltamivir prophylaxis have not been established in infants younger than 1 year, the CDC recommends its use for this purpose from 3 months of age onward.[188] Guidelines for minimizing risk to infants born to mothers who are actively infected have been provided by the CDC.[189] Pregnant women should receive treatment as soon as possible.[189] If tolerated, the mother should wear a mask during labor and delivery. After delivery, the newborn should be cared for away from the mother until she has received treatment for 48 hours, has become afebrile, and is able to control cough and secretions.[188] If desired, lactation should be facilitated, because the breast milk itself is not thought to be a means of viral transmission.[189]

Rotavirus

Although rare, epidemics of rotavirus diarrhea may occur in the nursery[190–193]; they are primarily caused by inadequate hand washing and cross-contamination between patients.[194] Standard and contact precautions should be followed throughout the duration of the illness. Some patients have prolonged fecal shedding of low concentrations of the virus; therefore some infection control experts recommend contact precautions for the duration of the hospitalization of such patients. Rotavirus is also an important cause of diarrhea in older infants. Live virus vaccines are now available for the prevention of rotavirus infection. Although the 2009 AAP statement recommended administration of the first dose of rotavirus vaccine at discharge from the NICU, several studies have documented the safety of pentavalent rotavirus vaccine administration during the hospital stay of preterm infants, with no concern for significant adverse events or symptomatic transmission from shedding.[195–198] Furthermore, one study noted that delaying administration of the first dose until discharge led to more than half of ELBW infants not receiving the rotavirus vaccine because they missed the window of postnatal age for vaccine administration.[199]

Enterovirus

There are numerous serotypes of enteroviruses, including polioviruses, Coxsackie viruses A and B, echovirus, and nonassigned subtypes.[200] Enterovirus infections have been described among neonates in the well-baby nursery and the NICU setting.[200–204] Both isolated cases and epidemics can occur. The clinical presentation associated with enteroviral infection is variable, ranging from asymptomatic to overwhelming multisystem organ dysfunction with poor prognosis.[201,204–207] The severity of the disease and risk of mortality are higher in perinatally acquired infections than nosocomially acquired cases, presumably related to the lack of maternal antibody present in the neonate.[201,208] Blood and cerebrospinal

fluid cultures should be obtained from any patient with clinical symptoms of disease. Polymerase chain reaction analysis is helpful in making a rapid diagnosis.[209,210] No antiviral agents are currently available to treat enteroviral infections in newborns.[209] Although commercially available intravenous immunoglobulin (IVIG) preparations have high levels of neutralizing antibodies to common enterovirus serotypes, there is no clear evidence that administration of immunoglobulin alters the process or outcome of enteroviral infection.[206,207,210,211]

Prevention of Healthcare-Associated Infection

Quality Improvement Efforts

In recent years, many NICUs and quality improvement collaboratives have described their success in decreasing the rate of CLABSI and other HAIs in the NICU.[16–20,46,212] Several guiding principles and common themes are present in these success stories. Monitoring, surveillance, and benchmarking of the HAI rates in the nursery are critical components of any prevention program. A key and powerful initial step is comparing institutional performance (or "benchmarking") to improve understanding of the performance of a NICU in comparison with peer institutions and to recognize opportunities for improvement.[213] Next, formation of "quality improvement collaboratives" helps institutions develop common sets of best practices and common definitions and attempts to minimize interhospital variation in outcomes, allowing lower-performing hospitals to learn from hospitals with lower rates of infections. Several statewide and other collaboratives have reported reductions in CLABSI rates with use of this strategy.[16–20,46,212,214,215] These significant successes have been accomplished with the development and implementation of care "bundles," incorporating a group of interventions aimed at standardizing care to minimize HAIs, education of staff on quality improvement principles, and developing a shared mental model of the preventable nature of most HAIs.

Overall Approach to Infection Control

The CDC recommends a tiered approach to infection control (Box 37.2). Standard precautions should be used with all patient contact, regardless of the underlying diagnosis or infectious status. These precautions consist of universal precautions (designed to prevent blood and body fluid contamination) and body substance precautions (designed to prevent contamination with moist substances). Transmission-based precautions are necessary when a patient is infected with a known or suspected pathogen that is associated with a high risk of contamination via airborne or droplet transmission or contact with the skin or contaminated surfaces.[216]

Guidelines for Hand Hygiene Practices

The importance of hand hygiene has been known since the 1800s, as elucidated by Labarraque and Semmelweiss.[217] Although optimal hand hygiene is the cornerstone of HAI reduction efforts, healthcare settings continue to face challenges in standardizing practices among caregivers.[218,219] Hand hygiene techniques are effective in decreasing the colonization rate of resident and transient flora and have been shown to reduce cross-contamination among patients. Direct patient contact and respiratory tract care seem to be particularly associated with contamination.[220] Organisms such as RSV, *S. aureus*, and gram-negative bacilli can survive on inanimate objects ("fomites"), so holding an infant infected with one of these organisms, changing diapers, and even touching items in the infant's environment can result in hand contamination.[44,217]

Recommendations on indications and techniques for hand hygiene were published by the CDC in 2002 and updated by the World Health Organization (WHO) in 2009 (Box 37.3).[221] These guidelines will be effective only if every healthcare provider performs hand hygiene before and after every patient contact. Reported barriers to compliance with hand hygiene recommendations include skin irritation, poor accessibility of sinks or cleansing agents, insufficient time, heavy workload, understaffing, and lack of information. Alcohol-based hand rub may increase compliance by increasing ease of accessibility.[222] Direct observation is the best method of measuring hand hygiene compliance. A common misconception is that use of gloves obviates the need for adequate hand hygiene. Leakage and contamination of gloves have been reported.[217,218] Disposable single-use gloves should be removed after each patient encounter, and hands should be washed before and after their use. The WHO has a number of excellent, publicly available educational resources to encourage appropriate hand hygiene in healthcare settings, including hand hygiene videos in many languages and tools for implementation of hand hygiene training and auditing programs.

Hand hygiene is extremely cost-effective. The additional hospital charges associated with a single HAI may almost equal the yearly hand hygiene budget. One study estimated the cost of a hand hygiene intervention program to be approximately $57,000 per year.[223] Assuming that 25% of the observed decrease in infections was attributable to improved hand hygiene practices, a saving of $2100 was estimated for every infection averted.

• BOX 37.2 Principles for the Prevention of Healthcare-Acquired Infection in the Neonatal Intensive Care Unit

Observe recommendations for standard precautions with all patient contact
Observe recommendations for transmission-based precautions (gowns, gloves, masks, isolation, as indicated)
Use good nursery design and engineering
Ensure appropriate cleaning and disinfection of environment of care
Appropriate nurse-to-patient ratio
Avoidance of overcrowding and excessive workload
Improve hand hygiene compliance (see Box 37.3)
Provide meticulous skin care
Encourage early and appropriate advancement of enteral feedings
Adopt care bundles targeted to prevention of each healthcare-associated infections (HAIs)
Targeted efforts may be required during outbreaks and to prevent HAIs in high-risk patients
Perform continuous monitoring and surveillance of healthcare-acquired infection rates in the neonatal intensive care unit
Provide education and feedback to nursery personnel

• BOX 37.3 Centers for Disease Control and Prevention Recommendations for Hand Hygiene

Healthcare personnel should use an alcohol-based hand rub or wash with soap and water for the following clinical indications:

- Immediately before touching a patient
- Before performing an aseptic task (e.g., placing an indwelling device) or handling invasive medical devices
- Before moving from work on a soiled body site to a clean body site on the same patient
- After touching a patient or the patient's immediate environment
- After contact with blood, body fluids, or contaminated surfaces
- Immediately after glove removal

Healthcare facilities should:

- Require healthcare personnel to perform hand hygiene in accordance with Centers for Disease Control and Prevention (CDC) recommendations
- Ensure that healthcare personnel perform hand hygiene with soap and water when hands are visibly soiled
- Ensure that supplies necessary for adherence to hand hygiene are readily accessible in all areas where patient care is being delivered

Unless hands are visibly soiled, an alcohol-based hand rub is preferred over soap and water in most clinical situations due to evidence of better compliance compared with soap and water. Hand rubs are generally less irritating to hands and, in the absence of a sink, are an effective method of cleaning hands.

Selection and handling of hand hygiene agents:

- Provide products with a low irritancy potential.
- Solicit input regarding skin tolerance, feel, and fragrance of products being considered.
- Determine known interaction between products used to clean hands, skin care products, and the types of gloves used in the institution.
- Ensure that dispensers are accessible at the point of care.
- Provide alternatives for individuals with adverse reactions to standard products.
- When alcohol-based hand rub is available in the healthcare facility, use of antimicrobial soap is not recommended.
- Soap and alcohol-based hand rub should not be used concomitantly.

Use of gloves

- The use of gloves does not replace the need for hand hygiene.
- Wear gloves when it can be reasonably anticipated that contact with blood or other potentially infectious materials, mucous membranes, or nonintact skin will occur.
- Remove gloves after caring for a patient; do not reuse them with other patients.
- Change or remove gloves during patient care if moving from a contaminated body site to either another body site within the same patient or the environment.

Other aspects

- Do not wear artificial fingernails or extenders; keep natural nails short.
- Hand hygiene promotion programs:
  - Focus specifically on factors with significant influence on behavior and not only on the type of hand hygiene product. The strategy should be multifaceted and multimodal and include education and senior executive support for implementation.
  - Educate staff about the types of patient-care activities that result in hand contamination and about the advantages and disadvantages of various methods used to clean their hands.
  - Monitor adherence to hand hygiene practices, and provide performance feedback.
  - Encourage partnerships between patients, their families, and healthcare workers.

Modified from Polin RA, Denson S, Brady MT, et al. Strategies for prevention of health care-associated infections in the NICU. *Pediatrics*. 2012;129:e1085–e1093; CDC guidance for hand hygiene (https://www.cdc.gov/handhygiene/providers/guideline.html).

Guidelines for Gloves and Gowns

Although gowning by healthcare workers is still a common practice in many countries, a meta-analysis of studies of gowning in newborn nurseries revealed that gowning by healthcare workers did not reduce rates of colonization, rates of infection, length of stay, or mortality in infants.[224] Moreover, an observational study noted that when increasing numbers of patients were placed under contact precautions, necessitating gowning and gloving, gowning actually led to a decrease in compliance with precautions.[225] However, some studies have shown that use of nonsterile gloves along with correct hand hygiene practices could reduce bloodstream infections in preterm infants compared with hand hygiene alone.[226,227]

Care of the Patient Environment

NICU design may have an impact on risk of nosocomial transmission, with emerging evidence regarding the benefits of single-occupancy rooms.[95,228,229] Airflow, ventilation, plumbing, building structure, and presence of isolation rooms are all important components of maintaining a safe environment of care. Protocols for daily cleaning of surfaces and patient equipment and periodic "deep" cleaning and disinfection are important to reduce bioburden in the immediate patient surroundings.[230] NICU visitation policies must be carefully designed based on risk of viral transmission, use of visitor screening policies, hand hygiene education to families, considerations around sibling visitation, and encouragement of influenza vaccination.[230]

Human Milk Feedings

Several studies have reported lower risks of sepsis and necrotizing enterocolitis with early enteral feeding with human milk.[231] The various mechanisms proposed for the beneficial effect of human milk feeding include its content of immunoprotective substances and prebiotics and probiotics that modulate the development of a protective infant gut microbiome.[232] Donor human milk is increasingly used as a substitute when mother's own milk is unavailable; however, the pasteurization process likely diminishes the activity of many of these immunomodulating components.[233] A Cochrane review comparing donor human milk to formula demonstrated moderate certainty evidence of lower rates of necrotizing enterocolitis (NEC) with donor breast milk but no impact on growth, neurodevelopment, or mortality.[234]

Skin Care

The skin of VLBW preterm infants is immature and an ineffective barrier to prevent transepidermal loss of water and invasion of bacteria. The stratum corneum has mechanical and chemical properties that decrease the risk of infection[235] and matures at approximately 32 weeks' gestation. In a prematurely born neonate, the maturation process is accelerated and is usually complete by 2 to 4 weeks after birth.[235] The most recent Cochrane meta-analysis found that studies of topical emollients or creams did not impact rates of invasive infections in high-income countries[236]; however, studies of topical vegetable oil emollients in low- and middle-income countries suggested a reduction in infections but no impact on mortality, with low certainty of evidence.

Topical antiseptics and bathing agents must be chosen with attention to gestational age and skin integrity.[237] Povidone-iodine

is commonly used for skin preparation prior to procedures such as umbilical line placement and lumbar punctures. For many other procedures, providers may choose between povidone and chlorhexidine for skin preparation.[238] Chlorhexidine is also widely used for daily bathing for CLABSI prevention, with some evidence of efficacy, and is also used as part of *S. aureus* decolonization efforts.[239–241] However, the FDA recommends use with care in infants younger than 2 months and premature infants due to concerns with systemic absorption. Chlorhexidine can cause irritant skin reactions and burns, especially when alcohol-based formulations are used on fragile or preterm skin.[242] There remains a lack of clear consensus across NICUs on adoption of chlorhexidine bathing and inclusion criteria for use, but its use should be considered when infection rates remain high despite high compliance with other infection prevention measures.[241,243] For preterm infants, serial bathing to decrease bacterial bioburden may be accomplished using soap and water alternatives.[237,243]

Surveillance for Resistant Pathogens and Control Measures

Units can use specific strategies to identify resistant pathogens such as MRSA and VRE and develop targeted control measures. Strategies include use of PPE, and contact isolation to limit horizontal transmission in the NICU, along with emphasis on diligent hand hygiene for family and healthcare personnel. Teams may consider routine active surveillance for MRSA, for example, to inform infection control measures and clinical management decisions. In an outbreak setting, additional active surveillance measures may be employed, along with strategies such as contact isolation and targeted decolonization when applicable.[244]

Additional Strategies in Limited Resource Settings

Use of topical antiseptics such as chlorhexidine for cord care, kangaroo mother care (skin-to-skin care), and topical emollient massage are interventions that have demonstrated some lowering of risk of sepsis and mortality in lower- and middle-income countries and could be additional considerations in these settings (Cleminson and McGuire, 2021).[230]

Prevention of Central Line–Associated Bloodstream Infection

The care and maintenance of CVCs are key to reducing CLABSIs. Most institutions currently adopt a "care bundle" of practices surrounding central line insertion and maintenance that have been shown to be effective at reducing CLABSI rates (Box 37.4).[16,18–20,46,212,213,215,245] The key elements during catheter insertion are hand hygiene, aseptic technique, skin antisepsis, and sterile dressing technique. Best practices during catheter maintenance include hand hygiene, daily review of line necessity, daily inspection of the insertion site and dressing, standardization of practices around IV tubing changes, and "scrubbing the hub (needleless connector)" of the central line to minimize contamination (see Box 37.4). In addition, NICUs have incorporated bathing strategies in patients with central lines as described earlier. Compliance with the maintenance bundle is of great importance, as NICU patients often have extended need of central lines, and most CLABSIs in NICU patients occur several days to weeks beyond insertion.[108]

Several NICUs have adopted practices targeting early removal of central lines, when infants advancing on enteral feedings demonstrate tolerance of 70% to 80% of goal volume feeds. Studies from a multicenter collaborative of Children's Hospital NICUs combined orchestrated testing with quality improvement efforts, to pinpoint individual bundle interventions that were likely to have the greatest impact on CLABSI reduction.[214] Standardized changes of IV tubing and practices around scrubbing the hub were found to be associated with the greatest benefit.[214] This is likely because contamination of the catheter hub is the most likely source of infection for neonates, and these practices directly address this risk. Specific guidelines around use of umbilical catheters have also been developed, emphasizing timely removal—not later than 14 days from placement for umbilical venous catheters and ideally not later than 5 days for umbilical arterial catheters.[219,246]

Vascular access teams of trained personnel have also been deployed in NICUs in recent years, composed of skilled personnel who ensure standardized line insertion and maintenance practices, and support education of staff and quality improvement initiatives; studies have demonstrated reduction in CLABSIs following deployment of these teams.[247,248]

A meta-analysis comparing skin antiseptic agents found that use of chlorhexidine gluconate for catheter site care reduced the risk of CLABSI by 50% compared with use of solutions containing povidone-iodine.[249] A randomized trial in VLBW infants showed that while chlorhexidine gluconate–containing dressings reduced colonization of central venous lines (compared with povidone-iodine), CLABSI rates were comparable with the two modes of catheter site care; of note, chlorhexidine was associated with high contact dermatitis rates in the most immature infants.[250]

There are some data describing the use of prophylactic vancomycin and antibiotic lock therapy with vancomycin in neonates, suggesting a reduction in CoNS bloodstream infection; however, these studies did not demonstrate reductions in length of stay or mortality. Given the concerns for potential development of antibiotic resistance with widespread use of prophylactic antibiotics, these practices are not recommended.[251–253] A similar concern regarding the possibility of fluconazole-resistant fungal infections, fluconazole-related toxicity, and lack of evidence regarding mortality or long-term morbidity reduction has limited the recommendation for prophylactic fluconazole to high-risk units, despite evidence of fungal CLABSI reduction with prophylactic fluconazole from randomized trials.[254]

Recent data suggest a plateauing of CLABSI rates despite continued use of CLABSI prevention bundles.[63] Future approaches may include additional customized interventions for high-risk subgroups, as well as strategies to more broadly decrease rates of bacteremia.[63,255]

Catheter Removal Following Central Line–Associated Bloodstream Infection

A retrospective review compared outcomes in patients in whom catheters were removed at the onset of infection with those in whom catheters were left in place.[256] Forty-six percent of infants in whom in-place catheter sterilization was attempted had complications, compared with 8% of infants whose catheters were removed. Infants with gram-negative infections were more likely to have complications if catheters remained in place.[256] A study of infants with CoNS CLABSIs found no difference in complications or mortality rate if catheter removal was delayed[160]; however,

• BOX 37.4 Central Line Insertion and Maintenance Bundle Elements

Components of Central Line Insertion Bundle

Component	Supporting Recommendations From 2011 CDC Prevention Guidelines
Utilize dedicated and trained personnel for central line insertion	• Designate only trained personnel who demonstrate competence for the insertion and maintenance of central lines. (Category IA) • Educate healthcare personnel regarding the indications for central line insertion, proper procedures for insertion and maintenance, and appropriate infection control methods. (Category IA) • Periodically assess knowledge and adherence to guidelines for all personnel involved in central line insertion and maintenance. (Category IA)
Perform appropriate hand hygiene	• Wash hands with conventional soap and water or with alcohol-based hand rubs before and after palpating catheter insertion sites, inserting, replacing, or dressing an intravascular catheter. (Category IB)
Utilize maximum sterile barrier precautions	• Sterile gloves should be worn for the insertion of central catheters. (Category IA) • Use maximal sterile barrier precautions, including a cap, mask, sterile gown, sterile gloves, and sterile full-body drape, for central line insertion. (Category IA)
Prepare skin with an appropriate antiseptic agent	• No recommendation can be made for the safety or efficacy of chlorhexidine in infants aged <2 months. (Unresolved issue) • Cutaneous antisepsis with a >0.5% chlorhexidine preparation with alcohol is recommended in most patient populations before central line insertion and dressing changes, but if there is a contraindication, tincture of iodine, an iodophor, or 70% alcohol can be used as alternatives. (Category IA) • Antiseptics should be allowed to dry according to the manufacturer's recommendation prior to placing the catheter. (Category IB)

Components of Central Line Maintenance Bundle

Component	Supporting Recommendations From 2011 CDC Prevention Guidelines
Assess and document daily whether or not central line placement or continued use is necessary: a. "Do we really need to place a central line?" b. "If there was no line in place today, would we place one?"	• Use a peripherally inserted central catheter instead of a short peripheral catheter when the duration of IV therapy will likely exceed 6 days. (Category II) • Promptly remove any intravascular catheter that is no longer essential. (Category IA)
Perform appropriate hand hygiene	• Wash hands with conventional soap and water or with alcohol-based hand rubs before and after palpating catheter insertion sites or dressing an intravascular catheter. (Category IB)
Assess central line dressing integrity and catheter insertion site daily (at minimum)	• Replace catheter dressing if damp, loosened, or visibly soiled. (Category IB) • Evaluate the catheter insertion site daily by palpation through dressing to discern tenderness and by inspection if dressing is transparent. If patient has local tenderness or other signs of possible CLABSI, opaque dressings should be removed and site inspected visually. (Category II)
Perform appropriate central line dressing change, when required: a. Two-person procedure b. Site cleansed with appropriate solution c. Cleansing solution allowed to air-dry completely	• Replace dressings on short-term central line sites at least every 7 days for transparent dressings, except in pediatric patients in which the risk for dislodging the catheter may outweigh the benefit of changing the dressing. (Category IB) • Antiseptics should be allowed to dry according to the manufacturer's recommendation. (Category IB)
Develop and use standardized intravenous tubing setup and changes	• In patients not receiving blood, blood products or fat emulsions, replace continuously used administration sets, including secondary sets and add-on devices, at no more than 96-h intervals, but at least every 7 days. (Category IA) • Replace tubing used to administer blood, blood products, or fat emulsions (those combined with amino acids and glucose in a 3-in-1 admixture or infused separately) within 24 h of initiating the infusion. (Category IB)
Maintain aseptic technique when changing IV tubing setup and changes	• Minimize contamination risk by scrubbing the access port with an appropriate antiseptic (chlorhexidine, povidone-iodine, an iodophor, or 70% alcohol) and accessing the port only with sterile devices. (Category IA)

Category IA: strongly recommended for implementation and strongly supported by well-designed experimental, clinical, or epidemiologic studies. Category IB: strongly recommended for implementation and supported by some experimental, clinical, or epidemiologic studies and a strong theoretical rationale; or an accepted practice (e.g., aseptic technique) supported by limited evidence. Category IC: required by state or federal regulations, rules, or standards. Category II: suggested for implementation and supported by suggestive clinical or epidemiologic studies or a theoretical rationale. Unresolved issue: represents an unresolved issue for which evidence is insufficient or no consensus regarding efficacy exists.

CDC, Centers for Disease Control and Prevention; *CLABSI*, central line–associated blood stream infection.

Data from Mobley RE, Bizzarro MJ. Central line-associated bloodstream infections in the NICU: Successes and controversies in the quest for zero. *Semin Perinatol.* 2017;41(3):166–174.

these patients were more likely to have persistently positive culture results when their lines were not removed with the first positive culture result (43% vs. 13% for immediate catheter removal). Patients with CLABSIs due to gram-negative or fungal pathogens should have their catheters removed as early as possible; patients with CLABSIs caused by CoNS should undergo catheter removal if the culture results are persistently positive or if the patient's condition is unstable.[160,256]

Prevention of Healthcare-Associated Pneumonia

Like CLABSI prevention efforts, best practice bundles for VAP prevention have been adopted in many hospitals. The key elements of VAP prevention as recommended by the CDC include surveillance for VAP (which does not include routine cultures), prevention of bacterial transmission, staff education, and risk reduction in the patient.[122,219] Key practices that may help reduce the risk of VAP include timely removal of tracheal tubes from patients, minimizing aspiration of pathogenic bacteria by elevating the head of the bed by 30 to 45 degrees, and performing oral hygiene with sterile water.[122] There is no clear evidence of the superiority of closed versus open suctioning systems in minimizing VAP risk.[122,123,126] Other important considerations include use of hand hygiene and gloves prior to management of ventilation equipment, developing standard practices for suctioning while minimizing contamination, and optimizing noninvasive ventilation strategies whenever feasible.[257]

Antibiotic and Adjunctive Therapies

Antibiotic choice should initially cover a broad spectrum of pathogens and should then be narrowed as soon as possible to cover the specific bacteria identified once sensitivities are known. Antibiotic use should be discontinued if infection is not proved and is not likely. Coverage for *Pseudomonas* spp. or other resistant gram-negative organisms should be considered in patients with rapid clinical deterioration.[2,160] However, empiric broad-spectrum antibiotic use should be limited as much as possible to avoid development of resistant infections.[258]

A recent meta-analysis on the prophylactic use of IVIG in preterm neonates included data on immunoglobulin M–enriched IVIG and found no reduction in mortality or adverse neurodevelopmental outcomes.[259] IVIG is therefore not recommended for routine use in suspected or proven sepsis.[259] Hemopoietic colony-stimulating factors (granulocyte and granulocyte-macrophage) are effective in raising the neutrophil count but have not consistently decreased HAI rates or mortality.[260,261]

The most recent meta-analysis of randomized controlled trials of enteral lactoferrin for prevention of sepsis and necrotizing enterocolitis provides low-certainty evidence of decrease in late-onset sepsis.[262] The meta-analysis also provides low-certainty evidence that lactoferrin supplementation in combination with probiotics decreases late-onset sepsis and NEC; however, there were few studies and concerns about methodological quality.[262]

Probiotic use has increased in NICUs over the past few years, with up to 10% of extremely preterm infants receiving some formulation, despite lack of FDA-approved options.[263] Meta-analyses of studies of probiotics are challenging to assimilate due to heterogeneity in studies; however, there does appear to be a benefit in reducing risk of NEC.[264–266] Conflicting results even between RCTs may in part be due to variability in probiotic composition, dosing, and target populations between studies.[267,268] The AAP, Canadian Pediatric Society, and ESPGHAN have issued statements advocating for caution regarding routine use of probiotics in preterm infants. The Committee on Fetus and Newborns states that current evidence does not support the routine, universal administration of probiotics to preterm infants, particularly those with a birthweight of less than 1000 g.[256,269]

Adverse Outcomes Related to Healthcare-Associated Infection

HAIs are a potentially modifiable contributor to a spectrum of adverse outcomes, across all gestational and postnatal ages, but especially in the most immature infants (Table 37.4). Studies from the NICHD NRN have demonstrated a striking increase in mortality in VLBW infants who experience late-onset infection (18% in infected infants vs. 7% in uninfected infants), with higher mortality rates for gram-negative or fungal sepsis.[2] Several studies have found the length of stay to increase because of sepsis: NICHD NRN data indicated that the mean length of stay increased from 60 to 79 days in VLBW infants, while the VON group found an increase in the length of stay of 4 to 7 days.[2,6,270] In the subset of infants with intestinal failure due to necrotizing enterocolitis, hospital length of stay and the duration of parenteral nutrition were greatly increased by the occurrence of infections.[96] A study of VAP in pediatric intensive care units and NICU populations noted an increased duration of mechanical ventilation (by 3 days).[271]

TABLE 37.4 Adverse Outcomes Associated With Bloodstream Infections in Very Low Birth Weight Infants in the Neonatal Intensive Care Unit

Adverse Outcome	Study	Adjusted Effect
Death	Stoll et al.[63]	2.4-fold increase (17% vs. 7%)
	Stoll et al.[2]	2.6-fold increase (18% vs. 7%)
	Makhoul et al.[94]	2.0-fold increase (17% vs. 9%)
Poor neurodevelopmental outcome	De Haan et al.[12]	OR 4.8 (1.5–15.9) (gram-negative BSI)*
	Mitha et al.[4]	OR 2.2 (1.5–3.1)†
	Schlapbach et al.[8]	OR 3.2 (1.2–8.5)†
	Stoll et al.[10]	OR 1.4 (1.3–2.2)*
Increased length of stay	Stoll et al.[63]	19–22-day mean increase
	Stoll et al.[2]	18.6-day mean increase
	Makhoul et al.[94]	27-day mean increase
	Atif[257]	9.2-day mean increase
Increased cost	Payne et al.[6]	$54,539 mean increase
	Donovan et al.[13]	$16,800 mean increase

*Bayley-II motor or cognitive score less than 85, blindness, deafness, or cerebral palsy.
†Cerebral palsy.
BSI, Bloodstream infection; *OR*, odds ratio.
Data from Cantey JB, Milstone AM. Bloodstream infections: epidemiology and resistance. *Clin Perinatol*. 2015;42:1–16.

There is also strong evidence that infections in VLBW infants are associated with an increased risk of adverse neurodevelopmental outcomes. Studies of ELBW infants have found that infants who experienced infection had impaired head growth, increased risk of cerebral palsy, lower Bayley mental and psychomotor development indices, and visual deficits.[5,7,10] An analysis by the NICHD NRN of trends from 2005 to 2012 noted a decrease in infections across all VLBW gestational ages in this time period, while also noting slight improvements in survival without major morbidity in infants born at 25 to 28 weeks' gestation; the authors suggest that at least some of this improvement may plausibly be related to the reduction in infection rates over the same period.[56]

Evidence regarding the adverse effects of excess antibiotic exposure continues to mount. Development of antibiotic resistance is a well-known concern, potentially facilitating the emergence and spread of resistant nosocomial pathogens within the NICU.[136,142,272–275] Several investigations into the impact of gut microbial alterations in early life have demonstrated associations between early antibiotic exposure, gut microbial dysbiosis, and the occurrence of GI dysfunction, necrotizing enterocolitis, sepsis, long-term immune dysregulation and GI disorders, and increased mortality.[276–279]

HAIs are also associated with significantly increased use of healthcare resources and healthcare costs.[6,280–282] One meta-analysis of the costs of HAIs in the United States estimated costs attributable to CLABSIs at $45,814 (95% CI $30,919 to $65,245), to VAP at $40,144 (95% CI $36,286 to $44,220), to SSIs at $20,785 (95% CI $18,902 to $22,667), and to catheter-associated UTIs at $896 (95% CI $603 to $1189).[282] A retrospective study of HAIs in NICUs calculated an incremental cost of $16,800 attributable to bloodstream infections,[13] whereas another study in VLBW infants found a more modest cost increase but still amounting to thousands of dollars ($1280 to $5875 per infection).[6] Attributable costs in a study of pediatric and neonatal VAP were estimated at $30,000.[271]

Decreasing HAI rates in the NICU can reduce the risk of adverse events in infants during their hospital stay, thereby decreasing the incidence of short-term and long-term adverse outcomes, length of stay, and direct healthcare costs. HAIs have therefore become a very important focus of quality improvement efforts in NICUs across the United States and the world.

Conclusion

Prevention of HAI is a cornerstone of excellence in NICU care. Although it is encouraging that HAIs in NICUs have greatly decreased over time, rates have plateaued in the past decade. CLABSIs and other HAIs continue to cause significant morbidity and death in the most vulnerable NICU patients in many countries of the world, as well as contribute to antibiotic use and development of antimicrobial resistance. Considerable work remains to be done in the United States and across the world to move closer to eliminating hospital acquired infections. Collaborative quality improvement approaches, ensuring adequate staffing of nurseries, and emphasizing "bundles" of good practices are key to accomplishing these goals. Clinicians must continue to focus on effective prevention strategies, including adherence to strict hand hygiene policies, minimal use of invasive devices, promotion of enteral nutrition especially breastmilk, surveillance of infection patterns, and education of all nursery staff members. Additional specialized prevention strategies may need to be considered in certain high-risk populations to maximize effective prevention.

Suggested Readings

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38 Lung Development

ERIN PLOSA AND JENNIFER SUCRE

KEY POINTS

- Lung organogenesis is organized into five stages, beginning at embryonic day 25 in humans.
- The mechanisms of lung organogenesis, including branching morphogenesis, stretch/mechanotransduction, alveolarization, microvascular maturation, and cellular differentiation, depend on transcriptional regulation of cell–cell and cell–extracellular matrix signaling networks.
- Alveolarization and lung growth are primarily postnatal processes that extend to early adulthood to establish a surface area that matches growing metabolic needs.
- Disruption in the lung developmental program results in congenital lung malformations.
- The transition from intrauterine to extrauterine life requires the maturation of the surfactant system and the development of lung defense systems against infection and environmental injury.

The primary function of the lung is to exchange oxygen for carbon dioxide to meet the demands of aerobic cellular respiration. The oxygen consumption of the adult human ranges from 250 mL/min at rest to 2630 mL/min at peak exercise.[1] To accommodate these metabolic needs, a large surface area and a thin alveolocapillary membrane are required to enable efficient diffusion of oxygen, more so than carbon dioxide. Ultimately, the zone of gas exchange will attain a surface area of 50 to 100 m^2 and a volume of 2.5 to 3.0 L in the adult human. Therefore, the lung must develop in such a way to maximize the alveolar surface area to meet these needs. While a substantially large surface area is critical for oxygen uptake, the diffusion distance from the alveolus to the red blood cell must be relatively short to facilitate release of carbon dioxide. Meanwhile, a protective aqueous barrier protects the delicate alveolar epithelium, working in tandem with surfactant to mitigate surface tension and alveolar collapse. The result of normal lung development is a thin, expansive alveolar epithelial surface area intermingled with a well-approximated capillary network that facilitates the exchange of oxygen and carbon dioxide.

The trachea, airways, and alveoli are in constant contact with the external environment. Every inhalation brings large numbers of microorganisms, as well as potentially toxic particles and gases, into direct contact with epithelial surfaces. Lung organogenesis must also incorporate mechanisms for clearance of microorganisms and allergens that may result in epithelial infection or injury. Similarly, the lung must defend against nonparticulate gases that are potentially harmful. Oxygen, so critical to cellular function, can be the source of harmful reactive oxygen species that require detoxification, as do inhaled pollutants. The appropriate development and maintenance of these lung functions are critical to the health and survival of newborns.

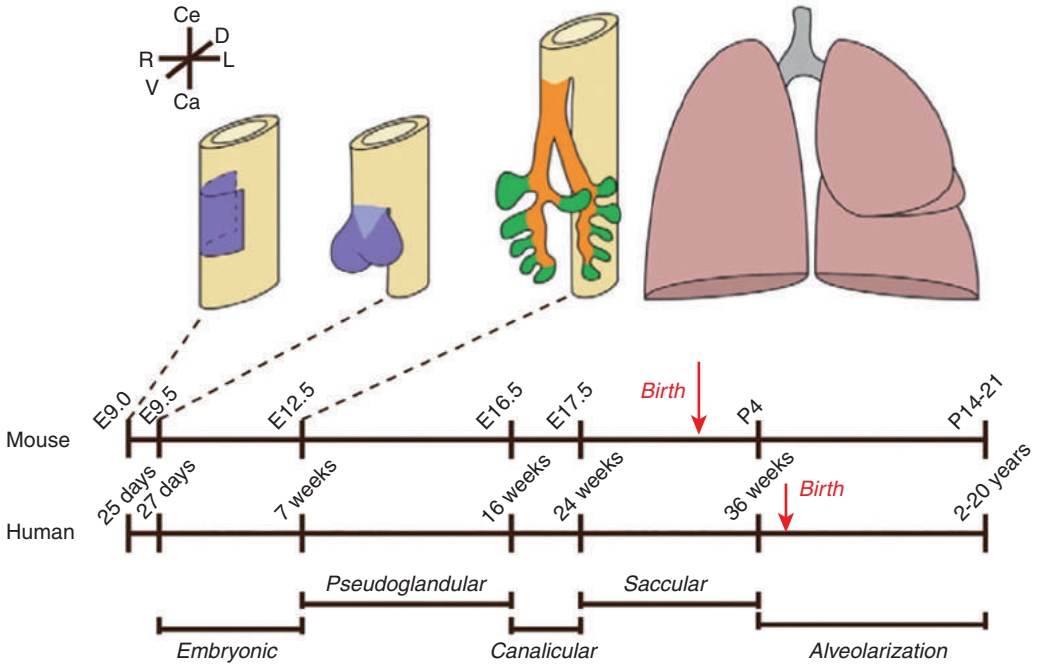
Key Events in Lung Development

Lung organogenesis begins early in human gestation (by day 25), and growth extends well into early adulthood.[2,3] Lung development is organized into 5 sequential stages (embryonic, pseudoglandular, canalicular, saccular, and alveolar), although the timing of these stages is somewhat imprecise, and considerable overlap may occur. Fig. 38.1 shows a timeline of fetal and postnatal lung development in the mouse lung versus the human lung.

Development of Airways and Gas Exchange Surfaces

The initial phase of lung development, the *embryonic phase*, is marked by the formation of the lung bud and the initial branching events. The lung bud is first recognizable as a laryngotracheal groove of the ventral foregut at 25 days of human gestation. The lung bud consists of epithelium and surrounding mesenchyme and begins the first in a series of dichotomous divisions that give rise to the conducting airways and five primordial lung lobes (two on the left and three on the right in humans). Tracheoesophageal fistulae, tracheal atresia, and tracheal stenosis result from errors in separation of the laryngotracheal groove, whereas failure to form the initial branches can result in pulmonary agenesis, most typically of the right lung.[4] Branching continues into the *pseudoglandular stage* of lung development. By 7 weeks of human gestation, the trachea, segmental bronchi, and subsegmental bronchi are evident. By the end of 16 weeks, all bronchial divisions are completed. It is important to remember that although the conducting airways will certainly enlarge as the fetus and newborn grow (airway diameter and length increase twofold to threefold between birth and adulthood), large airway branching ceases after 16 weeks of human gestation.

Closure of the pleuroperitoneal folds is a critical event of the pseudoglandular phase, reaching completion by 7 weeks and resulting in separation of the thoracic cavity from the peritoneal cavity. Failure to close the pleuroperitoneal folds results in congenital diaphragmatic hernia. Continuity is retained between these cavities, resulting in herniation of abdominal contents into the



• **Fig. 38.1** Comparison of Lung Development Events in Mice and Humans. Lung endoderm specification begins at embryonic day 9 in the mouse, and the development stages in the mouse are depicted associated with key transcription factors. Comparisons are provided with the timing of equivalent events in human lung development, beginning at day 25 of gestation in the human. *Ca*, Caudal; *Ce*, cephalic; *D*, dorsal; *L*, left; *R*, right; *V*, ventral. (Modified from Herriges M, Morrisey EE. Lung development: orchestrating the generation and regeneration of a complex organ. *Development*. 2014;141:502–513.)

thorax when the midgut returns to the peritoneal cavity from the umbilical cord at 10 weeks of human gestation. The structural consequence of congenital diaphragmatic hernia is pulmonary hypoplasia of the lung ipsilateral to the diaphragmatic defect as the bowel and solid viscera migrate into the thorax. Pulmonary hypoplasia may also extend to the contralateral lung as the mediastinum shifts because of accumulating abdominal viscera in the thorax.

The *canalicular phase* is marked by completion of the conducting airways through the level of the terminal bronchioles. Terminal bronchioles give rise to the pulmonary acinus, the rudimentary gas exchange unit, which is in turn comprised of a respiratory bronchiole and all of its associated alveolar ducts and alveoli. A terminal bronchiole and all the distal acinar structures constitute a lobule. Branching of these distal air spaces continues on a more limited basis during the canalicular phase, finally achieving a total of 23 airway subdivisions.

The *saccular phase* of lung development (24 to 38 weeks of human gestation) refines the relationships between the air spaces, capillaries, and mesenchyme, enabling an alveolocapillary membrane sufficient to participate in gas exchange (0.6 μm) by approximately 24 weeks of human gestation. Beyond this point, the efficiency of gas exchange is determined by the available surface area. Lengthening and widening of the terminal sacs expand the gas-exchange surface area. Each saccule consists of smooth-walled air spaces with thickened interstitial spaces containing a double capillary network. These will give rise to two or three alveolar ducts, further expanding the available surface area. Expansion of these acinar structures continues well into the third trimester of human gestation.

Postnatal lung development can be subdivided into additional stages.[3] True alveoli become evident as early as 36 weeks of gestation in the human fetus, initiating the *alveolar phase* of lung development. The development of primary alveoli is followed by a further expansion of the gas-exchange surface area through the formation of septa, or secondary crests (described further later). Postnatal alveolarization extends from term through 2 years of age. An initial phase of "*bulk alveolarization*" occurs within the first 6 months postnatally, with a more modest addition of secondary alveoli through the remainder of this period. The alveoli of the infant lung are different from adult alveoli. These immature secondary alveoli contain a double capillary bed, whereas adult alveoli are invested by a single capillary bed. *Microvascular maturation*, the next phase of postnatal lung development, occurs between the first few postnatal months of life through 3 years of age (discussed later).

There is considerable controversy regarding when the lung ceases to add alveoli. Estimates have ranged from as early as 2 years to as late as 20 years of age in humans.[5] This is further complicated by the observation that alveolar expansion can occur in response to pneumonectomy in adult animals[6] and humans.[7] The acquisition of alveoli after the maturation of the microvasculature has been termed *late alveolarization*. This activity has been most often demonstrated in subpleural regions of the lung and likely invokes mechanisms similar to secondary crest formation.

The addition of alveoli is not the only means for expanding the surface area of the lung. While alveolarization wanes over the first 3 years of life in the human, growth of the lung continues to expand the gas-exchange surface area. Between 2 years of age and adulthood, lung tissue expands with lung volume proportionately to the increase in body weight of the child. From four years of age to adolescence, lung volume increases at a pace disproportionately greater than body weight despite a slowed rate of alveolarization, suggesting that increased size of alveoli makes a significant

contribution to expanding lung volume in later childhood.[8] Thus owing to the combined processes of prenatal lung development, postnatal lung development, and lung growth, there is tremendous potential for expansion of the gas-exchange surface area that is developmentally programmed into the fetal lung to account for the growing needs of the infant, child, and adult for aerobic cellular respiration. The extent to which these developmental mechanisms can be harnessed after premature birth, with or without superimposed lung injury, is a topic of active investigation that relies on extrapolation of experimental data from mice.

Composition of Airways and Alveoli

As branching morphogenesis proceeds, the airway and alveolar epithelium gives rise to specialized cells that participate in gas exchange, surfactant production, mucociliary clearance, detoxification, and host defense through a process called differentiation. Differentiation occurs in proximal air spaces first, then proceeding to distal air spaces, lagging behind branching. Temporal as well as contextual signals foster the regionalization of epithelial cell types.

Proximal Airways

The proximal airway epithelium is tall and columnar, decreasing in height to a more cuboidal appearance more distally.[9] Four different epithelial cell types line the trachea and bronchi: undifferentiated columnar, ciliated, secretory/goblet, and basal cells. Undifferentiated columnar epithelial cells are joined by multiciliated cells between 11 and 16 weeks of human gestation. These cells are more prevalent in proximal airways and possess multiple motile cilia at the apical surface that beat in a coordinated fashion to clear mucous. Secretory cells can be seen as early as 13 weeks of human gestation and contain either mucous or serous granules, or both. The number of secretory cells with mucous granules peaks at midgestation during fetal lung development and declines in the third trimester relative to adulthood. Finally, immature basal cells expressing epidermal keratin have been noted as early as 12 weeks of human gestation. Basal cells play a critical role in regenerating injured large airway epithelium.

Cartilaginous support of the tracheobronchial tree begins and also proceeds in a centrifugal fashion beginning in the primitive trachea at 4 weeks, reaching the main bronchi by 10 weeks, and proceeding to the most distal terminal bronchioles by approximately 25 weeks of human gestation. Cartilaginous investment of airways is complete by the second month postnatally. Submucosal glands found between the cartilaginous tissue and surface epithelium play a major role in airway host defense. Submucosal gland development can be characterized by five stages: epithelial budding and invasion of the lamina propria, development of a lumen, initiation of tube branching, and repetitive dichotomous branching. The airways of infants and children contain relatively more submucous glands than those of adults. The glands are lined by mucous cells proximally and serous cells more distally, the latter constituting 60% of the total epithelial cell content of the glands. Serous cells secrete water, electrolytes, and proteins with antimicrobial, anti-inflammatory, and antioxidant properties, while the mucous cells produce primarily mucins. In addition to this host defense role, submucosal glands also contain a population of basal cells that respond to injury of the airway by replenishing the airway epithelium.

Muscular investment of the airways begins as early as 6 to 8 weeks of gestation as smooth muscle cells are identifiable around the trachea and large airways. Fetal airway smooth muscle is innervated and able to contract during the first trimester. Muscularization increases throughout fetal life and childhood such that there is an increased amount of smooth muscle relative to airway size when compared with adult airways. Moreover, the rapid increase in the amount of bronchial smooth muscle immediately after birth occurs regardless of the timing of delivery, term or preterm.

An additional airway cell deserves mention because of its association with a wide variety of pediatric diseases. Pulmonary neuroendocrine cells (PNECs) are found throughout the airways, often in innervated clusters known as *pulmonary neuroepithelial bodies* (NEBs) located at branch points in the bronchial tree.[10,11] Although they arise from foregut endoderm, the cell of origin is distinct from other epithelial components of the lung. PNECs have large numbers of dense core vesicles containing neuropeptides, including serotonin and calcitonin gene–related peptide/bombesin, and regulate bronchial tone by releasing their contents in response to stretch- and hypoxia-mediated stimuli. Recent evidence suggests that PNECs also function as airway sensors that trigger immune responses.[12] Pathologic conditions associated with PNEC/NEB hyperplasia include bronchopulmonary dysplasia (BPD), disorders of respiratory control (congenital central hypoventilation syndrome and sudden infant death syndrome), cystic fibrosis, chronic obstructive pulmonary disease, congenital diaphragmatic hernia, and pulmonary hypertension. Neuroendocrine hyperplasia of infancy is a rare form of interstitial lung disease of infancy associated with expansion of the number of PNECs and NEBs. Although the associations are strong, it remains unclear whether PNECs/NEBs play a primary role in these diseases or a responsive secondary role.

Distal Airways

The bronchiolar epithelium differs from the more proximal airway epithelium. In addition to being more cuboidal in appearance, the epithelium contains progressively fewer ciliated cells and goblet cells, which are ultimately absent from the terminal bronchioles. Instead, the nonciliated, secretory club cell is found in increasing numbers and density down the conducting airways, such that the club cell is the most abundant cell of the terminal bronchiole.[9] Club cells are first evident by 16 to 17 weeks of human gestation, initially exhibiting large glycogen stores that are replaced by secretory granules. Between 23 and 34 weeks of gestation there is a dramatic increase in club cell numbers in distal bronchioles. Club cells are critical to host defense and detoxification functions of the lung by producing high levels of cytochrome P450 and flavin monooxygenases in the lung. The club cell also plays an important role in immunoregulation in the distal airways. Important host defense products of the club cell include club cell secretory protein, surfactant protein A (SP-A), surfactant protein D (SP-D), leukocyte protease inhibitor, and a trypsin-like protease. Club cells produce a precursor form of surfactant protein B (SP-B) that may contribute to host defense. The secretion of antiproteases from club cells suggests that they modulate the protease–antiprotease balance in the distal part of the lung.

Alveolar Epithelium

During the fourth through sixth month of human gestation the epithelial cells lining the acini begin to differentiate further.[13] The cuboidal epithelial cells accumulate large glycogen stores and develop small vesicles containing loose lamellae. The large glycogen pools provide a ready source of substrate required for the production of increasing amounts of surfactant phospholipids, and they decrease in size as surfactant production advances

in the fetal lung. In cells destined to become type 2 cells, lamellar bodies become larger, more numerous, and more densely packed with surfactant phospholipids and proteins, whereas those cells destined to become type 1 cells, on losing their prelamellar vesicles and becoming progressively thinner, adopt a phenotype more suitable for gas exchange. Type 1 and type 2 alveolar epithelial cells are readily identified early in the saccular stage of fetal lung development. There remains considerable controversy regarding the origin of type 1 epithelial cells. These cells in culture demonstrate very slow turnover, with a doubling time estimated to be between 40 and 120 days, suggesting that functionally they are terminally differentiated in vivo. Furthermore, in response to epithelial denudation occurring with lung injury, type 2 cells rapidly proliferate to reestablish epithelial continuity and then lose phenotypic features such as lamellar bodies and acquire markers of type 1 cells, suggesting that rapid repopulation of type 1 cells requires a type 2 cell intermediary. More recent studies in animals have suggested that alveolar type 1 cells can be induced to exit their terminally differentiated state and proliferate.[14]

There is increasing appreciation for type 1 alveolar epithelial cells as more than just a passive membrane for gas exchange.[15] While a large surface area and small cytoplasm-to-nucleus ratio provides a thin alveolocapillary membrane to facilitate gas exchange, it also provides a large absorptive surface in the lung. The presence of water and ion channels, some distinct from those in type 2 cells, facilitates the maintenance of a relatively dry alveolus. Type 1 cells may also regulate cell proliferation locally, signal macrophage accumulation, and modulate the functions of local peptides, proteases, and growth factors.

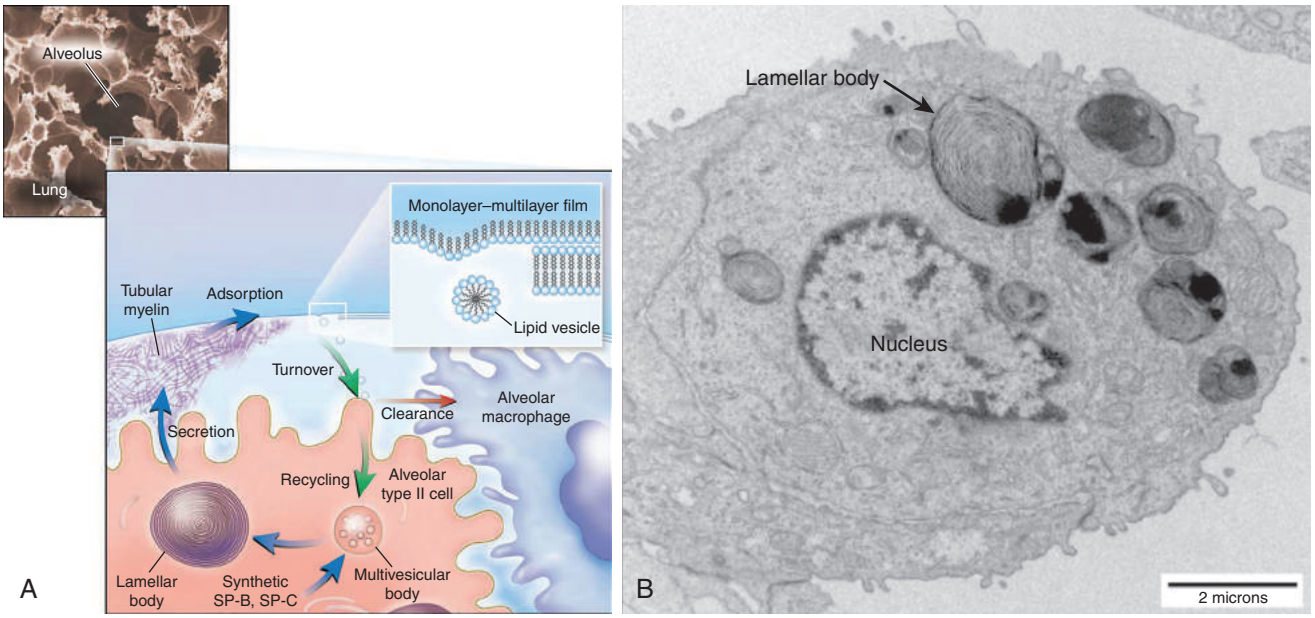
While most notable for its role in surfactant production (discussed later), the type 2 alveolar epithelial cell provides other important functions in the alveolus.[16] Type 2 cells are local progenitor cells and, like type 1 cells, contain ion and water channels as well as ion pumps that contribute to the movement of water and ions across the epithelium. Type 2 cells also contain and secrete important antioxidants (superoxide dismutases 1, 2, 3 and glutathione) and molecules of innate host defense (SP-A, SP-D, and lysozyme) to participate in detoxification and sterilization of the alveolar microenvironment.

More recently, it is becoming clear that alveolar type 2 cells may play a part in exacerbating alveolar disease. The type 2 cell participates in the coagulation–fibrinolysis cascade through the production of fibrinogen, urokinase-type plasminogen activator, and tissue factor, especially under pathologic circumstances. Type 2 cells are increasingly recognized as a source of cytokine and chemokine production in the lung, as well as growth factors that can promote fibrosis. Finally, cross talk between epithelial cells, cell matrix, interstitial cells, and local inflammatory cells can foster the resolution of injury and inflammation or prolong lung remodeling after injury, with detrimental effects such as lung destruction and fibrosis. Therefore, while previously heralded as the defender of the alveolus, type 2 alveolar epithelial cells play a much more complex role in alveolar health and disease.

Surfactant

Pulmonary surfactant is essential for maintenance of alveolar health. The alveolar epithelial cells secrete a thin layer of liquid to protect the gas-exchange surface. The surface tension generated by this aqueous layer opposes alveolar inflation and promotes alveolar collapse at the end of expiration owing to the law of Laplace, whereby the collapsing pressure on the alveolus is directly proportional to the surface tension and inversely proportional to the radius of the alveolus. The film of pulmonary surfactant at the air–liquid interface lowers surface tension as the alveolar surface area decreases with exhalation, thereby preventing end-expiratory atelectasis, maintaining functional residual capacity, and lowering the force required for subsequent alveolar inflations.

Pulmonary surfactant is a complex mixture of phospholipids, neutral lipids, and proteins that is synthesized, packaged, and secreted by type 2 alveolar epithelial cells.[17] The life cycle of surfactant is depicted in Fig. 38.2. Storage of surfactant occurs in



• **Fig. 38.2** (A) The life cycle of surfactant. (B) Electron micrograph of a type 2 alveolar epithelial cell showing the prominent lamellar bodies near the apical surface. *SP-B*, Surfactant protein B; *SP-C*, surfactant protein C. (From Whitsett J, Weaver T. Hydrophobic surfactant proteins in lung function and disease. *N Engl J Med*. 2002;347:2141–2148.)

the lamellar body, a lysosome-derived membrane-bound organelle that undergoes regulated secretion in response to a variety of stimuli, including stretch. In the alveolus, surfactant phospholipids transition through an extracellular storage form, tubular myelin. Phospholipid and protein components are recycled out of the surfactant monolayer at the air–liquid interface and taken back into the alveolar type 2 cell, where they can be repackaged into lamellar bodies. Alternatively, alveolar macrophages are able to engulf and degrade surfactant components.

The predominant surfactant phospholipid is saturated dipalmitoyl phosphatidylcholine (DPPC), with the remaining phospholipids consisting of monounsaturated phosphatidylcholine, phosphatidylglycerol, and other phospholipids (Table 38.1).[18] DPPC is the only surface-active component of lung surfactant capable of lowering surface pressure to nearly zero. The presence of unsaturated phospholipids and other lipid components such as cholesterol enables the monolayer to remain fluid at body temperature during the respiratory cycle. Phospholipid content in the fetal lung increases with advancing gestation because of increased activity of enzymes responsible for phospholipid synthesis within type 2 cells. The expression and activity of enzymes of the choline incorporation pathway, the predominant pathway for surfactant phospholipid synthesis, are not only developmentally regulated but are also induced by hormones. The inductive hormones that have direct clinical relevance are glucocorticoids and agents that increase intracellular cyclic adenosine monophosphate (cAMP) levels such as the β-adrenergic agonist (and tocolytic) terbutaline.

Surfactant contains a group of specific proteins with importance for surfactant function and host defense. The four surfactant proteins, SP-A, SP-B, surfactant protein C (SP-C), and SP-D, are subdivided on the basis of their physical characteristics into either hydrophobic (SP-B and SP-C) or hydrophilic (SP-A and SP-D) proteins. The hydrophobic surfactant proteins play a major role in the surface-active properties of surfactant, whereas the primary roles of the hydrophilic surfactant proteins are in host defense, immunomodulation, and surfactant clearance and metabolism.

Together the hydrophobic proteins, SP-B and SP-C, facilitate the mobilization of surfactant phospholipid from tubular myelin to the surface monolayer, promote spreading of phospholipids in the surfactant film, and assist in film stability at the end of expiration. SP-B plays a central role in alveolar health because of its critical function in surfactant homeostasis. SP-B is a secretory protein that exhibits strong association with membranes, unlike SP-C, which contains a membrane-spanning domain and covalently attached fatty acids (palmitate) that render it integral to phospholipid membranes.[19] Both SP-B and SP-C are synthesized as large precursor proproteins that undergo extensive posttranslational processing as they pass through the secretory pathway, ultimately reaching the lamellar body. SP-B is essential for the process of lamellar body formation, and the type 2 alveolar epithelial cells of infants with an inherited deficiency of SP-B are devoid of lamellar bodies. Because the lamellar body is where SP-C proteolytic processing is completed, infants with inherited deficiency of SP-B are also deficient in mature SP-C, instead accumulating a larger, nonfunctional precursor of SP-C. Thus, patients with inherited deficiency of SP-B, despite having relatively normal surfactant phospholipid profiles, make a pulmonary surfactant with very poor surface tension properties because of the combined defects in SP-B and SP-C. Conversely, because SP-C does not play either a direct or an indirect role in SP-B processing, animals with SP-C deficiency have normal SP-B, have normal lamellar bodies, and do not exhibit perinatal lethality due to surfactant dysfunction.

Like the enzymes of surfactant phospholipid production, SP-B and SP-C exhibit developmental and hormonal regulation of expression.[20] In human fetuses, SP-C messenger ribonucleic acid (mRNA) is detected as early as 12 weeks of gestation and SP-B mRNA by 14 weeks, yet the mature proteins are not detectable in fetal lung tissue until after 24 weeks. SP-B protein is not detectable in amniotic fluid until after 30 weeks of gestation, the amount increasing toward term.[21] This is due to developmental regulation of posttranslational events in the proteolytic processing of proSP-B and proSP-C.[22] Consequently, infants delivered prematurely have reduced levels of both surface-active components of surfactant—phospholipid and hydrophobic surfactant proteins. The rate of type 2 cell differentiation, and secondarily surfactant production, is modulated by the levels of endogenous corticosteroids and can be accelerated by prenatal administration of glucocorticoid to women in preterm labor. The response of the surfactant system to prenatally administered glucocorticoids involves all key lipid and protein components, and occurs primarily through increased gene expression, thus representing precocious maturation mimicking the normal developmental pattern. Endogenous thyroid hormones, prostaglandins, and catecholamines also have stimulatory effects on type 2 cell maturation as well as on clearance of lung fluid at birth. Certain proinflammatory cytokines (e.g., tumor necrosis factor [TNF]-α and transforming growth factor [TGF]-β) inhibit surfactant production in experimental systems and may downregulate surfactant in conditions such as sepsis and inflammation.

TABLE 38.1 Composition of Pulmonary Surfactant

Component	Percentage (by Weight)
Lipid	92
Saturated phosphatidylcholine	41
Unsaturated phosphatidylcholine	25
Phosphatidylglycerol	9
Other phospholipids	4
Cholesterol	8
Neutral lipids	5
Protein	10
Surfactant proteins	8

Development of the Pulmonary Vasculature

The primary role of the pulmonary vasculature is to supply blood flow to the acini for gas exchange.[23] During early fetal life the airways act as a template for pulmonary blood vessel development. The earliest pulmonary vessels form de novo in the tissue surrounding the lung bud in a process known as *vasculogenesis*. Mesodermal cells within the mesenchyme investing the developing lung tube differentiate into endothelial cells, proliferate, organize into chords, and develop a central lumen. As each new airway buds into the mesenchyme, a new plexus forms that adds to the pulmonary circulation, thereby extending the network of arteries and veins. By the fifth week of human gestation, a capillary network surrounds each bronchus, and circulation of blood between the right ventricle and the left atrium via this network is evident.

During the canalicular stage of lung development, new blood vessels form from preexisting vessels, a process known as *angiogenesis*. In contrast to vasculogenesis, angiogenesis is initiated by endothelial cell proliferation and sprouting from established vessels, resulting in extension of a vascular network into undervascularized regions. Vasculogenesis is the primary mode of pulmonary vascular development until the 17th week of gestation, when all preacinar airways and their accompanying vessels are present, whereas angiogenesis becomes the predominant mode of vascular development in the later stages of lung development. Although originally thought to be sequential processes, it is generally accepted that both occur concurrently during lung development, with angiogenesis dominating in the central part of the lung and vasculogenesis dominating in the periphery.[24] Interconnections between vascular networks arising from both angiogenesis and vasculogenesis increase in the saccular phase of lung development.

In the human lung a second circulatory system, the bronchial circulation, arises from the dorsal aorta and nourishes the cellular constituents of the lung itself. The bronchial vasculature develops after the pulmonary circulation, with bronchial vessels first apparent by 8 weeks of gestation. The network of bronchial vessels is extensive, with bronchial arteries demonstrated as distal as the alveolar ducts in the adult respiratory tree.

Vasculogenesis and angiogenesis are the primary mechanisms of vascular development throughout intrauterine life. The human lung at term contains only a small portion of the adult number of alveoli, and the air space walls are represented by a thick "primary septum" consisting of a central layer of connective tissue surrounded by two capillary beds, each of them facing one alveolar surface.[3] As alveolar architecture changes with the appearance of secondary septa, or secondary crests, folding of one of the two capillary layers occurs within the secondary septa. Microvascular maturation involves fusion of the juvenile double capillary network into a single capillary system present in the adult lung. Fusion is facilitated by the expansion of alveolar surface area and luminal volume, which compresses the interstitium, bringing the capillary networks in close proximity. This process is evident in the third postnatal week, during which lung volume increases by 25%, with a concomitant 27% decrease of the interstitial tissue volume. Subsequently, preferential growth of areas with a mature, fused capillary system continues.

Lastly, it is well known that lung volume increases about 23-fold between birth and young adulthood, while capillary volume expands 35-fold. It has been shown recently that this increase in capillary volume occurs by a third mechanism of vascular development: intussusceptive microvascular growth. This new concept in capillary network growth involves the formation of transluminal tissue pillars within capillaries that then expand, resulting in a net increase in capillary surface area.[3]

Muscularization of the pulmonary arteries begins early in development.[25] Initially the muscular investment of the vasculature is derived from the migration of bronchial smooth muscle cells from adjacent airways. Muscularization of the pulmonary arteries begins in the canalicular stage and continues through the remainder of gestation. Smooth muscle cells develop from the surrounding mesenchyme, altering their cellular shape and initiating expression of α-smooth muscle actin, a marker of their transformation into smooth muscle cells. A third phase of vascular muscularization, largely restricted to the distal part of the lung, involves the process of endothelial–mesenchymal transition, marked by endothelial cell division, separation and migration away from the endothelial layer, and expression of smooth muscle cell markers.

Muscularization of pulmonary arteries normally extends to the level of the terminal bronchiole and is minimal to absent in blood vessels surrounding respiratory bronchioles. Abnormal extension of smooth muscle along arterioles supplying acinar structures occurs in infants dying of persistent pulmonary hypertension of the newborn, as well as in infants with congenital diaphragmatic hernia and severe BPD.

Development of Pulmonary Host Defense

Every minute, the adult human lung takes in approximately 7 L of air contaminated with a variety of potential pathogens that can cause lung injury. The continuous exposure of the epithelial surface of the conducting airway to inhaled pathogens requires the presence of an efficient innate immune response system as a first line of defense against infection. The proximal and distal airway epithelial cells play a major role in clearing pathogens by secreting antimicrobial as well as anti-inflammatory molecules into the airways and alveoli. In the proximal airways, components of mucociliary clearance appear as early as 11 weeks of human gestation, including ciliated epithelial cells, goblet cells expressing mucins, and submucosal glands as described previously. The relative increase in the number of airway goblet cells over ciliated cells in prematurely born infants compared with term infants renders premature infants more prone to enhanced mucus production and obstruction caused by poor mucociliary clearance.

A number of microbial defense molecules are produced and secreted by epithelial cells into the airways.[26] They include lysozyme, C-reactive protein, lactoferrin, collectins, β-defensins, and the cathelicidin CAP-18/LL-37.[27] Two lung collectins originally identified as surfactant-related proteins, SP-A and SP-D, play a larger role in lung host defense than in surfactant biophysics. Although originally identified as products elaborated by epithelial cells lining airways (club cells) and alveoli (type 2 cells), they are secreted at other epithelial surfaces exposed to the external environment.[28–31] These collectins interact with microorganisms and inflammatory cells to facilitate microorganism clearance and modulate inflammatory and apoptotic responses.[32]

The basis for the interactions of the lung collectins with microbes and antigens centers on the binding of sugars by the carbohydrate recognition domains of these proteins.[33] Interactions between collectins and gram-negative bacteria depend on the ability of SP-A and SP-D to bind lipopolysaccharide, whereas interactions with gram-positive bacteria, including group B β-hemolytic streptococcus, depend on recognition of the gram-positive outer membrane component lipoteichoic acid.[34] The collectins bind a variety of fungi as well as *Pneumocystis carinii* and play an important role in inhibiting a variety of respiratory viruses, including influenza A virus and respiratory syncytial virus. Differences in the structure of the carbohydrate recognition domain provide SP-A and SP-D with altered affinities for different sugar molecules, allowing complementary functions and improving the diversity of microbial interactions.

The lung collectins also modulate the functions of a variety of immune cells, including macrophages, neutrophils, eosinophils, and lymphocytes.[35–37] The collectins enhance the local production of chemotactic factors to attract macrophages and neutrophils and cytokines that activate macrophages and eosinophils, as well as attenuate lymphocyte responses by inhibiting T-cell proliferation. Other direct effects on inflammatory cells include modulating the production of reactive oxygen and nitrogen species used in killing microorganisms.

Like the hydrophobic surfactant proteins, SP-A and SP-D exhibit both developmental and hormonal regulation of expression.[20] In human fetuses, SP-A mRNA is undetectable before 20 weeks of gestation, and SP-A is first detectable in amniotic fluid by 30 weeks of gestation, with the amount increasing toward term.[21] The SP-A gene promoter is induced by cAMP and glucocorticoids, although the response to glucocorticoids is biphasic, showing attenuation of SP-A expression at higher doses. Retinoids, insulin, and growth factors, including TGF-β and TNF-α, inhibit SP-A gene expression. Like SP-A, SP-D expression in the developing lung is quite low during the second trimester[38] but is detectable in amniotic fluid in the third trimester, increasing toward term. The levels of both SP-A and SP-D increase markedly in the first few days after preterm birth and can be modulated by the local microenvironment of pathogens, toxins, and reactive oxygen species.[39,40]

Development of Detoxification Systems

Although oxygen is an essential component of cellular processes, concentrations beyond the physiologic limits may be hazardous to cells. The lung is particularly susceptible to reactive forms of oxygen and free radicals since it is the organ with the highest exposure to atmospheric oxygen. The fetal lung is exposed to oxygen tensions of 20 to 25 mmHg in utero, and the transition to air breathing is associated with a fourfold to sevenfold increase in oxygen tension, presenting a significant oxidant stress. Oxygen free radicals arise from endogenous production through metabolic reactions or by exogenous exposure, such as air pollutants and cigarette smoke. Free radicals injure the lung through oxidation of proteins, DNA, and lipids. Therefore, an antioxidant detoxification system is especially critical during the transition of the fetal lung to air breathing.

Oxygen free radicals are highly toxic substances and exist in several different forms. Superoxide is produced by the reduction of molecular oxygen through the addition of an electron, hydrogen peroxide is produced from the transfer of a single electron to superoxide, and hydroxyl radicals are produced through the interaction of hydrogen peroxide with superoxide. The free electrons of free radicals promote peroxidation of membrane lipids, alter sulfhydryl and other groups on exposed amino acids in proteins, and cause direct damage to DNA. All of these changes have been described in the lungs of animals and humans exposed to high levels of oxygen, although the dose response differs by species and with age, leading to altered epithelial integrity, interstitial and air space edema, and infiltration of inflammatory cells. Reactive oxygen species have also been implicated in enhancing production of proinflammatory mediators by lung epithelial and resident inflammatory cells, through chromatin remodeling, triggering signal transduction pathways, and activating transcription factors.[41,42]

Antioxidants, both nonenzymatic and enzymatic, attenuate the effects of oxygen free radicals in the lungs. The major nonenzymatic antioxidants are glutathione, vitamin C (ascorbate), vitamin E (primarily α-tocopherol), β-carotene, and uric acid. Enzymatic antioxidants include superoxide dismutases (1, 2, and 3), catalase, thioredoxins, and a variety of peroxidases. Animal studies indicate that many of the antioxidant enzymes are induced before term delivery, and limited data suggest that the same is true for human fetuses.[43] However, premature animals fail to induce antioxidant enzymes in response to oxidative lung injury.[44] Thus, preterm infants are at significantly higher risk of oxidant lung injury owing to both increased need for oxygen in the treatment of respiratory distress syndrome and underdeveloped antioxidant defenses.

Mechanisms of Lung Development

Fetal and postnatal lung development depend on several key developmental processes: branching morphogenesis to promote branching of the lung bud into the surrounding mesenchyme, static and cyclic stretching of the lung that assist in promoting sacculation, alveolarization to enhance the expansion of the gas-exchange surface area, and vasculogenesis and angiogenesis to ensure that the developing epithelial surface area is invested with both a gas-exchange and a nourishing vascular supply.

Branching Morphogenesis

Branching morphogenesis is a fundamental mechanism of lung development. Branching is mediated by the accelerated growth of epithelial cells along the stalk of a branching airway with concomitant growth arrest at the branch tip.[45] This process requires extensive communication between epithelial cells and with the adjacent mesenchyme, as well as integration of microenvironmental cues from the extracellular matrix.[46] Classic tissue recombination experiments in which mesenchyme from proximal airways was transplanted to distal airways (and vice versa) indicate that the mesenchyme has an important inductive role in dictating the branching pattern and cell fate of the expanding epithelium.[47] More recently, murine modeling indicate that three modes of branching—domain branching, planar bifurcation, and orthogonal bifurcation—are the basic mechanisms that characterize development of the complex three-dimensional respiratory tree through the pseudoglandular phase.[48,49] These branching patterns are used repetitively during lung development and appear to be governed by a genetic clock orchestrating side branch formation, then planar bifurcation of a side branch, and planar rotation dictating the orientation of the bifurcation, thereby establishing a complex, three-dimensional structure.

Stretch and Mechanotransduction

The role of physical factors in modulating lung size is well established: normal lung growth requires adequate space in the chest cavity and appropriate tonic and cyclic distending forces. Genetic defects that compromise the thoracic skeleton and space-occupying lung masses such as congenital cystic adenomatoid malformations are associated with pulmonary hypoplasia due to the restriction of intrathoracic space. Denervation of the diaphragm to eliminate fetal breathing movements is associated with pulmonary hypoplasia, as is oligohydramnios.

Static Stretch: Fetal Lung Fluid Production

Fetal lung fluid is a product of the epithelial lining of the developing lung,[50,51] averaging 4 to 6 mL/kg/h. The resistance imparted by laryngeal abduction results in fluid accumulation to a total volume of 20 to 30 mL/kg during gestation, which generates an end-expiratory pressure of approximately 2 to 4 cmH_2O. The composition of fetal lung fluid is distinct from that of both amniotic fluid and plasma, as illustrated in Table 38.2. The increased chloride content of fetal lung fluid as compared with serum is the result of active chloride secretion by the tracheal and distal pulmonary epithelium, largely because of the chloride channel CLC-2/CLCN2. A variety of growth factors, hormones, and lipid mediators influence

TABLE 38.2 Composition of Human Fetal Lung Fluid Compared With Other Body Fluids

Component	Lung Fluid	Interstitial Fluid	Plasma	Amniotic Fluid
Sodium (mEq/L)	150	147	150	113
Potassium (mEq/L)	6.3	4.8	4.8	7.6
Chloride (mEq/L)	157	107	107	87
Bicarbonate (mEq/L)	3	25	24	19
pH	6.27	7.31	7.34	7.02
Protein (g/dL)	0.03	3.27	4.09	0.10

the production of fetal lung fluid during gestation, including enhancers (prolactin, keratinocyte growth factor, prostaglandin E_2, and prostaglandin F_2) and inhibitors (β-adrenergic agonists, vasopressin, serotonin, and glucagon).

While fetal lung fluid is an essential component of lung development, it presents a significant obstacle to the transition to air breathing on delivery. Three important events must occur to minimize the amount of fetal lung fluid and its potential impact on alveolar surface tension before the transition to air breathing: absorption, bulk removal, and maturation of pulmonary surfactant. Conversion of the pulmonary epithelium from a secretory to an absorptive surface is a critical event during the third trimester. Enhanced sodium transport across the alveolar epithelium is in part responsible for this change. Much evidence suggests that increasing expression of components of the epithelial sodium channel (ENaC) in the third trimester is a major factor in promoting sodium reabsorption from alveoli, with water passively following the movement of sodium.[51,52] Transgenic mouse pups that do not express the α subunit of ENaC die quickly after birth because of failure of fetal lung fluid clearance. Induction of ENaC subunits occurs at a transcriptional level in response to changes in extracellular matrix components, glucocorticoids, aldosterone, and oxygen. Drugs that increase intracellular cAMP levels (i.e., β-adrenergic agonists, phosphodiesterase inhibitors, and cAMP analogues), while not increasing the number of ENaC channels, increase the probability of a channel being open to sodium transport. In addition, glucocorticoid and thyroid hormones play an important role in priming the lung epithelium to be responsive to the actions of β-adrenergic agonists on sodium transport across lung epithelia near term. Thus, the prenatal use of glucocorticoids and tocolytics may improve lung function in prematurely born infants through enhanced lung fluid reabsorption as well as by increasing surfactant production. Epithelial water channels, consisting of aquaporin proteins, are also induced during the late fetal period.[53] While water channels are clearly essential for fluid movement across epithelial cell membranes, their importance for fetal lung fluid clearance is less certain in the face of perinatal survival of transgenic mouse pups that do not express aquaporin 5 and/or aquaporin 1.

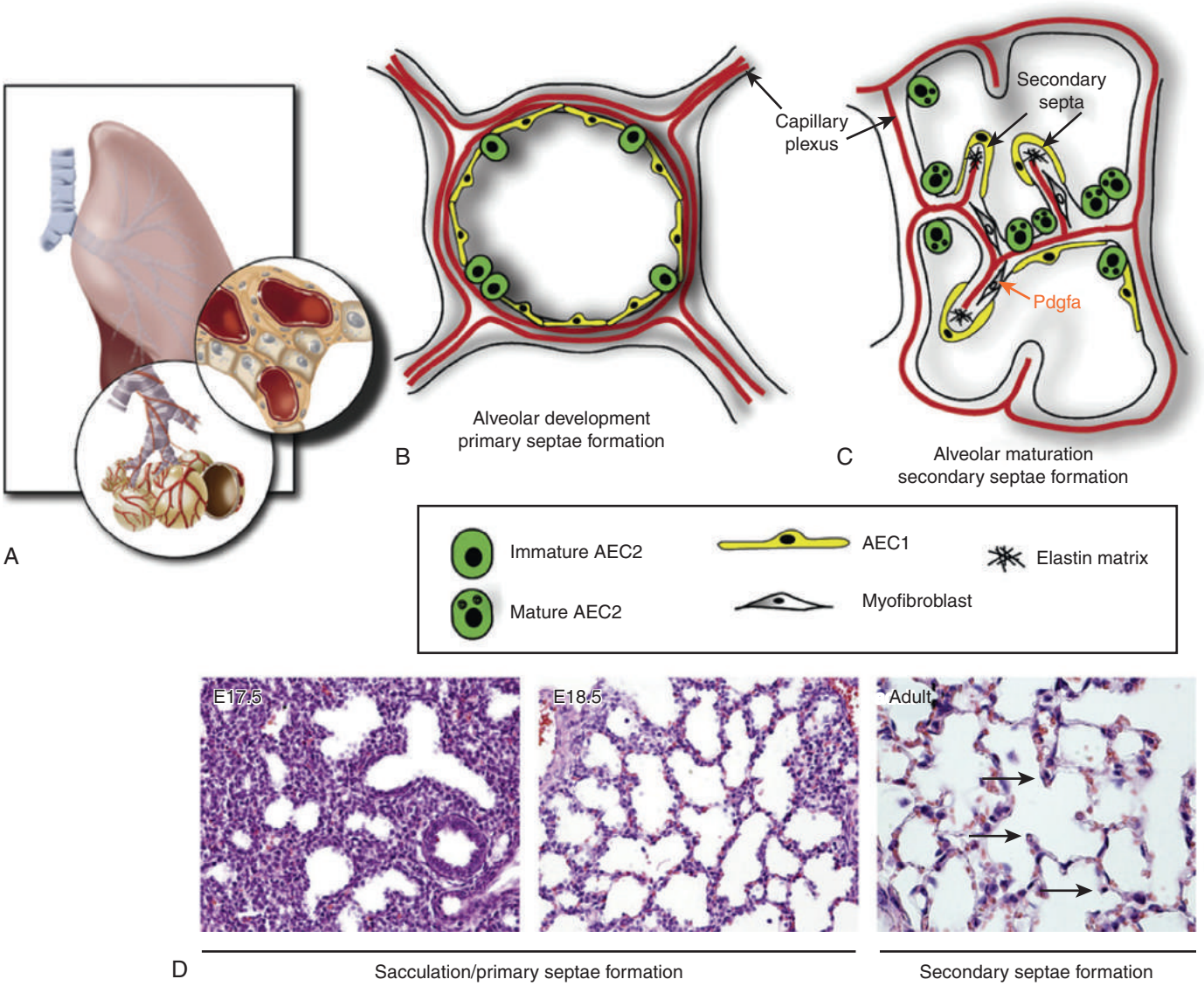
Conversion to an absorptive surface is not enough to minimize the amount of fetal lung fluid at the time of term delivery. The absence of uterine contractions is associated with an increased incidence of retained fetal lung fluid in infants born by cesarean delivery without the benefit of labor. On delivery of the head and neck, continued uterine contractions on the fetal thorax promote expulsion of bulk fluid from the fetal lung. However, animal studies suggest that the magnitude of the benefit of thoracic compression during labor is modest.[54] Instead, the primary mechanism by which labor facilitates clearance of lung fluid is through hormonal effects on fluid clearance, especially through catecholamine-induced changes in the open probability of ENaC channels. The onset of air breathing, associated with increased intrathoracic negative pressure, assists in the clearance of residual fetal lung fluid into the loose interstitial tissues surrounding alveoli. Fluid is then reabsorbed via lymphatics and pulmonary blood vessels. It is generally accepted that the amount of residual liquid in the lung after transition is complete is approximately 0.37 mL/kg body weight.

Cyclic Stretch: Fetal Breathing Movements

Fetal breathing movements, detectable as early as 10 weeks of gestation in the human fetus, is an essential stimulus for lung growth.[55] Sustained periods of fetal breathing increase in duration with advancing gestation. Fetal breathing occurs for 10% to 20% of the time at 24 to 28 weeks, increasing to 30% to 40% after 30 weeks of gestation. Originating from the diaphragm, fetal breathing is erratic in frequency and amplitude. The volume of fluid moved is small and insufficient to be cleared from the trachea. Respiratory rates range from 30 to 70 breaths per minute, while periods of apnea of up to 2 hours have been recorded. The frequency of fetal breathing varies with sleep state (inhibited during quiet sleep) and exhibits diurnal variation, with the lowest rates recorded early in the morning. Fetal breathing is hormonally responsive, and the inhibition of fetal breathing with the onset of labor is attributed to the action of increased levels of circulating prostaglandins. Maternal medications can influence the frequency of fetal breathing movements. Central nervous system stimulants are associated with increased fetal breathing (i.e., caffeine, amphetamines), whereas depressants are associated with decreased fetal breathing (i.e., anesthetics, narcotics, nicotine, ethanol). Animal studies have clearly shown that permanent cessation of fetal breathing, regardless of the cause, is associated with impaired fetal lung growth. However, the impact on fetal lung development of short-term alterations in fetal breathing frequency and amplitude is unknown. Together, constant distending pressure from the production and retention of fetal lung fluid and episodic cyclic fetal breathing are important mechanisms for lung growth during fetal life.

Alveolarization

While branching morphogenesis is the primary developmental program that establishes the conducting airways of the lung, it is important to remember that alveolarization is the developmental program that will establish the large surface area involved in gas exchange.[56] This process will result in a 20-fold increase in surface area between birth (with between 0 and 50 million alveoli) and adulthood (>300 million alveoli). The established dogma suggests that primitive saccules develop low ridges (primary septa) that subdivide the saccule into an alveolar duct containing primary alveoli and outpouchings between the ridges (secondary septa/crests) that establish secondary alveoli (Fig. 38.3), however these secondary septa have also been described as having a three-dimensional ring structure.[57] Septation is critical for the process of



• **Fig. 38.3** Alveolarization. (A) Alveolar development begins in late gestation as the endothelial plexus becomes tightly associated with the epithelium of the distal saccules. (B) Early alveoli contain several important cell types, including the flattened type 1 alveolar epithelial cells *(AEC1)* used in gas exchange and the cuboidal type 2 alveolar epithelial cells *(AEC2)* that make surfactant, which lie close to a double capillary network. (C) Maturation of the alveolar compartment is marked by the generation of secondary septa, which involves the development of alveolar crests, a process dependent on the development of myofibroblasts and the deposition of elastin. (D) Histologic sections stained with hematoxylin and eosin demonstrating the changes in distal lung morphology of the developing mouse lung, with secondary septa denoted by *arrows*. Pdgfa, platelet derived growth factor subunit A, found in myofibroblasts in mature alveolae. (From Morrisey EE, Hogan BL. Preparing for the first breath: genetic and cellular mechanisms in lung development. *Dev Cell*. 2010;18:8–23.)

microvascular maturation and also leads to the development of the pores of Kohn, allowing gaseous continuity between acini and a route for macrophages to move freely between alveoli.

The topic of alveolarization is rapidly evolving, receiving increasing attention because of observations that infants who die after severe BPD exhibit alveolar simplification with little evidence of secondary septation. It remains unclear to what extent the alveolarization potential may be permanently altered by preterm birth alone—in the absence of other injuries—and whether the developmental program can be resumed after preterm birth.

Interdependence of Alveolar and Vascular Development

Recent evidence suggests that the pulmonary capillary bed actively promotes normal alveolar development, maintains alveolar structures throughout life, and can contribute to degenerative lung disease in adulthood.[23] The observation that combined abnormalities in the airways and vasculature occur in BPD supports this hypothesis. Intraacinar arteries and veins continue to develop after birth by angiogenesis as long as alveoli continue to increase in number and size. This may well be a reciprocal process because vascular

growth around the distal air spaces suggests an inductive influence from the alveolar epithelial cells as well.

Molecular Basis for Lung Development

The developmental processes that contribute to lung organogenesis are under the regulation of interdependent signaling pathways mediated by secreted growth factors that are themselves under the control of large networks of transcription factors controlling gene expression. Gene regulatory networks common to other organs that depend on branching morphogenesis for organogenesis, most notably in the kidney and mammary gland, are also found in the lung. Selected regulatory networks are highlighted in the following sections.

Growth Factors in Lung Development

The initiation of branching morphogenesis is the result of the interplay of signals between the developing lung epithelial tube and its surrounding mesenchyme. Central to this process is the family of fibroblast growth factors (FGFs) that are produced and secreted by mesenchymal cells and bind to receptors on the plasma membrane of epithelial cells, setting up a system of mesenchymal–epithelial cross talk. In particular, FGF10, secreted by mesenchymal cells, binds to its receptor, FGF receptor 2 isoform IIIb (FGFR2IIIb), on nearby epithelial cells. This signal is strongest in the epithelial cells at the tip of branching airways owing to a focused FGF10 gradient within the mesenchyme. Binding of the FGF10 ligand to the FGFR2IIIb receptor results in activation of the mitogen-activated protein kinase (MAPK) pathway within epithelial cells, setting off a cascade of downstream signaling events that regulates cell adhesion, cytoskeleton, and cell polarity, all essential elements of cell migration.[58] One of the genes induced by FGF10/FGFR2IIIb signaling, *SPRY2*, inhibits the MAPK pathway, resulting in feedback inhibition of further FGF10/FGFR2IIIb signaling. Thus, a signal propagated by the mesenchyme has an effect that is tightly regulated within the epithelial cell.

Proper lung development requires exquisitely precise FGF10 signaling. Animals expressing reduced levels of FGF10 develop pulmonary hypoplasia with reduced numbers of large airways.[59,60] Furthermore, increased FGF10 signaling during fetal lung development in mice by intrapulmonary injections of recombinant FGF10 produced cystic structures with epithelial characteristics dependent on the location of the injection: proximally with club cells, distally with type 2 alveolar epithelial cells.[61] Together these data provide strong evidence that FGF10 signaling has diverse responses—from initiation of branching to differentiation of epithelial cells—depending on the temporal and/or spatial context of signaling.

Like branching morphogenesis, lung vascular development is a complex and highly organized process that requires multiple vascular signaling molecules to interact in a specific temporospatial sequence. Vascular endothelial growth factor (VEGF) is a critical growth factor in angiogenesis and vasculogenesis. The expression of the VEGF ligand by epithelial cells and VEGF receptors (VEGFRs) by endothelial cells of the developing human fetal lung reinforces the interdependence of the air space and vascular development. The expression of VEGF mRNA and protein is localized to the epithelial cells at the distal tips of developing lung branches, and the expression levels increase with time.[25,56] VEGF gene expression is induced in epithelial cells by the hypoxic environment of the growing fetal lung through the actions of the oxygen-sensing hypoxia-inducible factor family of transcription factors. From the single VEGF gene, five different VEGF protein isoforms are possible, although VEGFA (VEGF165) is the most studied. Each isoform has different affinities for each of the three VEGFRs (Flt-1/VEGFR1, Flk-1/kinase insert domain receptor/VEGFR2, and Flt-4/VEGFR3). Vascular endothelial cells express primarily VEGFR1 and VEGFR2, whereas VEGFR3 is expressed on the plasma membrane of the lymphatic endothelium. VEGFRs are expressed on the plasma membrane of endothelial cells surrounding the developing airways from very early in gestation, and expression of VEGFR2 is considered the earliest marker of an endothelial progenitor cell. In vitro and in vivo experiments have shown that VEGFA induces endothelial cell proliferation and migration, both key elements of vascular sprouting, as well as tube formation through interactions with VEGFR2. VEGFR1 appears to have more importance in transforming primitive endothelial tubes into stabler vascular networks, in part by reducing endothelial proliferation through downregulation of VEGF production. The embryonic lethality of animals with reduced VEGF expression attests to the critical importance of VEGF/VEGFR signaling to vascular development in the fetus, though not limited to the developing pulmonary vasculature.

Transcription Factors in Lung Development

The ligand–receptor interactions, important for branching morphogenesis and pulmonary vascular development, are in part determined by the actions of transcription factors on facilitating or reducing gene expression. Transcription factors are also critical in the differentiation of the lung epithelium in the airway epithelium as well as differentiation of type 1 and type 2 alveolar epithelial cells in the distal lung.

The most important transcription factor in the lung is thyroid transcription factor 1 (TTF-1), a product of the *NKX2-1* gene. TTF-1 is considered a master regulator of lung development, as transgenic mice null for *NKX2-1* exhibit complete absence of lung branching.[62] *NKX2-1* plays a prominent role in establishing cell fate proximally to distally along the branching lung epithelium, with expression ultimately becoming more restricted to club cells and alveolar type 2 cells. TTF-1 is critical for the expression of genes that are unique to differentiated epithelium, such as *SCGB1A1* expression in club cells and surfactant proteins in type 2 alveolar epithelial cells. DNA binding sites are found in the promoter regions of all four surfactant genes, *SCGB1A1*, and *NKX2-1* itself, setting up a positive feedback loop for sustained TTF-1 expression. TTF-1 function is highly dependent on phosphorylation of critical amino acids, although it remains unclear which kinase is involved in this process.[63] VEGFA expression is reduced in animals unable to phosphorylate TTF-1, providing another important link between epithelial and vascular development. TTF-1 is itself regulated by other transcription factors that bind to the promoter region of the *NKX2-1* gene, specifically hepatocyte nuclear factor 3β and GATA-binding protein 6. Thus the ability of networks of transcription factors to bind to gene regulatory elements of DNA in a coordinated fashion during fetal lung development enables the temporospatial expression of growth factor networks that foster branching morphogenesis, the process of differentiation that ultimately gives rise to the approximately 40 cell types that constitute the human lung and the coordination of both epithelial and vascular development.

Disorders of Lung Development

The normal developmental lung program usually proceeds in a stereotypical manner, but defects in these morphogenic processes during specific developmental windows result in recognizable human lung malformations. The most common congenital lung malformation is a collection of intrapulmonary airway branching lesions known as congenital pulmonary airway malformations (CPAMs), with an incidence of approximately 1 in 2500 to 8000 live births[64]. These lesions communicate with the tracheobronchial tree, retain normal blood supply and are divided into 5 subtypes, termed Stocker's classification, based on presumed site of origin, size, and nature of the epithelial lining (Table 38.3).[65,66] There is great variability in the clinical presentation for fetuses and newborns with CPAMs from asymptomatic to development of non-immune hydrops, with type 1 associated with development of bronchoalveolar carcinoma in adolescence or adulthood, and type 2 with 50% to 60% incidence of associated extrapulmonary major congenital anomalies.[65] For those CPAMs detected prenatally, the CPAM-volume ratio (CVR) on prenatal ultrasound has been used as a tool to predict clinical outcomes, although no clear parameters have been established for neonatal outcomes other than development of hydrops.[67,68] The specific molecular defect that results in CPAMs is unknown but proposed mechanisms include ectopic application of proximal branching morphogenesis programs in a distal location or focal obstruction during development that irreparably disrupts the branching.[69]

Less common than CPAMs is congenital lobar emphysema. These lesions represent 10% of congenital lung malformations, are connected to the tracheobronchial tree and consist of hyperinflation of one or more of the pulmonary lobes. They most commonly affect the left upper or right middle lobes and have the potential for air trapping and compression of neighboring lung tissue.[70]

Unlike CPAMs or congenital lobar emphysema, bronchopulmonary sequestrations do not communicate with the tracheobronchial tree and are supplied by systemic arterial circulation, typically the thoracic aorta or one of its divisions. These islands of non-functioning lung tissue are more commonly extralobar (85%) with systemic venous drainage, with a less common intralobar variant (15%) that drains through the pulmonary system to the left atrium.[70,71]

The most notable congenital lung malformation of the distal lung airspace is alveolar capillary dysplasia/ misalignment of the pulmonary veins (ACD/MPV). This rare, almost universally lethal lung malformation is diagnosed by the presence of distinct pathologic findings including (1) immature lobar development, (2) pulmonary capillaries located remote from the alveolus, and (3) pulmonary veins "misaligned" and contained within the same adventitial sheath as the small pulmonary arteries.[72,73] Infants with ACD/MPV become progressively and relentlessly hypoxic beginning at 24 to 48 hours of age. The majority of these infants (50% to 80%) will have major extrapulmonary congenital anomalies, including congenital diaphragmatic hernia, congenital heart disease, or intestinal atresias. This lung malformation is associated with mutations in FOXF1, and known chromosomal deletion of the region containing this gene in an infant with unexplained hypoxia and other anomalies should prompt consideration of ACD/MPV as a diagnosis.[74,75]

TABLE 38.3 Stocker's Classification of Congenital Pulmonary Airway Malformations

Type	Presumed Site of Development	Notable Features
0	Tracheobronchial	Dense non-cystic lesion
1	Bronchial/bronchiolar	Usually a large cyst(s) involving a single lobe, commonly symptomatic in newborn period, associated with bronchoalveolar carcinoma in older pediatric or adult patients
2	Bronchiolar	Smaller cysts 0.5–2 cm lined by simple cuboidal or columnar epithelial cells; associated with extrapulmonary anomalies
3	Bronchiolar/alveolar	Non-cystic mass of glandular tissue
4	Distal acinar	Cysts of variable size (often large) lined by type 1 and 2 alveolar epithelial cells; pneumothorax in older children

Novel Concepts in Lung Development

Stem/Progenitor Cells in the Lung

The ability of lung epithelium to replace cells damaged by normal aging or injury has become the focus of increasing attention.[76,77] Stem cells are undifferentiated and have an unlimited capacity for self-renewal. Asymmetric divisions allow self-renewal through one daughter cell while enabling the other daughter cell to become more terminally differentiated. Progenitor cells are more committed, and although capable of self-renewal, they exhibit more restricted cell fates. Thus far, experiments combining lineage tracing with animal models of lung injury have identified several epithelial progenitor cell types having the capacity for both self-renewal and replacement of a variety of specialized lung epithelial cells. Basal cells in large airways and submucosal glands, identified by the expression of tumor protein p63/p63 and cytokeratin 5, are able to self-renew and give rise to ciliated and secretory cells. Club cells in smaller airways, identified by the expression of club cell secretory protein, seem to be a more committed progenitor cell since they are able to self-renew but differentiate only into club cells or ciliated cells. A subset of club cells, termed *bronchoalveolar stem cells* (BASCs), located at the bronchoalveolar duct junction and resistant to certain forms of lung injury, appear to have a role in epithelial repair. BASCs are both club cell secretory protein and SP-C positive and thus have the potential to produce either club cells or alveolar type 2 cells. Also in the smaller airways, neuroendocrine cells can self-renew and give rise to club cells and ciliated cells following exposure to injury. The most limited lung epithelial progenitor cell is the alveolar type 2 cell, which divides rapidly to reestablish epithelial continuity in damaged alveoli and then transdifferentiates into alveolar type 1 cells. Less well studied are the mesenchymal progenitor cells that provide vascular and muscular components of the developing lung. Candidate cells have been identified that give rise to endothelial cells in the process of vasculogenesis and airway smooth muscle cells along the branching lung epithelial tubes.

Evidence for the existence of stem/progenitor cells in the lung is strong but limited largely to mouse models of lung development and lung repair. Therefore extrapolation to humans should be done with caution, as the murine transition between terminal bronchioles and alveoli is not equivalent to the transition in the human

lung. While the capacity for self-renewal is tantalizing, it necessarily means that such cells are at risk of autonomous growth such as cancer. Controversy exists around the potential to harness these populations of cells as a means for correcting abnormalities of lung development (pulmonary hypoplasia), genetic diseases of the lung (cystic fibrosis), and abnormal repair of injured lungs (BPD).

Epigenetic Regulation of Lung Development and Maturation

Regulation of normal development and the consequences of abnormal development are at the heart of understanding the implications of preterm birth and the development of potential lung protective therapies. The central dogma of DNA to RNA to protein is being challenged by increasing recognition of epigenetic mechanisms—histone modifications, modification of DNA and RNA, silencing RNA, and microRNA (miRNA) —for regulating gene expression. The evidence that miRNAs play an important role in normal fetal lung development is clear.[78] MiRNAs are small, noncoding RNAs (generally 19 to 22 nucleotides in length) found within cells that target genes for RNA degradation or inhibition of protein synthesis. There are more than 500 recognized miRNAs, some of which are particularly enriched in lung cell populations. MiRNAs are generated from a process that begins in the nucleus. Long primary miRNA is transcribed, processed, and exported from the nucleus, where further cytoplasmic maturation occurs before the mature miRNA is able to interact with regions of messenger RNA, usually in the 3′ untranslated region.

Emerging evidence indicates that miRNAs are essential for normal lung development, since targeted deletion of dicer, a key enzyme in miRNA processing, results in abnormal airway development and excessive apoptosis in the lungs.[79] The miR-17-92 cluster of miRNA is highly expressed in embryonic mouse lung, decreasing into adulthood as lung development progresses. Altered expression of miR-17-92 suggests a primary role for these miRNAs in maintaining E-cadherin expression in airway epithelial cells during branching morphogenesis. A novel class of noncoding RNAs, the long noncoding RNAs (or lncRNAs), similarly regulate lung development. These large nucleotides (>200 nucleotides in length) are less stable than mRNA, have fewer posttranslational modifications, and play a role in stabilizing chromatin, thereby regulating gene transcription. The expression of several lncRNAs is spatially and temporally correlated with transcription factors critical to lung development.[80] The best characterized is *NANCI* (Nkx2.1-associated noncoding intergenic RNA), which regulates neighboring Nkx2.1 expression. Knockdown of *NANCI* in vivo results in lung sacculation and epithelial differentiation defects.

Because of their role in regulating developmental and pathologic processes, noncoding RNAs are increasingly seen as targets for therapeutic interventions. The obstacles to using miRNA or lncRNA as therapeutic agents are similar to the obstacles encountered in other gene therapies, including mode of delivery, cell and tissue specificity, and the potential for off-target effects.

Summary

Lung branching morphogenesis is coordinated with pulmonary vascular development to provide a large surface area and thin alveolocapillary membrane to accomplish adequate gas exchange in the transition to air breathing and to meet the needs of a growing infant and child. Although occurring late in fetal lung development, maturation of the surfactant system is similarly critical in the transition to air breathing and the maintenance of patency of the gas-exchange surface. Although the fetal lung developmental program requires an array of transcription factors, hormones, and growth factors promoting branching morphogenesis, lung growth is equally dependent on intact neural input to modulate fetal breathing, stability of an appropriately sized thorax, and the presence of adequate lung and amniotic fluid. Furthermore, the maturation of the host defense and detoxification systems minimizes the effects of increased oxygen tension and exposure to potential pathogens accompanying the transition to air breathing. Premature birth impacts all of these functions, as illustrated in Table 38.4. BPD is the net result of multiple injuries to the underdeveloped lungs of premature newborns that compromise postnatal growth and development, thus impairing function. Integrated approaches to therapy that reflect the interdependency of these lung functions have the most promise for minimizing the impact of premature birth on childhood and, ultimately, adult lung function.

TABLE 38.4 Potential Impact of Premature Birth on Lung Development and Maturation

Event	Effect of Preterm Birth	Potential Consequences
Development of conducting airways	Branching: no effect; completed to the level of the respiratory bronchi by 24 weeks	None
	Tone: increased secondary to lung disease in part reflecting developmental deficiency of nitric oxide	Increased airway resistance
Alveolarization	Decreased in severe bronchopulmonary dysplasia; may also be compromised by excess glucocorticoids	Reduced lung growth and lung surface area with increased alveolar size; impaired pulmonary function
Development of alveolocapillary membrane	Minimal effect: reaches adult diameter by 24 weeks of gestation; glucocorticoids induce precocious thinning	Gas exchange largely dependent on surface area, not alveolocapillary diameter
Type 1 cell differentiation	Variable depending on timing of delivery	Gas exchange largely dependent on surface area

Continued

TABLE 38.4 Potential Impact of Premature Birth on Lung Development and Maturation —cont'd

Event	Effect of Preterm Birth	Potential Consequences
Type 2 cell differentiation	Variable immaturity and deficient surfactant production depending on timing of delivery; improved with prenatal administration of glucocorticoids	Developmental deficiency of surfactant content and composition results in respiratory distress syndrome.
Hydrophobic surfactant proteins (SP-B, SP-C)	Variable effect depending on timing of delivery and other factors such as infection that can impair gene transcription	High alveolar surface tension; respiratory distress syndrome
Hydrophilic surfactant proteins (SP-A, SP-D)	Variable effect depending on timing of delivery; both proteins appear relatively late in the third trimester	Compromised host defense: impaired ability to clear microorganisms from airways and/or alveolar space; impaired ability to modulate inflammatory responses
Club cell differentiation	Variable effect depending on timing of delivery; these cells appear in the middle of the second trimester, but antioxidant products appear late in the third trimester	Impaired antioxidant and antimicrobial defenses; may contribute to chronic lung disease and pneumonia
Mucociliary clearance	Variable effect depending on timing of delivery; goblet cells decrease in number toward term	Increased mucus production may obstruct small airways
Development of the pulmonary capillary bed	Variable effects depending on timing of delivery in parallel to alveolar development	Variable degrees of impaired gas exchange commensurate with impaired alveologenesis and any superimposed lung injury; pulmonary hypertension
Pulmonary arteries	Variable effects depending on presence and severity of associated lung disease	Pulmonary hypertension associated with chronic lung disease
Fetal lung liquid	Fluid loss: variable effects depending on magnitude and duration of fluid loss (i.e., prolonged premature rupture of membranes) as well as timing of delivery	Pulmonary hypoplasia
	Fluid retention: variable effects depending on timing of delivery because hormone surges near term and in labor promote reabsorption before delivery	Transient tachypnea of the newborn
Fetal breathing movements	Variable effects depending on timing of delivery but also depending on maternal exposure to substances that reduce fetal breathing movements	Unlikely to have effects in preterm infants unless coexisting conditions severely limit fetal breathing
Respiratory drive	Variable depending on timing of delivery	Apnea of prematurity

SP-A, Surfactant protein A; *SP-B*, surfactant protein B; *SP-C*, surfactant protein C; *SP-D*, surfactant protein D.

Suggested Readings

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39

Neonatal Pulmonary Physiology

WILLIAM E. TRUOG AND WINSTON M. MANIMTIM

KEY POINTS

- Understanding the movement and maintenance of a volume of gas in and out of the lungs forms the basis of pulmonary physiology.
- Maintenance of functional residual capacity (FRC) is vital to adequate lung mechanics and gas exchange.
- There are multiple methods and techniques for measuring respiratory mechanics in healthy newborns and sick infants. However, to be clinically relevant, the results of these measurements must be interpreted within the context and limitations of these techniques.
- Understanding ventilation perfusion matching is key to understanding overall pulmonary gas exchange.
- Bronchopulmonary dysplasia (BPD) is characterized by complex and heterogenous pathophysiology that affects gas exchange involving large and small lung units and pulmonary vasculature.

Lung Volumes and Lung Mechanics

Knowledge of the functional components of lung volume and the mechanical properties of the lungs plays an important role in the understanding of neonatal respiratory physiology. The lungs have physical and mechanical properties including elastic recoil, airway resistance and inertance that resist inflation. The dynamic interactions between these properties are responsible for the effort required during normal spontaneous tidal breathing. A driving pressure is required to move a volume of gas into and out of the respiratory tract during a respiratory cycle that results in measurable airflow and volume changes. Functional residual capacity (FRC) is the volume of air in the lungs at the end of expiration of a normal, resting breath when alveolar pressure (P_A) equals atmospheric pressure (P_{atm}; Fig. 39.1A). Establishment of the FRC is vital to maintain adequate lung mechanics and gas exchange. FRC is maintained by the opposing forces of lung elastic recoil and chest wall outward recoil. Changes in the elastic properties of either the lung or the chest wall alter FRC.

Respiratory System Compliance

The lung contains elastic tissues that pull the lung to its deflated state. This property is called elastic recoil. Hooke's law requires that the pressure needed to inflate the lungs must be in proportion to the volume of inflation. The change in volume divided by the change in pressure is the lung compliance (Fig. 39.1B).

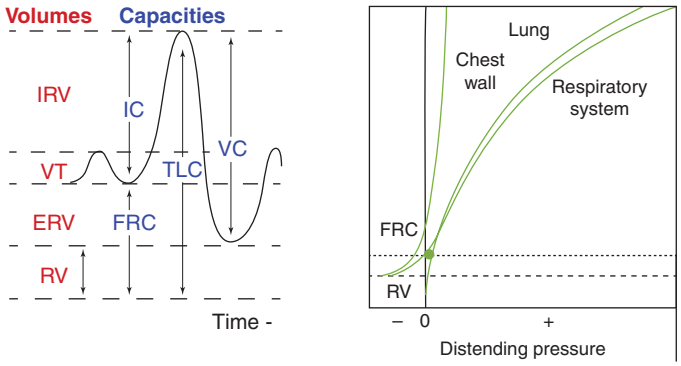
Conventionally, the volume of inflation is plotted on the *y*-axis, and the distending pressure is plotted on the *x*-axis. In this way the constant of proportionality is volume divided by pressure, or lung compliance. Throughout the range of tidal breathing the relationship between pressure and volume is linear. At higher lung volumes, as the lung reaches its elastic limit (i.e., total lung capacity), this relationship plateaus, making the pressure–volume relationship nonlinear. Static compliance is the change in volume for a given pressure during static, non flow conditions. On a static compliance curve, ventilation occurs at the steep portion where large changes in volume occur for small changes in pressure.[1] Dynamic compliance is the change in volume and pressure during spontaneous or mechanical breathing while there is airflow. It is predominantly influenced by the rate of respiration.

The total compliance of the respiratory system (C_{RS}) is comprised of the compliance of the lung (C_L) and chest wall (C_{CW}), where $1/C_{RS} = 1/C_L + 1/C_{CW}$. The tendency for the lung to collapse inward at the end of exhalation is balanced by the negative (subatmospheric) intrapleural pressure resulting from the outward recoil of the chest wall. FRC is maintained when these opposing forces are at equilibrium.[2] Inflation of the respiratory system above FRC requires a positive distending pressure that must overcome the elastic recoil of both the lung (alveolar pressure minus intrapleural pressure) and the chest wall (intrapleural pressure minus atmospheric pressure).[3] Deflation below FRC requires an active expiratory maneuver. Residual volume (RV) is defined as the volume of air that cannot be expired even with a forced deflation.

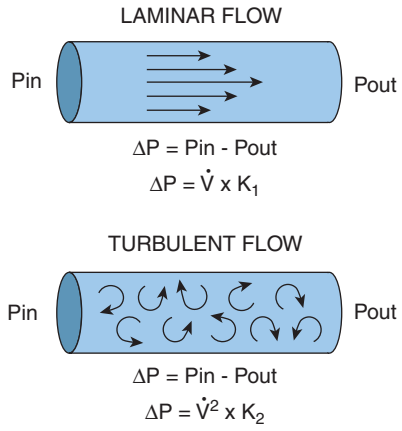
As depicted in Fig. 39.1B the infant's chest wall is composed primarily of cartilage. Therefore, C_{CW} is greater in infants, and the pleural pressure is less negative or only slightly sub atmospheric. As a result of the more compliant chest wall with very little outward distending pressure, the neonatal lung is more prone to collapse at the end of exhalation.[4] In newborn infants, FRC is maintained during spontaneous breathing by increasing expiratory resistance through laryngeal adduction (glottic narrowing), by maintaining inspiratory muscle activity throughout expiration, making the chest wall stiffer, and by initiating high breathing frequencies to limit the expiratory time, and causing the lungs to retain gas (gas trapping).[5,6]

Airway Resistance

A gradient of pressure (ΔP) is required for gas flow (V) to occur to overcome the nonelastic resistance of the lungs. Mathematically, resistance (R) = $(\Delta P)/V$. Physical resistance to gas flow arises due to friction between gas molecules against the walls of airways (i.e., airway resistance) and due to friction between the tissues of the lung and the chest wall (i.e., viscous tissue resistance). Resistance depends on the length and diameter of the airway as well as the density and viscosity of the gas. Airway resistance represents



• **Fig. 39.1** (A) Components of total lung capacity *(TLC)* include residual volume *(RV)*, expiratory reserve volume *(ERV)*, tidal volume *(VT)*, and inspiratory reserve volume *(IRV)*. Functional residual capacity *(FRC)* = RV + ERV; inspiratory capacity *(IC)* = VT + IRV. FRC is the volume of gas that remains in the lungs at the end of expiration when alveolar and airway pressures are at equilibrium. (B) An idealized plot of volume as a function of distending pressure for the lung, chest wall, and respiratory system (lung plus chest wall) of normal infant. The compliance curves are derived by instillation or removal of a measured volume of gas from the lung and allowing the respiratory system to come to rest against a shuttered airway. At this point only elastic forces are acting on the respiratory system, and airway pressure is equal to alveolar pressure. Intrapleural pressure can be measured with an esophageal balloon. Because airway pressure is equal to alveolar pressure, the distending pressure for the lung can be measured as *airway pressure minus intrapleural pressure*. The distending pressure for the chest wall is *intrapleural pressure minus atmospheric pressure*, and the distending pressure for the respiratory system is *airway pressure minus atmospheric pressure*. Compliance is the change in volume divided by the change in distending pressure. At FRC, the lung and chest wall distending pressures are equal but opposite and therefore the total respiratory system distending pressure is zero. The *shaded area* is the resting intrapleural pressure at FR.



• **Fig. 39.2** Gas flow ($\dot{V}$) through tubular structures occurs only in the presence of a pressure (P) gradient (Pin > Pout). For laminar flow, P is directly proportional to $\dot{V}$:

$$\Delta P = (\dot{V} \times 8 \times L \times \mu)/(\pi \times r^4).$$

In this case the constant of proportionality (K_1) is directly related to the length of the airway (L) and the viscosity of the gas (μ) and is indirectly proportional to the fourth power of the radius of the airway (r). When flow exceeds the critical velocity, its pattern changes from laminar to turbulent. For turbulent flow, ΔP is proportional to $\dot{V}^2$. The constant of proportionality (K_2) is directly proportional to the length of the airway and the density of the gas and inversely proportional to the fifth power of the radius of the airway.

approximately 80% of the total resistance of the respiratory system; tissue resistance and inertial forces account for the remaining 20%.[7] In the newborn, the small size of the airways makes them significantly more prone to high airway resistance with nasal resistance representing almost half of the total airway resistance.[8] In addition, neonates are preferential nose breathers. Therefore, any mucus or edema in the nasal passages can offer significant resistance to air flow.

The movement of gas through the airway is modeled by two basic patterns of flow, laminal and turbulent (Fig. 39.2). During laminar flow the pressure difference needed to move gas through the airway is directly related to the flow rate times a constant (airway resistance). During turbulent flow, however, this pressure is directly proportional to a constant multiplied by the flow rate squared. Gas flow becomes turbulent at branch points in airways, at sites of obstruction, and at high flow rates. Turbulence occurs whenever flow increases to a point that the Reynolds number exceeds 2000. This dimensionless number is derived from fluid dynamics and is calculated as $\mathrm{Re} = [p \times d \times V]/[u]$ where V is the volumetric flow rate, p is gas density, d is radius of the tube, and u the gas viscosity.[9] Obviously, turbulent flow is most likely to occur in the central airways, where volumetric flow is high, rather than in peripheral airways where flow is distributed across a large total cross-sectional area. Both types of flow exist in the lung, so the net pressure drop is calculated as follows[10]:

$$\Delta P = (K_1 \times \dot{V}) + (K_2 \times \dot{V}^2) \quad (1)$$

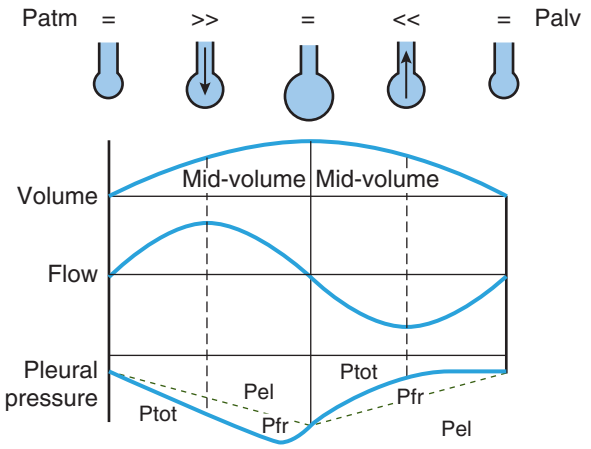
Inflation of the lung increases the length of airways and might therefore be expected to increase airway resistance; however, lung inflation also increases airway diameter. Because airway resistance varies with the fourth to fifth power of the radius of the airway, the effects of changes in airway diameter dominate, and resistance is inversely proportional to lung volume.[11] Airway resistance is lower during inspiration than during expiration, because pleural pressure becomes more negative during inspiration, and a greater distending pressure is applied across the lung. This distending pressure increases airway diameter as well as alveolar diameter and decreases the resistance to gas flow. During expiration, pleural pressure increases and airways are compressed. Collapse of airways is opposed by their cartilaginous support and by the pressure exerted by gas in their lumina. During passive expiration these defenses are sufficient to prevent airway closure. When intrapleural pressure is high, during active expiration, airways may collapse, and gas may be trapped in the lung causing dynamic hyperinflation. This problem may be accentuated in the small preterm infant with poorly supported central airways.

Inertance

Gas and tissues in the respiratory system also resist accelerations in flow, which is determined by gas density and the ratio of airways length to surface area. Inertance represents opposition to these accelerated forces and is assumed to be negligible during quiet breathing and physiologically significant only at rapid respiratory frequencies and high flow rates.

Dynamic Interaction

Compliance, resistance, and inertance all interact during spontaneous breathing (Fig. 39.3). This interaction is described by the equation of motion for the respiratory system:



• **Fig. 39.3** Gas flows from the atmosphere into the lung only if atmospheric pressure (P_{atm}) is greater than alveolar pressure (P_{alv}). At the end of exhalation, when P_{atm} equals P_{alv}, there is no gas movement in or out of the lung. During a spontaneous inspiration, the diaphragm contracts, the chest wall expands, and the volume in the intrathoracic space increases. As a result, pleural pressure (P_{pl}) decreases relative to P_{atm}, and a gradient is created between P_{pl} and P_{alv}, distending the lung, increasing alveolar volume, and decreasing P_{alv}. A gradient is also created between P_{atm} and P_{alv}, and gas flows from the atmosphere into the alveolar space. The rate of gas flow increases rapidly, reaches a maximum (peak flow), then decreases as the alveolus fills with gas and P_{alv} approaches P_{atm}. At peak inspiration, P_{alv} equals P_{atm}, and lung volume is at its maximum, as is P_{pl}. The *curved solid line* connecting the end of expiration to the end of inspiration is the total driving pressure for inspiration (P_{tot}). The *dotted line* represents the pressure needed to overcome elastic forces alone (P_{el}). The difference between the two lines is the pressure dissipated overcoming flow resistive forces (P_{fr}). During exhalation, this cycle is reversed.

$$P(t) = (V[t] \times 1/C) + (\dot{V}[t] \times R) + (\ddot{V}[t] \times I) \quad (2)$$

where $P(t)$ is the driving pressure at time t, $\dot{V}[t]$ is the lung volume above FRC, C is the respiratory system compliance, $\dot{V}[t]$ is the rate of gas flow, R is the resistance of the respiratory system, $\ddot{V}[t]$ is the rate of acceleration of gas in the airways, and I is the inertance of the respiratory system. If I is disregarded, the equation simplifies to

$$P(t) = (V[t] \times 1/C) + (\dot{V}[t] \times R) \quad (3)$$

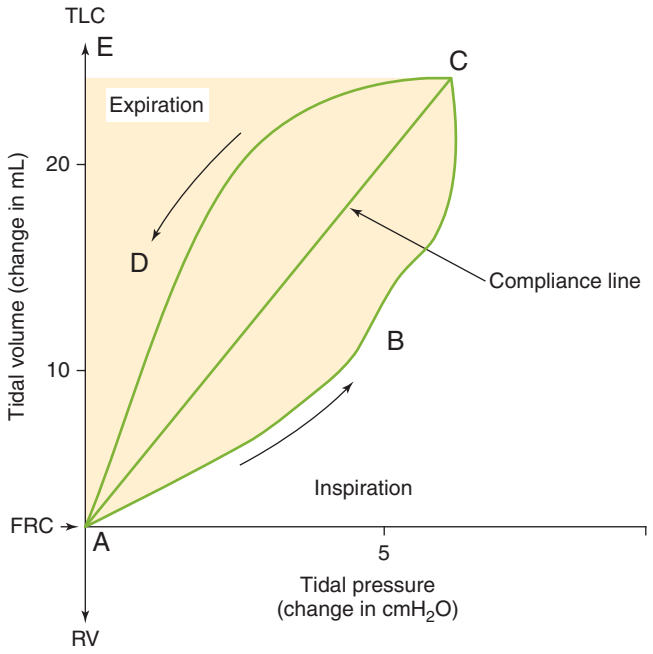
At times of zero gas flow (end expiration and end inspiration), the equation further simplifies to

$$P(t) = (V[t] \times 1/C) \text{ and } C = V(t)/P(t) \quad (4)$$

This series of equations and Fig. 39.3 demonstrate that at points of no gas flow (end of expiration and end of inspiration), only elastic forces are operating on the lung. During inflation or deflation of the lung, however, both elastic and resistive forces are important.

Work of Breathing

The work of breathing (WOB) is the amount of energy required to overcome the elastic and resistive elements of the respiratory system and move gas into and out of the lung during spontaneous breathing. WOB is defined as the cumulative product of distending pressure and the given volume displaced during inhalation or exhalation (Fig. 39.4):



• **Fig. 39.4** Inspiratory/expiratory pressure–volume loop showing the compliance line (*AC*, joining points of no flow); work done in overcoming elastic resistance (*ACEA*), which incorporates the frictional resistance encountered during expiration (*ACDA*); work done in overcoming frictional resistance during inspiration (*ABCA*); the total work of breathing during respiratory cycle is the sum of *ACEA* + *ABCA* equals *ABCEA*, or the entire *shaded area*. *FRC*, Functional residual capacity; *RV*, residual volume; *TLC*, total lung capacity. (From Wood BR. Physiologic principles. In: Goldsmith JP, Karotkin EH, eds. *Assisted Ventilation of the Neonate*. 4th ed. Philadelphia: Saunders; 2003:15–30.)

$$\text{WOB} = \int P \mathrm{d}V$$

where P is pleural pressure (in time) above resting pleural pressure and V is the volume (in time) relative to the resting thoracic volume.

In healthy infants, most of the WOB is done by the diaphragm during inhalation. Approximately one-third of the total inspiratory WOB is related to overcoming the resistance to gas flow in the airways.[12] Exhalation is usually passive because of the potential energy stored in the lung and the chest wall at the end of inhalation. The WOB is increased with increase in resistance, decrease in compliance or increased respiratory frequency when lung volumes decrease below FRC.

Time Constants

The distribution of gas within the lungs depends largely on the dynamic interaction between the compliance and resistance of the entire respiratory system. This relationship is called the time constant (Trs) and is the product of the resistance and compliance ($R \times C$). There are challenges to measuring changes in lung volumes and distending pressure of the respiratory system. Because of the difficulties associated with trying to achieve a steady state to measure static compliance, many investigators calculate the "dynamic compliance" and take advantage of the fact that gas flow in and out of the lung is transiently equal to zero at the end of inspiration and the end of expiration (see Fig. 39.3). Measuring

lung volume and distending pressures at these two points of rest allows calculation of compliance by division of the change in volume by the concomitant change in distending pressure. For passive mechanics, the behavior of the respiratory system during passive exhalation can be obtained relatively easily with the use of the occlusion technique by giving a positive pressure breath, then the airway is occluded, invoking the Hering Breuer reflex and a brief apnea.[13] Airway pressure is measured when the occlusion is released. The expired gas flow is measured with a pneumotachometer and integrated to volume; the flow is then plotted as a function of volume (Fig. 39.5A).

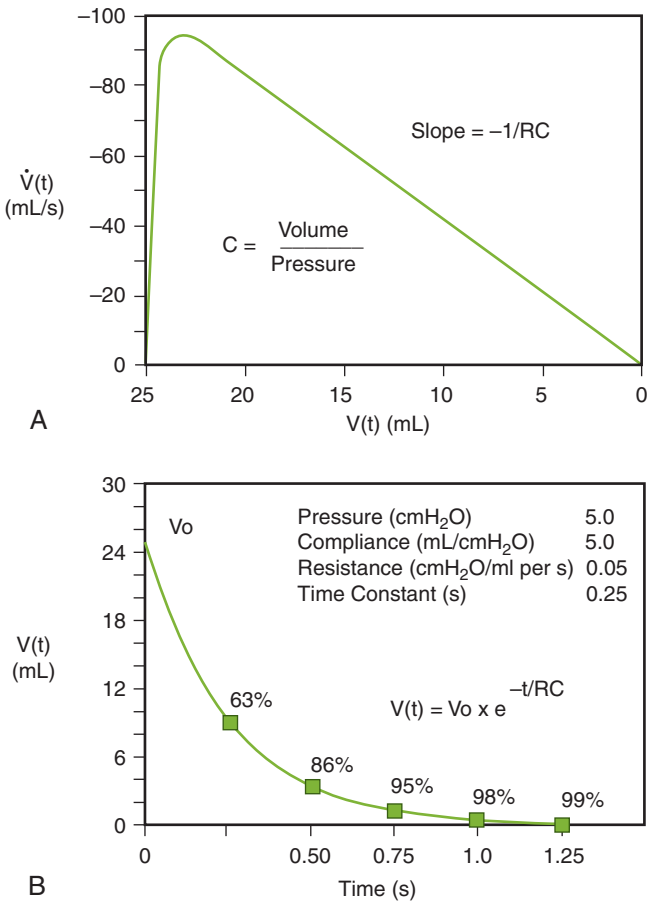
During a passive exhalation, no external forces are acting on the respiratory system [airway pressure or $P(t) = 0$], so the equation of motion simplifies to a first-order differential equation:

$$(V[t] \times 1/C) + (\dot{V}[t] \times R) = 0$$

Rearrangement yields the linear equation:

$$\dot{V}(t) = -\left(\frac{1}{RC}\right) \times (V[t]) \qquad (5)$$

where the slope is $-1/RC$, which can be determined by a linear regression of $\dot{V}(t)$ versus $V(t)$. This equation states that during passive exhalation, flow plotted against volume is a straight line with slope $-1/RC$. Since the volume can be calculated from the flow–volume curve, the pressure measured during the inspiratory hold can be used to calculate compliance and the slope of the line to calculate resistance. The quantity RC, i.e., the respiratory system time constant (Trs), in units of seconds, describes the rate at which the lung deflates during passive exhalation. It also implies the time it takes for the pressure within the alveoli to equilibrate (Fig. 39.5B). Time constants affect the rate of lung inflation in much the same manner.



• **Fig. 39.5** (A) Flow of gas out of the lung versus volume of gas remaining in the lung, $V(t)$, for a passive exhalation. Flow of gas out of the lung is negative by convention. After an initial sharp increase, flow decreases linearly as the lung empties. Static compliance of the respiratory system is obtained by division of the exhaled volume by the airway pressure at the beginning of the passive exhalation. Resistance is calculated from the slope of the flow–volume plot ($-1/RC$) and the compliance. This technique has the advantage of not requiring measurements of pleural pressure and being relatively unaffected by chest wall distortion. (B) $V(t)$ as a function of time for a passive exhalation. The graph is an exponential with the equation

$$V(t) = V_0 \times e^{-t/RC},\ V(t) = V_0 \times e^{-t/RC},$$

where V_0 is the starting volume, and e is the base of the natural logarithm (roughly 2.72). For this example, the time constant of the respiratory system (Trs) is roughly 0.25 s. Calculations show that when exhalation persists for a time equal to the time constant (t = 0.25 sec = 1 × Trs), 63% of the gas in the lung is exhaled. For t = 2Trs, 86% of the gas is exhaled; for t = 3Trs, 95% of the gas is exhaled; for t = 4Trs, 98% of the gas is exhaled; and for t = 5Trs, 99% of the gas is exhaled. If expiration is interrupted before t = 3Trs, gas is trapped in the lung. where V_0 is the starting volume, and e is the base of the natural logarithm (roughly 2.72). For this example, the time constant of the respiratory system (Trs) is roughly 0.25 s. Calculations show that when exhalation persists for a time equal to the time constant (t = 0.25 sec = 1 × Trs), 63% of the gas in the lung is exhaled. For t = 2Trs, 86% of the gas is exhaled; for t = 3Trs, 95% of the gas is exhaled; for t = 4Trs, 98% of the gas is exhaled; and for t = 5Trs, 99% of the gas is exhaled. If expiration is interrupted before t = 3Trs, gas is trapped in the lung.

Measurements of Respiratory System Mechanics

Respiratory system mechanics are objective measurements used to determine the severity of lung disease, changes in pathophysiology, response to therapeutic interventions, and progression of lung growth and development. Table 39.1 shows a summary of lung volumes and mechanics values obtained by different investigators using different techniques for healthy and sick infants and premature infants.

Lung mechanics in infants are typically measured during spontaneous breathing or during assisted ventilation or during short periods of apnea induced by taking advantage of the Hering–Breuer reflex. As previously described, a driving force is measured during a brief airway occlusion, when pressures equilibrate across airways during periods of no flow. The occlusion is then released and the flow and volume of gas are measured during the passive expiration. Calculations of compliance are affected by lung size; therefore, if lung compliances are to be compared, they must be corrected by dividing compliance by resting lung volume or FRC. The resulting value is called the *specific compliance*. For the normal newborn, resting lung volume is approximately 100 mL. In addition, lung compliance changes with volume history, meaning that it decreases with fixed tidal volumes and increases with periodic sigh that recruit poorly ventilated or collapsed air spaces resulting in improved lung compliance and oxygenation.[14] Variations in static compliance measurements are also associated with achieving steady state due to gas redistribution. Therefore, many investigators have suggested calculating dynamic compliance, which takes into account the movement of the gas in and out of the lungs, with the flow of gas transiently equaling zero at end of inspiration and end of expiration (see Fig. 39.3). The lung volumes and

TABLE 39.1 Lung Volumes and Respiratory Mechanics of Well and Sick Infants

Measurements	Units	Well Infant	Well Preterm Infant	RDS	BPD
Tidal volume	mL/kg	5–7	7.1–7.8	4–6	4–10
FRC	mL/kg	22–30	21–26	20–33	16–30
Compliance	mL/cmH_2O	1–2	1.1–1.6	0.3–0.6	0.2–1.0
Resistance	cmH_2O/L/s	25–50	52–97	60–160	30–170
Work of breathing	g cm/min/kg	500–1000		800–3000	1800–6500

BPD, Bronchopulmonary dysplasia; *FRC*, functional residual capacity; *RDS*, respiratory distress syndrome.
Although there are significant overlaps in the ranges of values, there is a general trend for infants with RDS and BPD to have significantly diminished lung compliance, increased airway resistance and increased work of breathing. Data from Cook CD, Sutherland JM, Segal S, et al. Studies of respiratory physiology in the newborn infant: III. Measurements of mechanics of respiration. *J Clin Invest*. 1957;36:440–448; Polgar G, String ST. The viscous resistance of the lung tissues in newborn infants. *J Pediatr*. 1966;69:787–792; Reynolds RN, Etsten BE. Mechanics of respiration in apneic anesthetized infants. *Anesthesiology*. 1966;27:13–19; Polgar G, Promadhat V. Pulmonary Function Testing in Children: Techniques and Standards. Philadelphia, PA: WB Saunders; 1971:273; Gerhardt T, Bancalari E. Chestwall compliance in full term and premature infants. *Acta Paediatr Scand*. 1980;69:359–364; McCann EM, Goldman SL, Brady JP. Pulmonary function in the sick newborn infant. *Pediatr Res*. 1987; 21:313–325; Choukroun ML, Tayara N, Fayon M, Demarquez JL. Early respiratory system mechanics and the prediction of chronic lung disease in ventilated preterm neonates requiring surfactant treatment. *Biol Neonate*. 2003;83:30–35; Castille R, Filbrun D, Flucke R, et al. Adult-type pulmonary function tests in infants without respiratory distress. Pediatr Pulmonol. 2000;30:215–227; Hjalmarson O, Sandberg K. Abnormal lung function in healthy preterm infants. *Am J Respir Crit Care Med*. 2002;165:83–87. McEvoy CT, Schilling D, Go M, et al. Pulmonary function in extremely low birth weight infants with bronchopulmonary dysplasia before hospital discharge. *J Perinatol*. 2020;doi.org/10.1038/s41372-020-00856-z.

distending pressures at these points of zero flow provide measurements for *dynamic compliance*. In normal newborns, dynamic compliance is generally assumed to be equal to static compliance. However, many respiratory disorders result in non-homogenous areas of the lungs with variable compliance and airway resistance (see Table 39.1). Therefore, dynamic measurement may underestimate the compliance of the entire respiratory system.[15]

Several investigators have noted that with obstructive lung disease, the expiratory flow-volume plot may not be linear but concave and does not fit into a straight line.[16] These studies found that the plots fit much better in a biexponential function, with one exponent describing the behavior of well-ventilated lung units (fast compartment) and the other describing the behavior of poorly ventilated lung units (slow compartment). This compartmentalization of lung units also affects lung inflation. During lung inflation, the fast compartment with short time constant fills rapidly, while the slow compartment with long time constant fills much more slowly, causing highly variable inspiratory time constants of the entire respiratory system. This phenomenon is typically manifested in infants with severe bronchopulmonary dysplasia (BPD) with very diverse time constants within the same lung.[17,18]

At higher respiratory rates, only the lung units with short inspiratory time constants are inflated resulting in a smaller ventilated lung. With smaller ventilated lungs, the measured dynamic compliance decreases as the respiratory rate increases. This phenomenon is termed the *frequency dependence of compliance* and is highly suggestive of inhomogeneous lung due to small airway obstruction.

The resistance of the total respiratory system can be calculated from measurements of driving pressure and the rate of gas flow and can be measured only when the lung is moving. The choice of driving pressure determines the site of the measurement. Most commonly the driving pressure is airway pressure minus atmospheric pressure (resistance of the respiratory system) or airway pressure minus intrapleural pressure (total lung resistance). Gas flow rates are calculated from measurements obtained from devices placed in the infants' airway via a face mask, nasal prongs, or an endotracheal tube. In the spontaneously breathing infant, driving pressure is generated by respiratory muscles creating a negative intrapleural pressure that can be measured via a catheter, balloon or transducer in the esophagus. For the ventilated and paralyzed infant, the driving force is the positive pressure at airway opening measured within the ventilator circuit as close as possible to the airway opening. Flow and volume are measured continuously and simultaneously to driving force.[19] If there are no leaks, all of the gas flow in and out of the infant's lungs will travel through a pneumotachometer, which calculates flow as the pressure drop across a fixed resistance or a hot wire anemometer, which calculates flow by measuring the transfer of heat from a heated wire in the circuit just proximal to the infant. In both dynamic and passive techniques, flow, volume and driving force are used to solve the equation of motion of the lung and yield values for compliance and resistance. Traditionally, resistance of the total respiratory system is calculated from measurements of distending pressure, volume, and flow (see Fig. 39.3). For this calculation, points of equal volume are chosen during inspiration and expiration. The gas flow and the distending pressure are measured at each point. The pressure needed to overcome elastic forces should be the same for inspiration and expiration, and therefore the pressures should cancel out. Total resistance, consequently, is equal to distending pressure at the inspiratory point minus distending pressure at the expiratory point divided by the sum of the respective inspiratory and expiratory point gas flows. Subsequently, investigators simplified these calculations by measuring distending pressure, gas flow, and volume (see Fig. 39.3), then fitting these measurements to the equation of motion (Eq. 3) using multiple linear regression techniques and solving for the coefficients $1/C$ and R.[20] Calculations of pulmonary function by modern ventilators use much the same process.

Ventilator graphics yield valuable bedside real-time display of continuous patient ventilator interactions on a breath-to-breath basis. The measurements of pressure, flow, and volume changes, either as time-based scalar display or as flow-volume or pressure-volume loop graphics, provide clinicians with valuable data to customize ventilator settings based on underlying lung mechanics and pathophysiology, such as in small airway obstruction, excessive inflation, asynchrony, or pressure or gas trapping. Measurements of respiratory mechanics are limited by the inability to accurately

measure and differentiate delivered tidal volume during patient-initiated and ventilator-driven breaths. In addition, the one compartment single breath analysis by the ventilator software may substantially underestimate the variability in lung mechanics in the different regions of the lung of newborn infants. Despite these limitations, understanding ventilator graphics could provide additional bedside information about respiratory physiology and the interaction between the ventilator and the ventilated newborn infants.[21]

Volumetric capnography, in the form of continuous partial pressure of carbon dioxide (pCO_2) monitoring combined with continuous flow-volume graphics, provides estimates of anatomical and physiologic dead space, pulmonary capillary blood flow, and efficiency of ventilation. Pattern recognition of displayed waveform can identify pathophysiologic conditions such as airway obstruction, over ventilation or central hypoventilation conditions. In the spontaneously breathing infant, the end-tidal carbon dioxide ($EtCO_2$) measurements provide information on functional lung alterations such as in infants with BPD.[22,23]

Chest wall measurement techniques involve the assessment of tidal breathing by estimating change in chest volume at the chest wall, instead of airflow at the airway opening. One such technique, respiratory inductance plethysmography (RIP), measures changes in the cross-sectional area of the chest by changes in inductance in coils with weak currents around the chest and abdomen. This method could precisely measure thoraco-abdominal asynchrony.[19,24] In addition to providing measurement of the changes in overall tidal volume, thoraco-abdominal asynchrony is also used to detect airway obstruction, to monitor sleep apnea, and to indirectly measure the WOB.[22]

Another modality is electrical impedance tomography (EIT) which is a radiation-free dynamic imaging technique that can detect regional changes in lung volumes. This method is accomplished by transmitting very small alternating electrical currents through the lungs from a belt-like array of electrical transmitter/receivers around the chest wall. The resulting voltages are used to construct a two-dimensional image showing the distribution of electrical impedance throughout the chest. By repeated scans the relative distribution of air in the lungs is mapped out during tidal breathing, and a measure of total and regional ventilation of the lungs is obtained.[19] Clinical and experimental studies using this technique have shown usefulness in a variety of pulmonary conditions including ventilation inhomogeneity in infants with BPD and chronic lung disease of infancy.[25,26]

Forced deflation is a form of spirometry for sedated and paralyzed, intubated, and ventilated infants that inflates the lungs manually to near total lung capacity (+40 cm H_2O) and holding them static for 3 seconds followed by a forced expiratory maneuver by application of a constant negative pressure (−40 cm H_2O) to the endotracheal tube until expiratory flow ceases. A pneumotachograph or flow sensor attached to the breathing tube measures the maximum expiratory flow-volume curves, and the curve with the highest forced vital capacity is used for analysis. Normative data are available for healthy infants and young children up to 5 years of age.[27,28] Alternatively, a combination of comprehensive physiologically integrated spirometry using raised-volume passive and forced expirations as well as multiple-breath nitrogen washout has been developed for in-depth investigation of lung function in infants. Static lung volumes and lung capacities at an airway opening pressure of +30 cm H_2O and variables from the best forced expiratory flow-volume curve were found to be dependent on infant's age, weight and body length.[29] Another technique to produce full forced expiratory maneuvers in sedated infants has been described and uses lung inflation to near total lung capacity (TLC) to a pressure of +30 cm H_2O. Forced expiratory maneuver is produced by rapid thoracoabdominal compression with an inflatable jacket. The rapid deflation produces expiratory flow-volume curves that can demonstrate flow limitations. Fractional lung volumes can be estimated by combining this method of estimating vital capacity with a measurement of FRC obtained by plethysmography. Reproducibility and normative data for infants using this technique have been published.[30–32]

The use of this technique has recently identified three distinct phenotypes—obstructive, mixed, or restrictive—of severe BPD in former premature infants.[33]

Oscillometry, also called forced oscillation technique (FOT), is an alternative method for measuring respiratory system mechanics in which a pressure sine wave [$P(w)$] of a given frequency (w) is superimposed on the infant's tidal breathing, either spontaneously or ventilator-driven, and a resulting flow [$V(w)$] is measured. The resulting high-frequency changes in flow and volume are separated out from the tidal flow/volume changes by signal processing. Pressure, flow, and volume changes are then used to calculate the mechanical impedance of the respiratory system (Zrs = resistance + compliance + inertance) at the imposed frequency. The oscillation frequency used determines the mechanical parameters measured by FOT.[34,35] Technical standards regarding oscillometry measurements, including hardware, software, testing protocols, and quality control, have been recently updated by both the American and European Thoracic Societies. Reference values are available for children but not in infants, and its application in newborns and infants remains experimental.[36]

FRC, a static lung volume, is usually measured by inert gas dilution techniques using helium (He) or sulfur hydrofluoride (SF_6) dilution or inert gas nitrogen (N_2) displacement during a period of tidal breathing (spontaneous or ventilator) and hence is called multiple breath washout (MBW) technique. For He or SF_6 dilution, the gas is first washed into the lungs by adding it to the inspired air. For N_2 washout, breathing gas is switched to 100% oxygen during expiration and continued until the end-expiratory nitrogen concentration is below 2%. The inert gas concentration in the expired breath is measured continuously using a mass spectrometer or ultrasonic flow sensor, and the expiratory airflow and/or volume are measured using a pneumotachometer or ultrasonic flowmeter. FRC is calculated as the ratio of exhaled net tracer gas volume to the difference in the starting and ending end-tidal tracer gas concentrations. The lung clearance index (LCI) describes the number of lung turnovers necessary to clear the inert gas from the lungs. A high LCI suggests poor ventilation homogeneity, which can be a sensitive measure of small airway disease.[37] The total volume of gas in the thorax at the end of expiration (thoracic gas volume) can be measured with a body plethysmography and application of Boyle's law. As the measured thoracic gas volume is larger than the measured FRC by inert gas dilution, the difference between these two volumes represents the volume of trapped gas. A critical relationship exists between FRC and the volume of gas that remains in the lungs after maximal inhalation or exhalation. With the use of a N_2 gas washout technique during maximal inspiratory and expiratory maneuvers, the closing capacity (CC) can be identified as the point in which the conducting airways begin to collapse, resulting in areas of lung collapse that do not participate in gas exchange.[38]

Pulmonary Gas Exchange

Overview

Human life stops promptly when pulmonary gas exchange stops or slows substantially. The movement of oxygen from ambient air to alveolar air to blood to tissue and the reverse movement of carbon dioxide from tissue to lung to the exhaled gas cannot stop or be profoundly impaired for more than a few minutes before death ensues. The body of knowledge summarized above relating to lung volumes, lung mechanics and WOB has focused on air movement. Understanding lung mechanics and volumes is often of limited use when complex lung disorders undermine assumptions of the lung as a single functioning "black box." The reality is that even in healthy infants, the lungs function as multiple discrete gas exchanging units. Subdividing of lung areas becomes particularly problematic when the adverse impacts of preterm birth are superimposed.

Placental gas exchange operates until the moment placental circulation is separated from the mother's, with cord clamping and first breaths. There is no "in-between" time as placental blood-blood countercurrent system of gas exchange ends. Placental gas exchange and nutrient transfer is sufficient to allow a doubling of fetal weight between 28 and 32 weeks and a tripling by 37 weeks of gestation. Yet placental gas exchange is associated with a maternal uterine artery to fetal umbilical vein gradient of about 40 to 50 mmHg. This gradient allows for a relatively hypoxemic fetal situation. No other organ system other than the respiratory system undergoes a dramatic and irreplaceable physiologic change in minutes following birth. The lung, as the meeting place of alveolar ventilation and pulmonary perfusion, must convert from exchanging gas through the placenta with its blood–blood interface—an organ designed to maintain a hypoxic environment—into one in which the need for increased oxygen delivery requires an immediate increase in efficiency.

Gas movement into and out of the airways and the maintenance of gas volumes during the respiratory cycle promotes effective gas exchange. The juxtaposition of alveolar gas and pulmonary capillary blood establishes and maintains maximal diffusion gradients for oxygen and carbon dioxide. Pulmonary gas exchange can fail for many reasons (Box 39.1). The next section of this chapter will focus on development of normal and impaired pulmonary gas exchange.

Tools Available for Assessing Pulmonary Gas Exchange

Calculated oxygen gradient: Measurement of arterial PO_2 and PCO_2 in a person breathing ambient air at sea level reveals a great deal about gas exchange—but such measurements treat the lung as a single black box, not composed of many acinar or more proximal level areas of alveolar ventilation and pulmonary perfusion matching (V_A/Q). The $AaPO_2$ gradient can be calculated by making the assumptions that arterial and alveolar PCO_2 are very similar, ($PaCO_2 \cong PACO_2$); that steady state conditions apply; and that inspired gas is heated and humidified to body temperature pressure saturated (BTPS) conditions before arriving in the acinar spaces. The exact formula is as follows with R, the respiratory exchange ratio, typically assumed as close to 1.0 in healthy infants.

$$PAO_2 = PIO_2 - PACO_2 \times \left[FIO_2 + (1 - FIO_2)/R\right]$$

where

$$PIO_2 = FIO_2 \times (PB - PH_2O)$$

Calculated PIO_2 plotted against SpO_2: Venous admixture summarizes the contribution of shunt (zero ventilation of perfused areas), low V_A/Q areas, and diffusion disequilibrium on overall gas exchange. It is calculated by the following formula in which O_2 content is measured in systemic arterial blood (CaO_2), pulmonary venous blood (C_cO_2) and mixed venous blood (CvO_2). Simultaneous measurement of these oxygen contents is a difficult feat in sick neonates, which is rarely performed outside cardiac catheterization laboratories.

$$\frac{\dot{Q}va}{\dot{Q}t} = \frac{C_cO_2 - C_aO_2}{C_cO_2 - C_vO_2}$$

Potential treatments of hypoxemia depend on differentiating the contributions of intrapulmonary shunt, (areas of zero ventilation) and of low V_A/Q areas to venous admixture (Fig. 39.6).[39]

Changes in the shape of oxyhemoglobin disassociation curves, or in the position of the curves obtained at different calculated PIO_2, allows inferences about shunt versus "low V_A/Q" contributing to arterial hypoxemia (Fig. 39.7).[40,41] In complex pulmonary disorders of infancy, it is likely that both shunt and low V_A/Q areas contribute to venous admixture. Improving one contribution to low PaO_2 without distorting other aspects of gas exchange (e.g., raising end expiratory pressure to improve low V_A/Q areas) may exacerbate the respiratory problems. Knowledge of the potentially unstable and rapidly changing nature of gas exchange abnormalities, in real time, may allow physicians to optimize and individualize treatment applications, by means superior to trial and error.

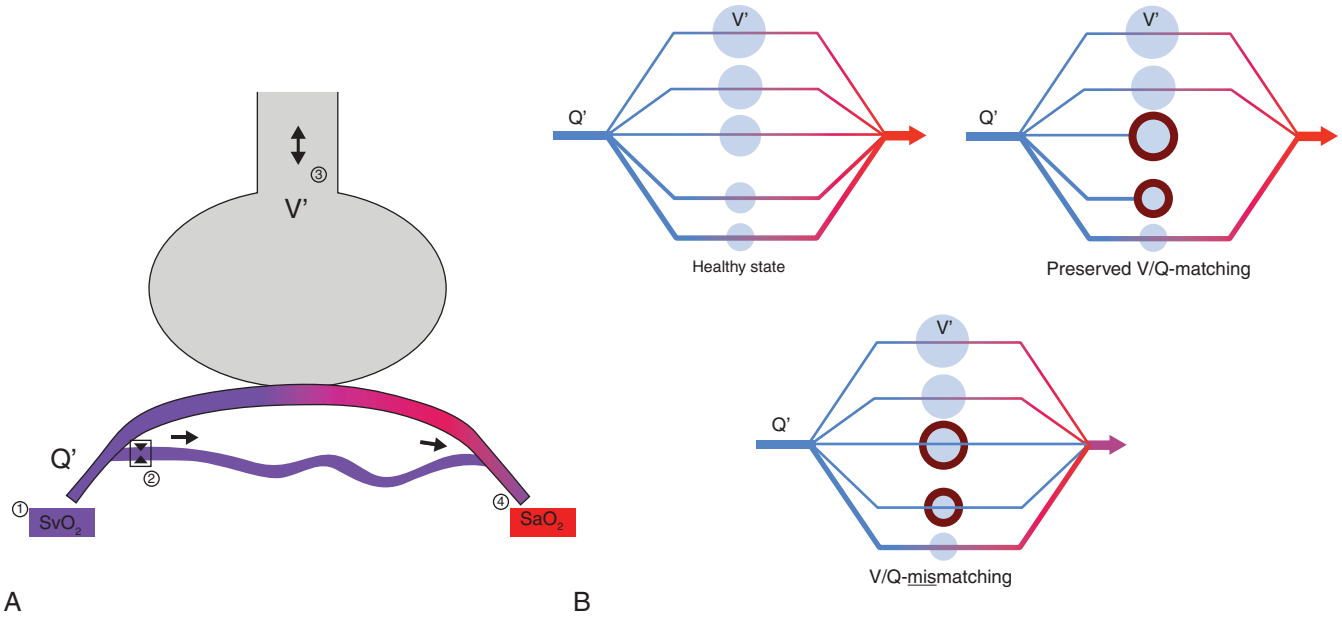
Nitrogen gradient: Because nitrogen does not participate in gas exchange directly, a gradient of arterial to alveolar nitrogen can develop (aADN2) if low V_A/Q areas are present. Assuming ideal alveolar gas composition, alveolar nitrogen is elevated in these areas and absorbed into pulmonary capillary blood. This gradient can be measured and provides another window into V_A/Q mismatch, without specifying the extent or location of these areas. Although nitrogen gradient has been used to measure low V_A/Q areas in respiratory distress syndrome (RDS), where it demonstrates that the major contribution to poor gas exchange is shunt, calculating aADN2 in complex disorders such as severe BPD has not been reported. Krauss et al. measured nitrogen gradients in both healthy term and preterm infants and infants with RDS and demonstrated that low V_A/Q areas do not appear to contribute substantially to gas exchange inefficiency.[42,43]

Trace inert gas technique: The shifting position of the neonatal oxy-hemoglobin dissociation curve and its sigmoidal shape make it difficult to use O_2 and CO_2 as tracers for V_A/Q inequality without many assumptions. Per Wagner PD et al., there is a method of infusing trace quantities of six inert gases of widely ranging solubility and measuring the retention of each gas in blood and excretion in expired gas.[44,45] Using the measured values,

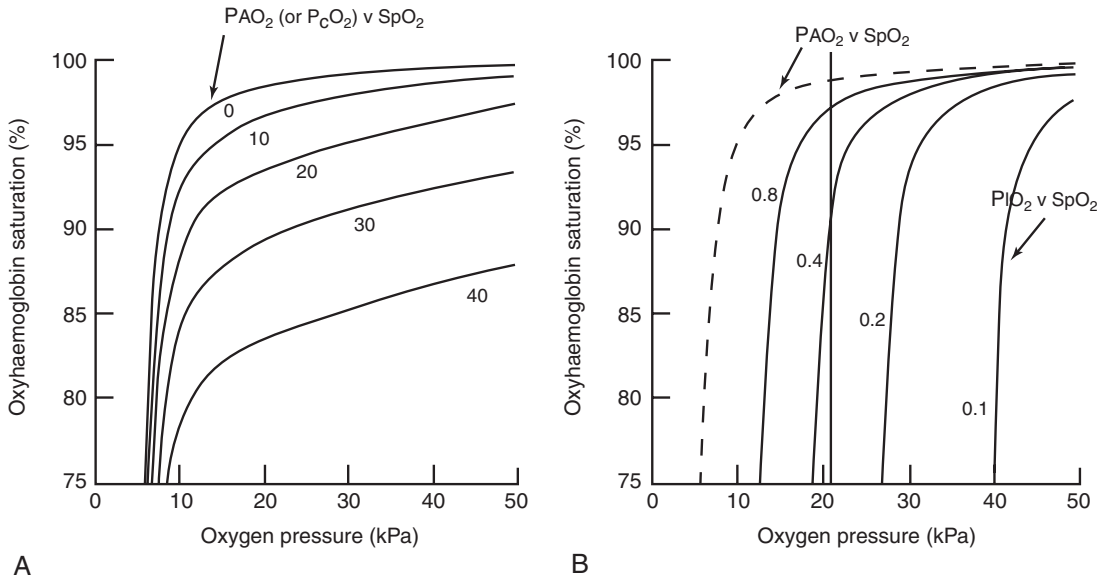
• BOX 39.1 Some Causes of Pulmonary Gas Exchange Failure

- Extreme inspiratory gas hypoxia
- Hypoventilation
- Intrapulmonary and/or extrapulmonary (cardiac) shunt
- Severe ventilation perfusion mismatch (in addition to any shunt present)
- Respiratory gas diffusion disequilibrium

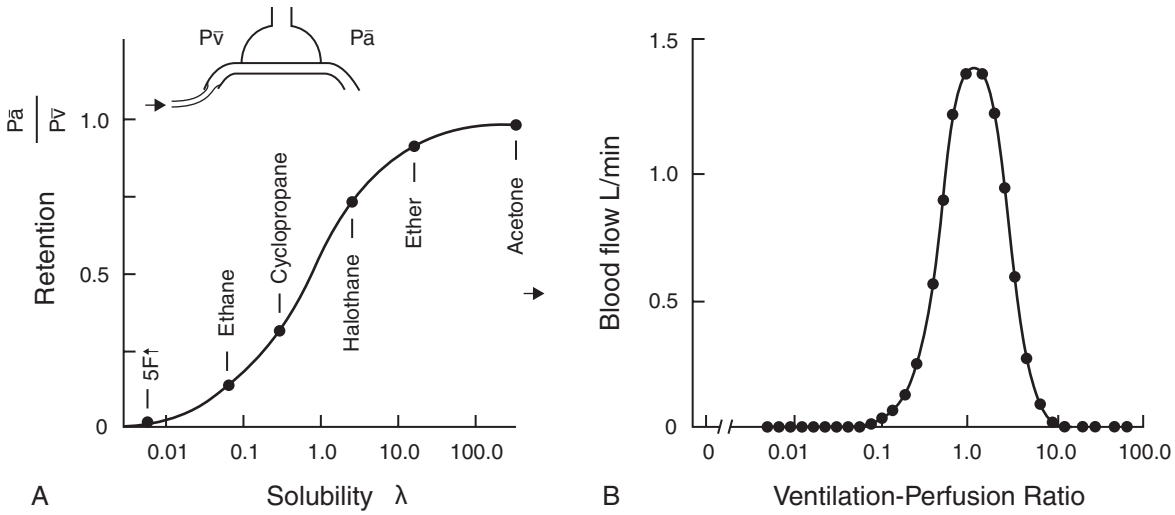
it is possible to estimate shunt, dead space, and a range of low and high V_A/Q lung areas (Fig. 39.8).[46] The technique offers a detailed way of understanding complex lung disorders and the impact of various therapies on V_A/Q matching (Fig. 39.9).[47] However, several assumptions are needed, including steady state conditions and the need to estimate or measure cardiac output. The technique is invasive and not easily applicable to bedside performance in critically ill infants.



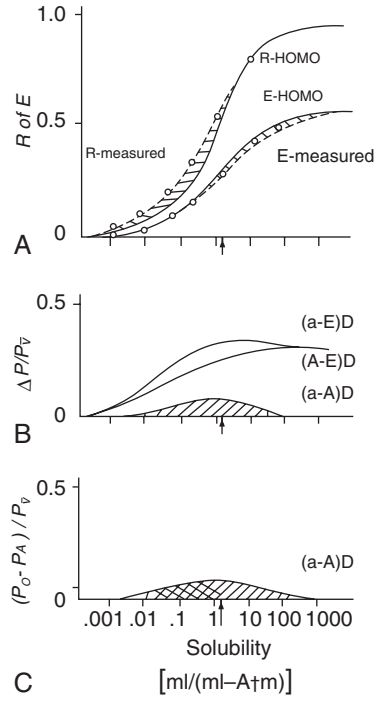
• **Fig. 39.6** (A) Simplified diagram showing factors that might impact on pulmonary gas exchange. SvO_2: Central venous oxygen saturation of hemoglobin as a marker for cardiac output and perfusion pressure (*1*); *Q′*: local pulmonary blood flow; SaO_2: arterial oxygen saturation of hemoglobin as the end result of the different factors (*4*) involved in pulmonary gas exchange; *V′*: local pulmonary ventilation (not shown are factors that can influence *V′* (e.g., broncho-obstruction, fluid accumulation in the depicted alveolus, etc.) (*3*); *2*: symbol to represent hypoxic vasoconstriction (HPV). (B) Schematic depiction of three different situation reflecting the overall pulmonary matching of blood flow (*Q′*) and *V′*. (From Amen EM, Becker BM, Truebell H. Analysis of VQ matching: a safety biomarker in drug development? *Biomarkers*. 2011;16:S5–S10, Fig. 1a and 1b.)



• **Fig. 39.7** Plots of oxyhemoglobin saturation (SpO_2, %) against inspired oxygen pressure (PIO_2, kPa).10 (A) Increasing shunt from 0% to 40% lowers the position of the upper part of the curve. (B) Reducing ventilation:perfusion ratio (VA:Q) from 0.8 to 0.1 shifts the curve to the right. The right shift of each PIO_2–SpO_2 curve from the position of the dissociation curve *(dashed line)* is the PIO_2–PaO_2 difference (kPa), which includes $PaCO_2$/R. The 0.8 curve represents the normal adult curve, which intercepts a PIO_2 of 21 kPa (vertical line) at 97% SpO_2. *R*, Respiratory gas exchange rate. (From Quine D, Wong CM, Boyle EM, et al. Non-invasive measurement of reduced ventilation: perfusion ratio and shunt in infants with bronchopulmonary dysplasia: a physiological definition of the disease. *Arch Dis Child Fetal Neonatal Ed*. 2006;91(6):409-414.)



• **Fig. 39.8** (A and B) Retention-solubility curve showing typical arterial retentions measured with the multiple inert gas technique. This retention-solubility curve can be used to derive a distribution of ventilation-perfusion ratios (B) that is compatible with it. *Pā*, Partial pressure in arterial blood; *Pv̄*, partial pressure in mixed venous blood. West JB. State of the art: ventilation-perfusion relationships. *Am Rev Respir Dis*. 1977;116:919–943.



• **Fig. 39.9** Measured and homogeneous retention (*R*) and excretion (*E*) curves plotted against solubility with *R* and *E* values shown as open circles (*A*). *Hatched areas* between measured and homogeneous *R* and *E* curves occur because of effects of $\dot{V}A/\dot{Q}$ heterogeneity. Their sum is equal to the area under the (a–A)D curve (*B*). As seen in *B*, this area under (a–A)D curve can be found by subtracting (A–E)D curve from (a–E)D curve. *Arrow on abscissa* indicates solubility that is numerically equal to mean $\dot{V}A/\dot{Q}$ of the lung. *C* shows area to the left of mean $\dot{V}A/\dot{Q}$ represented by *double crosshatched area* and is taken as an index of low $\dot{V}A/\dot{Q}$ area. AaDo2 was 14 Torr; calculated low $\dot{V}A/\dot{Q}$ area was 0.07, for this animal. (From Truog WE, Hlastala MP, Standaert TA, McKenna HP, Hodson WA. Oxygen-induced alteration of ventilation-perfusion relationships in rats. *J Appl Physiol Respir Environ Exerc Physiol*. 1979;47:1112–1117, Fig. 1.)

Imaging techniques for quantifying V_A/Q matching: All the above-described techniques are limited by inability to characterize spatial resolution, including cephalad-caudad, proximal-distal, or even segmental differences in gas exchange, using single photon emission computed tomography[48] (SPECT), measured V_A/Q in infants greater than 36 weeks postmenstrual age with moderate and severe BPD. The authors showed that the percentage of lung ventilation associated with relatively normal V_A/Q (between 0.6 and 1.4) correlated inversely with days of assisted ventilation. Recent advances in magnetic resonance imaging (MRI) including ultra-short performance characteristics may provide comparable or improved resolution compared to computed tomography (CT) while still providing accurate estimates of location and severity of V_A/Q abnormalities.[49]

Principles of Ventilation Perfusion Matching

In the healthy adult lung, the overall V_A/Q_p is ~0.8, with ~90% to 95% of the flow of the two gases to areas with V_A/O_p ratios between 0.7 and 1.2, thus allowing for efficient O_2 and CO_2 exchange (see Fig. 39.8). In complex pulmonary disorders, this harmonious overall ratio can be undermined by many factors.

Alveolar ventilation: Alveolar gas must take a circuitous route to the acinar spaces of the developed lung to be available for gas exchange. Inspired gas is diluted by mixing with gas in the anatomical dead space and the physiological dead space. Anatomical dead space includes the conducting airways which do not take part in gas exchange. Physiologic dead space includes areas of the lung that receive part of the minute ventilation but participate minimally in gas exchange. Thus, the term "physiologic" may be inappropriate.

Gas exchange occurs in the acinar units, cumulatively identified as the FRC. This volume of gas helps buffer the inter breath fluctuations CO_2 and O_2 in alveolar gas and hence in pulmonary capillary blood. Any condition limiting the volume or distribution of FRC can have a major adverse impact on efficient gas exchange. A simplified summary of dead space (VDS), dead space

ventilation (V̇Ds), alveolar ventilation (V̇A), total minute ventilation (V̇) and tidal volume (VT), is expressed as follows:

$$\dot{V}A = (VT - VD_S) \times (RR)$$

$$\dot{V}A = [VCO_2 \times (PB - 47)]/PACO_2$$

$$\dot{V}D_S = \dot{V} - \dot{V}A$$

A factor relevant to ventilation in infancy is the relative scarcity of channels of collateral ventilation. Their relative presence or absence (Fig. 39.10) can impact ventilation perfusion matching.[50] For instance, the adverse effect of scarce collateral channels magnifies the risks of creating areas of zero ventilation. Abundant collateral ventilation mitigates the development of diminished FRC but can lead to "series emptying" of the airspaces. Lung gas flow modeling typically assumes parallel emptying. Very slowly emptying compartments, as occur in many illnesses, worsen the tendency for series ventilation as inhaled gas with minimal CO_2 content mixes with still exhaling parts of the exhaled minute ventilation, potentially impairing CO_2 elimination.

Pulmonary perfusion: Forward flow from the right ventricle is continuous but variable in velocity through the cardiac cycle, unlike ventilation, which is discontinuous. One feature of pulmonary perfusion in healthy newborns is that chronotropy is the main mechanism for improving cardiac output. Lusitropy and increased ejection fraction are less useful to the neonatal heart for increasing output. With unchanging tissue O_2 extraction, increased pulmonary blood flow is one mechanism to increase PaO_2. The increase in PaO_2 occurs because mixed venous blood will have higher PvO_2, so there is a lower chance of incomplete pulmonary capillary oxyhemoglobin desaturation. Shorter transit times in some acinar beds may limit the impact of this cardiac lung interaction.

Diffusion gradient: Simultaneous diffusion of O_2 and CO_2 is the whole point of V_A/Q matching. Establishing and maintaining the thinnest possible barriers for diffusion is crucial. The gas exchanging surface can be impaired because of alveolar edema or loss of acinar volume with surfactant deficiency. Terminal airspaces are covered on the airway side with type 1 and type 2 pneumonocytes. Type 1 pneumocytes cover most of the surface area and their unique structure renders them exquisitely thinned. Recent evidence suggests that pulmonary endothelial capillary cells demonstrate the same capacity for extreme thinness.[51]

Diffusing capacity is a difficult measurement, both to perform and to interpret. As a measure of lung function, it is dependent on lung volume, capillary transit time, and intrapulmonary blood volume. The goal is to measure the intrinsic properties of the membrane(s) that pose the barrier. Using CO as the marker gas, the formula for measuring diffusion is:

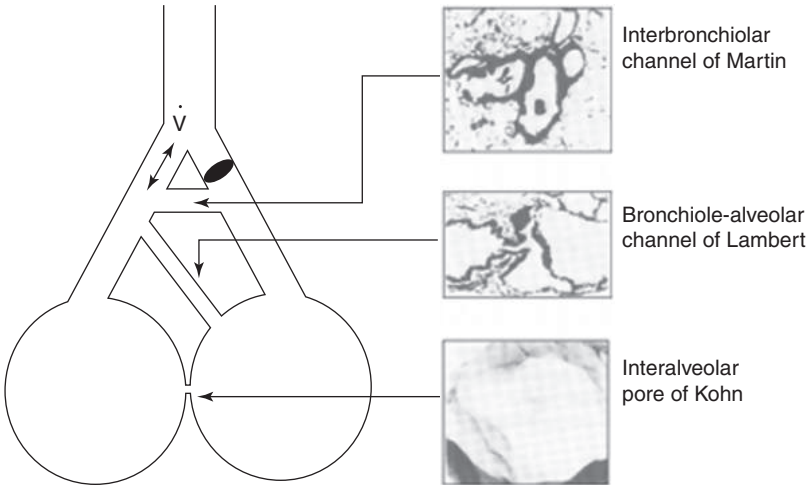
$$D_{LCO} = \dot{V}_A co/P_A co$$

Use of this formula and the technical challenges of measuring so-called steady state diffusing capacity have limited its usefulness in identifying specific conditions in infancy in which targeted therapies may be used. An example of this limited utility is that D_{LCO} can be influenced by changes in left atrial pressure and pulmonary blood flow.[52]

Impairments to infant pulmonary gas exchange: Tissue gas exchange cannot be accomplished at extreme altitude, no matter how efficiently ventilation and perfusion are matched, because of obligate alveolar hypoxia. Similarly, if hypoventilation results in inadequate removal of CO_2 and replenishment of alveolar stores of O_2, then gas exchange fails for reasons unrelated to ventilation perfusion matching. It also fails if profound anemia results in inadequacy of tissue delivery of oxygen no matter how efficient the matching of pulmonary perfusion and alveolar ventilation. Each of these factors can exacerbate gas exchange inadequacy associated with V_A/Q mismatching. Highlighted below are some but not all factors pertinent to infants with underlying complex pulmonary disorders.

Some Specific Conditions Impacting V_A/Q Matching

High altitude: The comparison of postnatal adjustment to birth in healthy infants was studied in a landmark paper by.[53] The authors noted a marked difference when comparing the transition of Tibetan infants born to mothers living for generations at high altitude to that of infants delivered to Han ethnic mothers who recently arrived at very high altitude. This finding suggests that the uneventful fetal to neonatal transition is influenced by genomic considerations and perhaps by epigenetic changes. How those population differences influence the exact mechanisms remain unclear.



• **Fig. 39.10** Pathways for collateral ventilation. (From Terry PB, Traystman RJ. The clinical significance of collateral ventilation. *Ann Am Thorac Soc*. 2016;13:2251–2257. Fig. 1.)

High FIO_2 inhalation and gas exchange: Differences exist in the effects of breathing high FIO_2 between adults and infants. Breathing FIO_2 of 1.0 or close to it has long been recognized as damaging to lungs, particularly to the capillary endothelium and alveolar epithelium. When healthy adults breathe an FIO_2 of 1.0, at least for brief periods, there is no interference with $\dot{V}_A/Q$ matching as judged by the inert gas technique.[54] However, in infant piglets conditioned with prior hyperoxic exposure, pulmonary hypoxic vasoconstriction is blunted, and shunt formation is increased compared to room air exposed control animals.[55] Impact on gas exchange will depend on the duration of the exposure to hyperoxia and the underlying state of maturity of the lungs and the capillary bed. Microvascular injury manifests itself as capillary leakage, raising the possibility of edema, surfactant inactivation and impaired gas exchange on multiple levels.

Elevated pulmonary vascular pressure and gas exchange: Neonatal pulmonary pressure is elevated at birth compared to later in infancy and demonstrates an exaggerated response to multiple stimuli. Increases in pulmonary pressure can induce profound increases in intrapulmonary shunt and extrapulmonary shunt (ductus arteriosus and foramen ovale, interatrial shunts). The increase in pulmonary pressure in response to alveolar hypoxia is more vigorous in lambs than in adult sheep, and the response begins at a higher PAO_2.[56] Vasopressor medications, used to elevate blood pressure systemically, can impair $\dot{V}_A/Q$ matching in lambs if the dose is sufficiently high to raise pulmonary arterial pressure and vascular resistance.[57]

Cardiac dysfunction and gas exchange: In addition to the anatomical locations that persist in their patency post-delivery as sites for right to left shunt, large left to right shunts can also impact $\dot{V}_A/Q$ matching and therefore gas exchange. Excessive pulmonary blood flow via a persistent ductus arteriosus (PDA) or ventricular septal defect (VSD) can have an adverse impact on $\dot{V}_A/Q$ matching as evidenced by lower values for arterial PO_2.

It is beyond the scope of this chapter to detail the impact of biventricular cardiac failure on the metabolic functions of the lung, However, adverse impacts on those functions may exacerbate gas exchange impairment. Enzymes found in abundance in the pulmonary microvascular endothelium include angiotensin converting enzyme and endogenous nitric oxide synthase. Alterations in any of these enzymes, among others, likely adversely impact $\dot{V}_A/Q$ matching.

Positive Pressure Ventilation and Gas Exchange

In the infant undergoing positive pressure ventilation, the degree to which lung inflation compromises venous return is related to the relative compliances of the lung and chest wall. If the infant's lung is poorly compliant and the chest wall is compliant, as in RDS, there is little effect of lung inflation on venous return. If the infant's lung is normally compliant but tight abdominal distention prevents descent of the diaphragm, intrathoracic pressure increases dramatically during positive pressure ventilation, and venous return and cardiac output can be impaired. This mechanism may help explain the circulatory instability of some infants after gastroschisis repair. A similar situation may arise in the preterm infant with pulmonary interstitial emphysema and massive lung overinflation. In such an infant, the heart is tightly compressed between the hyperinflated lungs and the other structures of the mediastinum and the diaphragm. Venous return may be severely limited and venous pressures so increased that massive peripheral edema often accompanies the reduction in cardiac output.

Pulmonary edema and gas exchange: Pulmonary edema is the abnormal accumulation of water and solute in the interstitial and alveolar spaces of the lung.[58,59] In the lung, fluid is filtered from capillaries in the alveolar septa into the alveolar interstitium and then into the lower-pressure extra alveolar interstitium. The extra alveolar interstitium contains the pulmonary lymphatics, which are crucial to maintaining a nonedematous microenvironment. The goal is to avoid net accumulation of interstitial fluid. Pulmonary edema results only when the rate of fluid filtration exceeds the rate of lymphatic removal. There are only three mechanisms by which pulmonary edema can occur: (1) the driving pressure for fluid filtration (filtration pressure) increases, (2) the permeability of the vascular bed (hence the filtration coefficient K_f) increases, or (3) lymphatic drainage decreases, or was insufficiently developed at birth.[60] All three mechanisms can contribute to complex lung disorders, especially BPD, with regionally variable effects on gas exchange.

Acknowledgment

The authors gratefully acknowledge the work of the previous authors of this chapter, Drs. Hansen, Richardson, and DiBlasi. Their contributions to earlier editions of this textbook made our task vastly easier, and we thank them for their previous work.

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Neonatal Respiratory Therapy

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KEY POINTS

- Most neonatal lung diseases are characterized by increased alveolar surface tension causing atelectasis. To offset this, pressure is applied to the upper airway through either noninvasive or invasive techniques. Regardless of the support technique applied, mean airway pressure (mPaw) is a good measure of the amount of support that is applied to offset these surface forces and improve ventilation/perfusion matching and oxygenation.
- There are multiple ways to provide noninvasive support. These include nasal cannula (NC) supplemental oxygen, high-flow nasal cannula (HFNC) which provides some level of positive pressure, nasal CPAP (NCPAP), and noninvasive ventilation modes, both synchronized and non-synchronized.
- There are multiple modes of mechanical ventilation, but all ventilator breaths can be characterized by when they start, how large they are, and the duration of the breath. Modes that support every breath and which provide consistent tidal volume (V_T) appear to be the best for most infants.
- High-frequency ventilation predominantly includes high-frequency jet ventilation (HFJV) and high-frequency oscillatory ventilation (HFOV). The mechanisms by which they provide gas exchange are complex, but the guidelines for adjusting them are simple. HFOV and HFJV are typically used for infants with severe lung disease.
- Noninvasive assessment of the respiratory status of an infant includes a physical exam, chest radiographs, and pulse oximetry. For ventilated infants, assessment of ventilator graphics plays a key role in optimizing ventilator support.

Respiratory Support

Respiratory support for most infants with immature and/or abnormal lungs falls into two broad categories: provision of supplemental oxygen and provision of pressure. Supplemental oxygen is one of the oldest therapies in modern neonatal care, while pressure applied to the upper airways and transmitted to the alveolar level is more recent but is fundamental to offsetting lung pathology. The techniques for providing these therapies can be thought of as a continuum extending from simple nasal cannula oxygen to nasal continuous positive airway pressure (CPAP) and noninvasive ventilation, to sophisticated forms of invasive mechanical ventilation. Moving through this continuum allows for the support of infants with more severe lung disease but is associated with concomitant complexity.

Supplemental Oxygen

Most neonatal lung diseases are characterized by atelectasis and ventilation/perfusion mismatch, resulting in impaired oxygenation. Other common neonatal conditions such as pulmonary hypertension and congenital cardiovascular anomalies can also result in right-to-left shunting and impaired oxygenation. Regardless of the cause, impaired oxygen delivery from the alveoli to the pulmonary capillary bed can lead to reduced oxygen content in the blood (hypoxemia) and tissue (hypoxia).

Administration of supplemental oxygen to newborns with lung disease has been part of routine neonatal care since the first part of the 20th century.[1] An increased F_IO_2 increases the alveolar O_2 tension (P_AO_2) in both well-ventilated and partially ventilated areas of the lung. The resulting increase in the alveolar-arterial O_2 gradient drives O_2 from the alveoli into the pulmonary circulation and partially compensates for the hypoxemia associated with ventilation/perfusion mismatch. However, supplemental O_2 does not improve the underlying atelectasis which may be causing the hypoxemia.

The primary goal of supplemental oxygen is to maintain adequate oxygen availability to the tissues, especially to the central nervous system and the heart. However, achieving the optimal level of supplemental oxygen support requires carefully balancing the toxicity inherent in oxygen supplementation and the potential damage to end organs caused by high arterial P_aO_2 levels against the potential damage caused by hypoxemia.

For infants with hypoxemia secondary to decreased ventilation/perfusion matching, correction of the ventilation/perfusion mismatch is at least as important as giving supplemental oxygen. In most acute neonatal lung diseases this means providing pressure to the upper airway to offset the surface forces leading to alveolar collapse and atelectasis. Thus, finding the ideal level of supplemental oxygen is almost always done in the context of simultaneously finding the ideal level of positive pressure support.

Early strategies to "normalize" oxygenation in preterm infants with the use of high F_IO_2 resulted in high rates of retinopathy of prematurity (ROP) and blindness. The early, liberal use of high levels of supplemental oxygen was then followed by a period when F_IO_2 was restricted, regardless of blood oxygenation level, and was associated with increased rates of neurologic damage and death.[2] The introduction of continuous oxygen monitoring, first with transcutaneous oxygen levels ($P_{TC}O_2$), then with saturation monitoring by pulse oximetry (S_pO_2), led to finer control of oxygenation and attempts to keep infants within tighter oxygenation target ranges.

Target Ranges of O_2 and CO_2

The ideal target ranges for oxygenation and ventilation are not certain and the ranges employed vary to some extent from center to center. General trends and guidelines are outlined below.

Target Ranges for P_aO_2 and S_pO_2

Infants, particularly preterm infants, have an inherently unstable respiratory system that makes it extremely difficult to maintain oxygenation saturation within a narrow range. In addition, preterm infants are easily destabilized by handling and procedures. Normal oxygen saturation for room air–breathing term or healthy preterm infants, after the immediate transition period, is greater than 93%, with P_aO_2 levels above 70 mmHg.[3] This is not surprising, since the mature and healthy infant lung has normal ventilation/perfusion matching and should be able to deliver "normal" amounts of oxygen to the blood.

The search for the ideal S_pO_2 target range in infants can be divided into three broad areas, each with its own body of research. The first is in delivery room resuscitation where recent research has focused on the rate at which the newborn's S_pO_2 should transition from the low intrauterine levels to higher postnatal levels. The second is the debate about the ideal S_pO_2 for infants with BPD, particularly to avoid or treat the pulmonary hypertension that often accompanies severe BPD. Both newborn resuscitation and BPD are addressed in more detail elsewhere in this textbook. The third area of research on ideal S_pO_2 target ranges is in preterm infants during the acute, post-delivery period. This has largely come down to a delicate balance between avoiding the higher S_pO_2 ranges which are associated with an increased risk of ROP and avoiding the lower S_pO_2 ranges which may be associated with increased mortality. Early data showed that ROP severity was associated with the duration of hyperoxemia and emphasized the importance of oxygen monitoring.[4] Higher rates of ROP and a more severe respiratory course appeared to occur in neonatal centers that tolerated higher S_pO_2 levels, and observational data indicated that decreasing the S_pO_2 target range could reduce the rates of ROP.[5–8] Subsequent randomized trials to compare the effects of targeting an S_pO_2 of 85% to 89% versus 91% to 95% had mixed results, with some studies suggesting an increased mortality in infants in the lower S_pO_2 range, and others not finding a difference between the ranges.[9–13] Taken together, these studies suggest that there is an increased risk of ROP but a decreased incidence of death or NEC in the higher target S_pO_2 range. However, the composite outcome of death or major disability does not differ between the groups.[14,15] The most recent European Consensus Guidelines for the Management of RDS suggest targeting S_pO_2 90% to 94%, with alarm limits set between 89% and 95%.[16]

Regardless of the exact target S_pO_2 range desired, maintaining infants within this range is often a challenge. Immature respiratory drive and a compliant chest wall, which increases the risk of atelectasis, frequently combine to make oxygenation inherently unstable. Preterm infants receiving supplemental oxygen appear to spend only half of the time with S_pO_2 within their target range.[17–19] The picture is also complicated because caregivers may tolerate higher than ideal S_pO_2 levels with the purpose of reducing the frequency of desaturation spells or attenuating their severity.[20] While S_pO_2 changes rapidly in response to the infant's oxygenation status, adjustments in F_IO_2 and/or level of positive pressure support may lead to gradual changes in gas exchange. Thus, there is always a risk of responding too slowly to changes in S_pO_2 and a simultaneous risk of over-correcting. In clinical practice, many infants experience hyperoxemia because F_IO_2 is not quickly returned to the basal level when a hypoxemic "spell" resolves.[20] In the end, optimal management of an individual infant's oxygenation status is usually a combination of clearly defined policies, careful S_pO_2 monitoring, and the judgment of experienced clinicians about how to respond to infants who stray from their desired target S_pO_2 range.

Policies of oxygenation monitoring should clearly identify both the intended range and the alarm limits of S_pO_2. Setting S_pO_2 alarm limits near the prescribed S_pO_2 target range can increase the proportion of time premature infants spend within the target range.[17] However, narrow alarm limit ranges will increase the number of times the monitor alarms and can lead to "alarm fatigue."[21] Staff compliance with S_pO_2 alarms, and their response to them plays an important role in keeping infants in the target S_pO_2 range. For this reason, staff education and communication are important components of maintaining infants within a target S_pO_2 range.[22–24]

Automated systems to adjust F_IO_2 to maintain S_pO_2 within a target range have been investigated in multiple trials and show consistent improvement in the maintenance of S_pO_2 within the target range as well as reductions in oxygen exposure and staff workload.[25–28] While theoretically attractive, this technology is not yet widely available for clinical use.

PCO_2 and pH Target Ranges

Tolerance to higher carbon dioxide levels may reduce the need for support and reduce the duration of ventilation. However, the results of clinical trials of permissive hypercapnia have been inconsistent. Initial trials suggested faster weaning of infants from mechanical ventilation when higher PCO_2 levels were tolerated.[29,30] On the other hand, there are data suggesting that hypercapnia impairs cerebral autoregulation in the newborn premature infant, suggesting a need for caution in tolerating acute hypercapnia.[31,32] The most recent large study of hypercapnia found no difference in the rate of death or BPD, and showed no difference in adverse sequelae at follow-up.[33,34] Taken together, these studies suggest that the optimal safe range of PCO_2 has yet to be determined. In clinical practice, most centers tolerate some degree of hypercapnia (e.g., PCO_2 in the 50s) in acute lung disease and tolerate a greater degree of compensated hypercapnia in infants with more chronic disease.

General guidelines:

- Rapid changes in PCO_2 should always be avoided. In particular, there is concern about the impact of PCO_2 changes on cerebral blood flow.
- Hypocarbia ($P_aCO_2 < 35$) should always be avoided. In most infants, hypocarbia represents excessive minute ventilation, with the associated risks of lung damage and cardiovascular compromise from excessive pressure and/or volume. Hypocarbia has also been associated with neurodevelopmental morbidity in multiple studies.[35] While hypocarbia was at one time used as a standard therapy for decreasing pulmonary vascular resistance in infants with pulmonary artery hypertension, that approach has almost disappeared with the ready availability of inhaled nitric oxide (iNO) as a far more safe and effective way to decrease pulmonary vascular resistance.
- The degree of hypercarbia which can be tolerated probably varies with post-natal age and with the degree of compensatory metabolic alkalosis. Most centers are comfortable tolerating PCO_2 levels in the 60s (with an acceptable pH) in infants with chronic lung disease.
- The target pH for most infants is usually around 7.25 to 7.35. Higher pH values typically represent hyperventilation and hypocarbia, whereas pH values less than 7.20 to 7.25 raise concerns about the potential impact of acidosis on cellular function, including adverse effects on myocardial function and cardiac output.

Types of Noninvasive Respiratory Support

In neonates, supplemental O_2 can be administered by itself or in conjunction with positive pressure techniques. The simplest ways of providing O_2 without positive pressure include the head box or tent, mask, and low-flow nasal cannula (NC). Other types of support, including high-flow nasal cannula (HFNC), NCPAP, noninvasive ventilation (NIV), and invasive mechanical ventilation all provide some level of positive pressure.[36]

Head Box or Tent O_2 Administration

With a head box or tent, gas at the desired F_IO_2 is delivered to a box or tent which is placed over the infant's head. The gas is typically heated and humidified and must be delivered at a flow rate that assures rapid exchange of gas in the hood and prevents accumulation of exhaled CO_2. This technique is simple but cumbersome, and somewhat limits access to the infant. Although widely used in previous years, O_2 supplementation via head box or tent has been almost entirely replaced by nasal canula oxygen.

Nasal Cannula O_2 Administration

NC devices deliver a constant flow of blended gas at a set F_IO_2 to the nostrils. Typically, the F_IO_2 is set with a blender that mixes 100% O_2 with room air, and the flow is set with a simple meter that can be adjusted to the desired rate, measured in L/min. At low flows, usually defined as less than 1 or 2 L/min, the gas is typically not heated or humidified. With low-flow NCs, the effective F_IO_2 is determined by the relative contribution to each breath of the gas from the NC and the amount of room air that the infant entrains or breaths from around the NC. During mouth breathing or crying, the infant is breathing exclusively room air and derives no supplementation from the NC. For any baby with a given inspiratory flow, increasing the NC flow will increase the amount of gas that the baby inhales from the NC and decrease the amount that is entrained from the surrounding room air, so that at some NC flow rate the baby's F_IO_2 is essentially the same as the F_IO_2 of the NC. However, the point at which the NC flow rate is sufficient to make the baby's F_IO_2 equal to the NC F_IO_2 is dependent upon multiple factors and is not easily measured.[37] In clinical practice, the exact inhaled F_IO_2 is not as important as the baby's oxygenation status, typically measured by pulse oximeter (S_pO_2). It is important to note that a NC at low flow rates does not provide any pressure to the lungs, so is most suited to infants who do not need some level of positive pressure.

High-Flow Nasal Canula

Depending on the size of the baby, cannula flows greater than 1 to 2 L/min can produce significant positive pressure at the nose, essentially providing a form of NCPAP. In general, NC flows above 2 L/min are considered HFNC, and the maximal HFNC flow used in NICUs is usually around 8 L/min. With HFNC most, or all, inspired gas reaching the baby is from the cannula rather than entrained room air. For this reason, HFNC systems should use gas that has been conditioned to avoid drying of the nose and mucosal damage. Conditioning of the gas includes heating and humidification, leading some to refer to this technique as HHHFNC (heated, humidified, high-flow nasal cannula).[38]

HFNC support is adjusted by varying the flow rate and F_IO_2. With HFNC, the effective pressure applied to the infant's upper airway increases as the HFNC flow increases. Depending on the HFNC flow rate, the size of the nasal cannula prongs, and the size of the infant, significant or even excessive levels of NCPAP can be delivered. The major advantage of HFNC is the simple nasal interface, typically with loose-fitting nasal prongs. This makes a system of support that is easy to manage and is generally perceived as comfortable for the infant. However, the significant disadvantage of HFNC as a method of providing NCPAP is that the level of NCPAP is not measured and is dependent on multiple factors. This leads to potentially under- or over-estimating the level of NCPAP support the HFNC is providing. HFNC may "wash out" CO_2 from the hypopharynx, so it can be effective in PCO_2 control if high or consistent levels of NCPAP are not required.[39]

Despite the widespread use of HFNC in preterm infants, the data on its efficacy compared to NCPAP is equivocal. The broad use of HFNC is largely due to ease of use, less risk of injury to nares, and perceived increased patient comfort with the use of an HFNC. In large trials comparing HFNC to NCPAP for support following extubation, there are no significant differences.[40,41] The data on the use of HFNC as a primary mode of support is mixed. Several trials have suggested that there is no difference between HFNC and NCPAP.[42,43] On the other hand, several large trials comparing HFNC to NCPAP as the initial mode of support concluded that HFNC has a significantly higher failure rate.[44–46] As suggested by recent reviews, the reduced nasal irritation seen with HFNC makes it an attractive option despite evidence that it is less efficient than NCPAP in preventing intubation.[47,48]

In most cases, the choice between HFNC and "real" NCPAP comes down to a balance between infant comfort, simplicity, and the ability to accurately measure and control the level of NCPAP. Given concerns about the effectiveness of HFNC in preventing intubation, it is probably most suited to infants at relatively low risk of requiring intubation or re-intubation.

Nasal Continuous Positive Airway Pressure

The care of preterm infants with respiratory distress syndrome (RDS) was revolutionized with the introduction of NCPAP in 1971.[49] Because RDS is characterized by decreased alveolar compliance and atelectasis, positive pressure applied to the upper airway will at least partially offset the surface forces leading to alveolar collapse. The prevention of alveolar collapse preserves functional residual capacity (FRC) and preserves lung compliance. This both improves ventilation/perfusion matching and decreases work of breathing. In addition to its effect at the alveolar level, NCPAP provides positive pressure to the entire airway. In patients with laryngomalacia, tracheomalacia, or bronchomalacia, positive pressure can also stabilize the "floppy airway" by preventing airway collapse during expiration. This is an important role for NCPAP in babies with congenital upper airway anomalies, as well as in babies with distal airway abnormalities associated with severe BPD.

There are several nasal interfaces for NCPAP, each with their own advantages and disadvantages. Probably the most common approach uses double nasal prongs which extend only a short distance into the nose, allowing relatively little leak and good transmission of pressure.[50] NCPAP can also be delivered through a mask that fits tightly over the nose, leaving the mouth uncovered. In many centers, alternating between the tight-fitting short prongs and the nasal mask is seen as an ideal compromise for effectively delivering the measured pressure, and providing an interface that does not cause nasal irritation or skin breakdown.

The positive pressure for NCPAP can be generated in several ways. With "ventilator CPAP," the CPAP circuit is attached to a conventional ventilator that is set to deliver a fixed CPAP level—essentially the same as ventilation with a frequency of 0 breaths/

min. The level of CPAP support and the F_IO_2 are set at the ventilator, and inspiratory gas heating and humidification are accomplished with a standard in-line humidifier. With "bubble CPAP," the pressure is generated by using a water column as an adjustable pop-off valve at the end of the expiratory limb of the respiratory circuit. The level of CPAP pressure is adjusted by the height of the water column above the end of the expiratory limb. This is an extremely simple system for monitoring the integrity of the CPAP device (lack of bubbles suggests there is an upstream leak or other problem) and for adjusting the level of CPAP (by adjusting the height of the water column). However, the depth of the water column is not an accurate predictor of the level of NCPAP delivered at the nasal interface.[51,52] There are commercial integrated bubble CPAP systems that incorporate heating and humidification and the "bubble" component. However, simple bubble CPAP systems can be easily assembled from standard NICU supplies.

One of the intriguing aspects of bubble NCPAP is the possibility that the bubbling at the end of the expiratory limb is actually advantageous. Bubbling results in small oscillations in pressure in the expiratory limb of the circuit which are transmitted "upstream" to the nasal interface and then to the airways.[53] It is not clear whether this oscillation has an actual clinical impact.

In most North American NICUs, the typical levels of NCPAP for preterm infants range from 5 to 8 cmH_2O. There is some evidence that the higher end of this range is more effective in preventing extubation failure in preterm infants who still need supplemental oxygen at the time of extubation.[54]

Bi-Level NCPAP

A variation of NCPAP provides two alternating levels of NCPAP support, with the theoretical advantage of optimizing lung recruitment and minimizing atelectasis without causing overdistension. This approach is frequently used in adults and is termed BiPAP (biphasic positive airway pressure). In infants, it is usually referred to as SiPAP and can be thought of as providing alternating levels of CPAP, or as providing CPAP with intermittent sigh breaths, usually only 1 to 3 cmH_2O above the lower baseline CPAP level. Although SiPAP/BiPAP has a set rate with alternating upper and lower pressures, it typically does not provide ventilation in terms of a set respiratory rate or tidal volume. Rather, it provides varying levels of NCPAP, with the patient breathing spontaneously above these NCPAP levels. There is conflicting evidence about whether BiPAP can increase CO_2 elimination and oxygenation compared with NCPAP.[55,56] There is also conflicting evidence about whether BiPAP improves respiratory outcomes. There is some evidence that synchronized BiPAP (done using a Graseby capsule, described below) decreases the duration of respiratory support compared to NCPAP.[57] However, un-synchronized BiPAP does not appear to be superior to NCPAP in sustaining extubation.[58]

Noninvasive Ventilation

The use of a nasal interface, rather than an endotracheal tube, to provide ventilatory support dates to the early days of neonatal mechanical ventilation and remains an active area of research.[36,59] It is an attractive option because it may provide a higher level of support than NCPAP while avoiding the potential complications of an endotracheal tube. It is particularly attractive for less mature infants whose respiratory status is compromised by a combination of RDS, immature and collapsible chest wall, decreased respiratory drive and apnea, and decreased respiratory muscle strength and endurance.

The nomenclature of NIV can be confusing, reflecting the fact that there are multiple ways to apply a mechanical ventilatory breath to a nasal interface. The acronyms NIMV (nasal intermittent mandatory ventilation or noninvasive mechanical ventilation) and NIPPV (nasal intermittent positive pressure ventilation) are sometimes used interchangeably with NIV.

In preterm infants, the cycling positive pressure of NIV at the upper airway produces an intermittent respiratory stimulus which could decrease central apnea. In addition to this stimulatory effect, it is possible that NIV also decreases apnea by facilitating lung recruitment and decreasing the work of breathing. At least one study has suggested that unsynchronized NIV can significantly reduce apnea.[60] Compared to NCPAP, synchronized NIV appears to decrease the work of breathing in infants with RDS.[61–63]

Clinical trials comparing NIV to NCPAP for prevention of extubation failure have shown mixed results, probably because some studies have used an unsynchronized form of NIV and others have looked at synchronized NIV. Synchronized NIV has been shown in small studies to reduce extubation failure compared to NCPAP.[64–67] However, the largest trial of NIV vs NCPAP combined synchronized and non-synchronized NIV modes and showed only a marginal decrease in the rate of post-extubation failure.[68] Recent meta-analysis suggests that NIV may be superior to NCPAP for preventing extubation failure, but does not reduce BPD or death, and that synchronization may be important for the successful application of NIV.[69,70]

NIV, both synchronized and unsynchronized, has also been used in early RDS to decrease the need for intubation. Synchronized NIV appears to be superior to NCPAP in reducing the need for intubation.[71] The data from trials comparing unsynchronized NIV to NCPAP are mixed, with some showing a decrease in the need for intubation,[72,73] and others showing no difference.[74,75]

The practical use of NIV is limited by several important factors. The nose and upper airway act as a filter so that pressures set at the ventilator are attenuated at the alveolar level. In essence, what is delivered to the lung may be significantly less than what is set at the ventilator and cannot be easily monitored. With unsynchronized NIV breaths delivered through small nasal cannula prongs (e.g., RAM cannula), there appears to be no significant transmission of tidal volume to the infant, suggesting that the main effect of NIV may be a simple increase in mean airway pressure.[76]

The simplest form of NIV is conventional time-cycled pressure-limited ventilation that uses the same circuits and gas-conditioning devices used for invasive ventilation. This approach, sometimes termed NIMV (nasal intermittent mandatory ventilation) has the advantage of simplicity, but the lack of synchrony with the infant makes it a poor form of support. The non-synchronized breaths are frequently delivered as the infant is attempting to exhale, leading to increased work of breathing and inefficient pressure delivery.

Synchronization of NIV is limited by the synchronization method. Many of the older studies of synchronized NIV used a small pressure sensor (Graseby pressure capsule) which was placed on the abdomen to detect abdominal movement at the onset of inspiration. However, this technique is not available with commercially produced ventilators in the US. Other approaches to synchronizing NIV which rely on flow sensing are not ideal because of the problems with leaking around the nasal interface leading to undetected breaths and/or "auto-cycling" of the ventilator.

Noninvasive Ventilation and Neurally Adjusted Ventilatory Assist

Neurally adjusted ventilatory assist (NAVA) is a mode by which the ventilator pressure is adjusted in proportion to the electrical activity of the diaphragm, as measured by electrodes embedded within a special nasogastric or orogastric tube. Although used for both invasive and noninvasive ventilation, NAVA is a particularly attractive way to provide synchronized NIV. With NAVA, the triggering of breaths is very sensitive to spontaneous inspiratory effort and is not affected by the leaks that make flow-synchronized NIV problematic. It also has the advantage of adjusting the size of the mechanical breath to the infant's respiratory effort. In clinical trials, NAVA appears to be an effective technique for synchronizing both invasive and noninvasive support.[77–83]

Noninvasive High-Frequency Ventilation

Several investigators have explored delivering high-frequency oscillatory ventilation (HFOV) through a nasal interface.[84] It is unclear whether nasal HFOV is superior to NCPAP in terms of CO_2 exchange.[85,86] At least one study suggests that it is not superior to NIV in reducing extubation failure, but is associated with less feeding intolerance.[87] A review of European NICU practices suggests that this technique has been used in a number of European NICUs.[88] In the US, it remains an intriguing investigational technique.

Nasal Interface

With any form of NIV, the nasal interface employed is usually short prongs or a nasal mask. NCPAP prongs are attached to tubing that provides both an inspiratory and expiratory limb. Prongs should fit snugly into the nares without blanching the surrounding tissue. If a nasal cannula is used, there is no expiratory limb and the prongs used must be smaller than those used with NCPAP to provide for exhalation around the nasal prongs. The cannula thus delivers significantly less pressure than is measured at the ventilator.[76] The risks of nasal damage and obstruction sometimes observed during the use of NCPAP are also present with NIV. Proper application and maintenance of the nasal interface and avoidance of excessive force on the nasal septum are important to avoid these complications.

Complications of Noninvasive Support

The complications of NCPAP or NIV are related to the gas pressure that is applied to the alveoli and to direct contact of the interface with the nose. With any of the noninvasive modalities that provide positive pressure, there is the risk of providing too much pressure and over-inflating the lung, both increasing the work of breathing and potentially causing both acute and chronic lung damage. Excessive pressure can also reduce venous return, increase pulmonary vascular resistance, and reduce cardiac output.

If nasal prongs are too large or are applied with too much pressure over the nasal septum, they can produce erosions or pressure necrosis that sometimes requires the interruption of NCPAP or NIV. Avoiding these complications while keeping the nasal prongs in place is a task that requires careful observation and skill.

During the application of NCPAP or NIV, there is also a chance of gas being pushed into the stomach. A nasogastric catheter is often used to avoid excessive gas accumulation and consequent gastric distention.

Choice of Noninvasive Support

Noninvasive support can be useful in preventing progressive atelectasis and respiratory decompensation, both early in the neonatal course and following extubation.[36] While NCPAP is the standard against which other techniques are measured, there may be advantages to HFNC and/or synchronized NIV in selected patients. HFNC is an attractive way of providing support because it is less likely to cause nasal injury than NCPAP. However, it has the significant disadvantage of not providing easily measured and adjusted levels of NCPAP, so it is probably most useful in post-extubation infants, and as a primary form of support for infants who are at relatively little risk of needing intubation. Synchronized NIV may offer advantages over NCPAP or un-synchronized NIV in preventing intubation and reducing extubation failure, although data are not yet available on its impact on long-term outcomes such as mortality or BPD.

HFNC and various forms of NIV are likely to become a larger part of NICU respiratory support as neonatal caregivers continue to search for the best ways to support infants with non-invasive techniques. As with most techniques in the NICU, well-designed protocols, rigorous training of staff, and meticulous attention to detail may well be as important, or more important, than the specific modality employed.

Weaning From Noninvasive Support

There are multiple approaches to weaning a baby from noninvasive support with supplemental oxygen to being unsupported in room air. While the most conservative approach would be a gradual progression from NIV to NCPAP to HFNC to NC to no support, this approach is probably not necessary for all infants. An argument can be made that preterm infants, with their compliant chest walls and tendency to atelectasis, should remain on some form of positive pressure support as long as they have any ventilation/perfusion mismatch and need for supplemental oxygen. With this strategy, infants would remain on NIV, NCPAP, or HFNC until $F_IO_2 = 0.21$, then be transitioned to room air without support.

There is relatively little published data to guide the decision of when to wean from positive pressure (HFNC, NCPAP, NIV) to supplemental oxygen via low-flow NC, or to room air. One randomized study examined strategies for managing infants who were stable on NCPAP 4 to 6 cmH_2O with F_IO_2 less than 0.25, and concluded the optimal strategy was to discontinue NCPAP, but resume it for at least 48 hours if the infant failed the transition, with failure defined as increased work of breathing, increased apnea/bradycardia, F_IO_2 greater than 0.25, or PCO_2 greater than 65.[89] However, a recent study suggested that continuing NCPAP for 2 weeks beyond the point where infants who were stable on NCPAP 4 to 6 cmH_2O and F_IO_2 less than 0.25 resulted in significantly improved FRC through hospital discharge.[90] This would suggest that the possible advantages of improving lung volume should be carefully weighed against the difficulty of continuing NCPAP in a maturing infant.

There are also multiple approaches to weaning from NCPAP, including "sprints" on and off NCPAP, a gradual decrease in NCPAP pressure, and abrupt discontinuation of NCPAP. All of these approaches work, and there does not appear to be a major impact of strategy on the outcome.[91–93] A strategy of abrupt discontinuation of NCPAP may lead to earlier discontinuation than a strategy of gradual weaning.[94]

Regardless of the strategy used in transitioning an infant from noninvasive positive pressure support, it is essential to observe the infant closely for changes in gas exchange and work of breathing.

Invasive Mechanical Ventilation

For some infants, supplemental oxygen and the positive pressure of noninvasive support are not sufficient to compensate for the degree of lung disease. For these infants, invasive (via an endotracheal tube) ventilation is required. Mechanical ventilation is designed to achieve two things: oxygenation (via optimal mean airway pressure) and CO_2 removal (via minute ventilation) while causing minimal lung injury. This can be accomplished with multiple types of "conventional" ventilators which mimic normal tidal breathing, and with high-frequency ventilators which cause very efficient mixing of gas between the alveoli and the airway without tidal breathing.

Intubation

Neonatal intubation is a technically challenging procedure that requires significant time to master, often requires multiple attempts, and is associated with a significant risk of complications.[95,96] Although usually a quick procedure when performed by a skilled practitioner, it is still uncomfortable for the infant and associated with significant autonomic changes, including blood pressure, oxygenation, and cerebral perfusion pressure. Both the US and Canadian recommendations for all non-emergent intubations include the use of analgesia or a combination of analgesia and muscle relaxation; simple sedation is not sufficient.[97,98] Even skilled practitioners find that paralysis increases success and decreases the potential trauma of intubation. For this reason, some centers perform all elective intubations, including for surfactant administration, after what is essentially a rapid sequence anesthesia induction. In this case, infants need to be supported until they are fully recovered from the medications, usually within 20 to 60 minutes.

Although not directly addressed in the US and Canadian guidelines, the recommendation for analgesia with each intubation could be extrapolated to other laryngoscopic procedures such as surfactant instillation with intubate/surfactant/extubate (INSURE) or less invasive surfactant administration (LISA) techniques. The discomfort and attendant physiologic changes of intubation are largely associated with the laryngoscopy and stimulation of the laryngeal area, not just the placement of an endotracheal tube (ETT). However, providing adequate levels of analgesia for INSURE or LISA decrease the ability to immediately transition to noninvasive support following the surfactant instillation.

A significant advance in intubation techniques is the introduction of more sophisticated laryngoscopes into the NICU. These allow visualization of the upper airway with a small camera at the tip of the laryngoscope and allow members of the team other than the one performing the intubation to see the anatomy and the procedure. This is useful both for teaching and for instrumentation of the difficult airway.

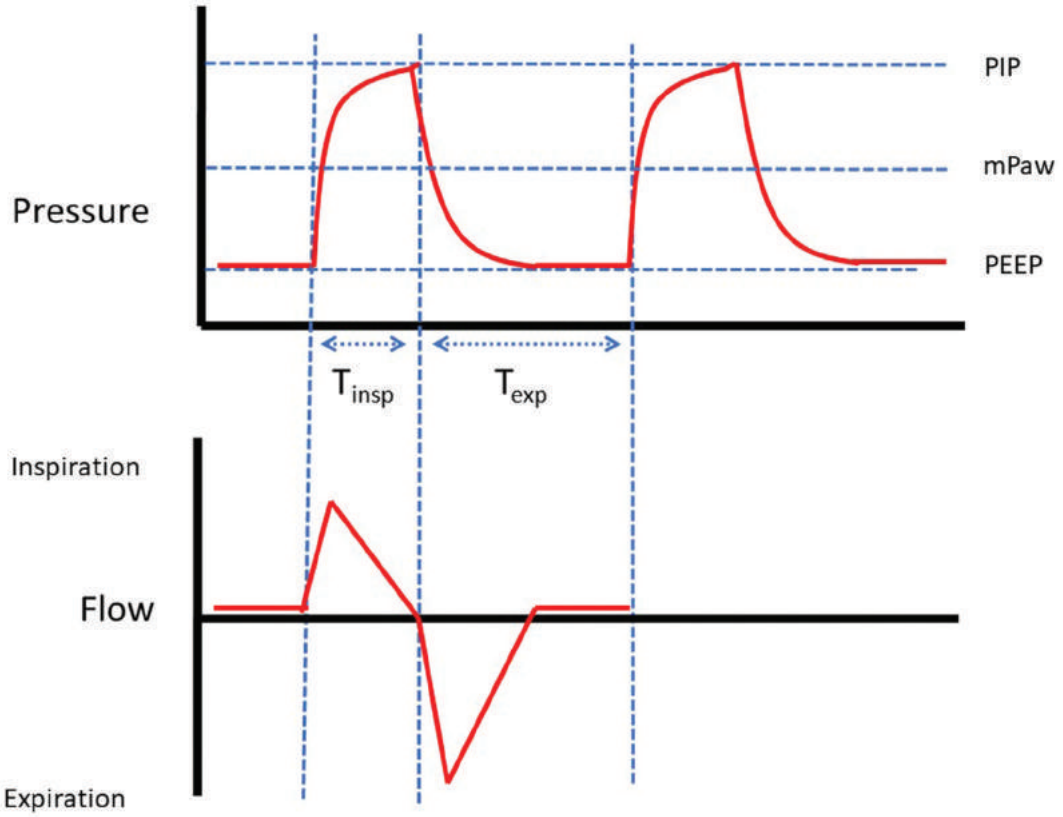
Conventional Ventilation

Despite the wide range of ventilator devices and strategies available, the fundamental physiology of mechanical ventilation follows a few simple rules. Most neonatal lung diseases are characterized by atelectasis, so providing optimal pressure to counteract surface forces is paramount for optimizing both oxygenation and ventilation. Basic principles and guidelines include the following:

- Oxygenation is impaired by the ventilation/perfusion mismatch which results from atelectasis. Assuming the lung is not over-distended, increasing mean airway pressure (sometimes abbreviated MAP, but more appropriately mPaw) will decrease atelectasis and improve oxygenation.
- Oxygenation can also be impaired by an over-inflated lung which reduces venous return to the heart.
- CO_2 exchange is impaired by inadequate minute ventilation which results from inadequate tidal volume and/or inadequate respiratory rate. Assuming adequate inspiratory and expiratory times (T_{insp} and T_{exp}), increasing minute ventilation will increase CO_2 exchange. For most infants with acute lung disease a V_T of approximately 5 mL/kg is appropriate.
- Synchrony between the infant and the ventilator will decrease the work of breathing and make both patient breaths and mechanical breaths more efficient. Synchronizing the ventilator with the patient includes matching the onset of the ventilator breath with patient inspiration, as well as matching the duration of the ventilator breath (T_{insp}) with the onset of patient exhalation. In lung diseases that are restrictive and without a significant obstructive component (e.g., RDS) the lung time constant ($R \times C_L$) is small, meaning there is rapid transmission of pressure from the upper airway to the alveolus, so short T_{insp} and short T_{exp} are effective. In small infants with significant RDS, T_{insp} as low as 0.2 to 0.3 seconds may be effective.

There are multiple neonatal ventilators on the market, each with multiple modes of ventilation, and each of these modes has multiple parameters which can be adjusted. To make matters more confusing, some modes have similar names but different mechanisms of action, or different names but similar modes of action. Although this leads to what seems like an unlimited number of choices about how to best ventilate an infant, these choices can be simplified by understanding the basic physiology and nomenclature of modern ventilation.[99,100] Regardless of the ventilator brand or mode of ventilation, it is helpful to think of a ventilator breath as characterized by several components which can be used to describe any conventional tidal mechanical breath (Fig. 40.1). Ventilator breaths can be described by the following:

- Onset of the breath, termed the *trigger*. Breaths that are initiated because a clock in the ventilator has determined it is time to deliver a breath are termed *time-triggered* breaths. Breaths that are initiated by the patient are termed *patient-triggered* breaths.
- Size of the breath, termed the *limit*. Historically most adult ventilators delivered a set volume with each breath (*volume-limited* breath) and most neonatal ventilators delivered a set inspiratory pressure with each breath (*pressure-limited* breath). More sophisticated neonatal ventilators can deliver both volume- and pressure-limited breaths. In addition, with some ventilator modes, the size of the breath is determined by constantly adjusting combinations of volume, pressure, and flow. There is good evidence that this technique of *volume-targeted* ventilation, compared to pressure-limited or volume-limited ventilation, reduces death, intraventricular hemorrhage (IVH), and BPD.[101]
- Duration of the breath or when the breath ends, termed the *cycle*. A breath that lasts for a fixed inspiratory time (T_{insp}) is termed *time-cycled*. A breath that is terminated when inspiratory flow drops below a certain point is termed *flow-cycled*. Because the decrease in inspiratory flow is dependent on lung



• **Fig. 40.1** Simplified Diagram of Pressure and Flow Versus Time With Time-Cycled Pressure-Limited (TCPL) Ventilation. T_{insp} begins when the breath is triggered, with flow rapidly increasing to maximum, then decreasing as pressure increases to peak inspiratory pressure *(PIP)*. At the end of inspiration, the breath cycles off and the patient is allowed to exhale. Early exhalation is typically at high flow, then drops as the lung approaches FRC which is maintained by positive end expiratory pressure *(PEEP)*. Note that mean airway pressure *(mPaw)* is influenced by every component of the pressure wave, including PEEP, PIP, T_{insp}, inspiratory and expiratory flow pattern, and rate.

compliance and respiratory effort, the duration of flow-cycled breaths is partially controlled by the infant.

- Inspiratory flow pattern which is either a fixed inspiratory flow *(constant-flow)* or can vary with patient demand *(demand-flow)*. With constant flow ventilators, there is the possibility that the flow is not sufficient to meet the patient's need during early inspiration, resulting in "flow starvation" which increases work of breathing and patient discomfort.

Below are some of the more commonly used neonatal ventilation modes, presented in the general order of development and clinical adoption.

Time-Cycled Pressure-Limited Intermittent Mandatory Ventilation

Time-cycled pressure-limited intermittent mandatory ventilation (TCPL IMV) is sometimes referred to as TCPL, IMV, or IPPV (intermittent positive pressure ventilation). It is the oldest form of neonatal mechanical ventilation, and is conceptually very simple, although of limited utility in most modern NICUs. Characteristics of TCPL IMV include:

- The size of the breath is pressure-limited, as the ventilator delivers a peak inspiratory pressure (PIP), then returns to end-expiratory pressure (PEEP). In general, adjusting PEEP affects FRC, and adjusting the driving pressure (PIP – PEEP) affects the tidal volume.
- The breaths are at a fixed rate and time-triggered—for example, at a rate of 30/min, a breath occurs every 2 seconds regardless of the infant's respiratory effort.
- The breaths are time-cycled so inspiration lasts a fixed period of time (T_{insp}) regardless of whether the infant is trying to inhale or exhale at the end of the ventilator breath.
- Bias gas flow is fixed at a set flow rate (L/min), which means there is a set maximal inspiratory flow rate and a constant flow of bias gas in the expiratory limb of the ventilator circuit.

The advantage of TCPL IMV is mechanical and conceptual simplicity, and it works well for paralyzed or heavily sedated patients for whom the ventilator is doing all the work of breathing. However, it is a difficult mode to use effectively in a spontaneously breathing patient. If the patient is trying to breathe spontaneously, the ventilator onset of inspiration and duration of inspiration do not synchronize with the patient's efforts. Not only are the baby's inspiratory efforts not supported but, more importantly, the baby may be trying to exhale while the ventilator is delivering a breath. In addition, the fixed flow of the gas on the inspiratory side of the circuit can be a limitation if the patient (particularly a large, strong infant) has a higher inspiratory flow

demand than the ventilator provides. For these reasons, TCPL IMV is rarely used except in anesthetized or paralyzed patients with no respiratory drive.

Synchronized Intermittent Mandatory Ventilation

The development of accurate flow sensors led to neonatal ventilators which can deliver breaths in synchrony with the patient's respiratory effort. The first of these modes to be developed was synchronized intermittent mandatory ventilation (SIMV). With SIMV, there is a fixed rate or number of breaths per minute (the "mandatory" part of the name) which last for a fixed duration (T_{insp}). However, the timing of these breaths coincides with at least some of the patient's breaths. For example, at a set SIMV rate of 30, if the baby is breathing 50 times a minute, the ventilator will sense the baby's respiratory rate and try to deliver 30 breaths that coincide with the onset of the baby's breath, rather than delivering a breath exactly every 2 seconds. The baby's other 20 breaths will be unsupported, with the baby inhaling from the bias gas flow which is maintaining PEEP. SIMV, which does not support all breaths, has been largely replaced by modes such as SIMV/PS or A/C which can support all breaths.[102] Characteristics of SIMV include:

- Size of the breath can be either volume-limited or pressure-limited.
- The breaths are delivered at a set rate, but the ventilator tries to synchronize these breaths with the patient's onset of inspiration. If the baby is apneic, the ventilator defaults to delivering time-triggered breaths at the set rate.
- Depending on the ventilator and the mode, breaths can be either time-cycled (fixed T_{insp}) or flow-cycled (duration of the breath varies according to the patient's inspiratory flow pattern). In general, if it is not specified as "SIMV with flow cycle," SIMV refers to time-cycled breaths with a fixed T_{insp}.
- One of the advantages of synchronizing the ventilator breath with the patient's breath is that a shorter T_{insp} is usually effective in delivering an adequate tidal volume since the ventilator is not "fighting" the baby to deliver the breath. Lower PIP and slightly shorter T_{insp} are often sufficient because the infant's inspiratory effort contributes to the generation of the V_T.

Assist/Control Ventilation

A logical extrapolation from SIMV, which supports some patient breaths, is a mode that supports all patient breaths. Sometimes referred to as "patient triggered ventilation" (PTV) in early studies, A/C ventilation supports every spontaneous patient inspiration with an *assisted* synchronous ventilator breath. Characteristics of A/C include:

- The size of the breath can be either volume-limited or pressure-limited, or in some ventilators, limited or targeted by a sophisticated combination of volume, pressure, and flow.
- The breaths are patient-triggered so the rate is set by the infant, as the ventilator delivers a breath with the onset of each inspiration. A backup rate is set so the ventilator will deliver a minimum number of breaths if the infant becomes apneic or does not trigger the ventilator.
- The breaths may be time-cycled (T_{insp} is set) or flow-cycled (T_{insp} is set by the patient's inspiratory flow).

The advantages of A/C ventilation include a reduction in spontaneous breathing effort relative to SIMV because A/C ventilation assists every inspiration.[103] Assisting every spontaneous inspiration in A/C mode ventilation appears to prevent diaphragmatic fatigue and can reduce the duration of weaning and ventilator dependency compared with IMV.[104,105]

One of the potential problems with A/C ventilation is *autocycling*. Successful A/C ventilation, if triggered by respiratory flow, depends on sensitive and accurate detection of when the infant has initiated a breath. If there is a leak around the endotracheal tube, the ventilator may be unable to distinguish this leak from the patient inspiratory effort and will deliver ventilator breaths at the maximal rate. Although there are ways to offset the effect of endotracheal tube leak ("leak compensation") careful observation of the infant and close attention to ventilator graphics is required to avoid autocycling. One of the clues that a ventilator in A/C mode is autocycling is a fixed respiratory rate at the minimum allowed T_{insp}. This is not a problem with A/C modes where the breaths are triggered by NAVA.

Pressure Support Ventilation

Pressure support ventilation (PSV) was originally developed as an adjunct to volume-limited SIMV in adult ventilators during weaning. As the rate of volume-limited breaths decreases, patients have spontaneous breaths between the mechanically supported breaths. The work of breathing for these unsupported breaths is increased because of the effort required to overcome the resistance of the ETT. Thus PSV *supports* these breaths with a small amount of *pressure* to compensate for the resistive load imposed by the ETT. That lead to the concept that PSV breaths should be small breaths between the larger breaths provided by SIMV.

Following are characteristics of PSV breaths:

- The breaths are patient-triggered. Each non-SIMV patient breath is supported with a PSV breath. PSV breaths are never delivered without patient inspiratory effort (i.e., there is no time-triggered PSV), so there is no "backup" or minimum PSV rate.
- The breaths are pressure-limited. There are modes similar to PSV where the breaths are volume-limited, but these are more correctly termed volume-support ventilation (VSV).
- The breaths are flow-cycled, so T_{insp} is determined by the infant's respiratory pattern.
- Because PSV does not have a set backup rate and will not support an apneic infant, it should be coupled with another form of ventilation with a set rate, typically SIMV.

In infants, when PSV is used as an adjunct to SIMV, it can reduce the spontaneous breathing effort compared with SIMV alone, even with PSV levels much lower than the peak pressures of the SIMV breaths.[106–108] More importantly, by reducing the need for high SIMV rates, PSV can facilitate weaning in comparison with SIMV alone.[109]

Although usually thought of as small breaths supporting the patient between larger SIMV breaths, PSV breaths can be set to approximate the size of the SIMV breaths they are between, in effect providing something like A/C.

Volume-Targeted Ventilation

Volume-targeted ventilation is a hybrid form of ventilation, combining pressure-limited and volume-limited features into a mode that automatically adjusts the peak positive pressure or the duration of the ventilator breath to maintain V_T within a narrow, targeted range. This requires accurate measurement of delivered tidal volume with each breath and breath-by-breath adjustment of inspiratory pressure and/or T_{insp}. While different ventilator models have different ways of providing (and names for) volume-targeted ventilation, they all have the advantage of providing consistent V_T to the infant. Volume-targeted breaths can be delivered in multiple triggering modes, including SIMV, PSV, and A/C.

Volume-targeted ventilation modes have been compared to pressure-limited modes in at least 20 clinical trials, involving over 1000 patients. Although many of these were small studies or had methodological problems, a recent Cochrane meta-analysis suggests that volume-targeted ventilation is superior to pressure-limited modes in terms of rates of death or BPD, pneumothoraces, severe IVH, and duration of ventilation.[101]

Choice of Conventional Ventilator Modes

Despite the wide variety of neonatal ventilators, each with multiple modes, and each with purported advantages, most babies can be managed with any modern neonatal ventilator and a few ventilator modes. Regardless of the device or the mode, several guidelines greatly simplify the management of infants supported with conventional ventilation:

- V_T should be maintained within a narrow range, typically around 5 mL/kg, to maximize the efficiency of ventilation and minimize the risk of pressure and volume-related over-distension injury. Some infants who seem to do well with V_T are slightly less than this. Very small infants may require V_T as large as 6 mL/kg as the in-line sensor will contribute to the dead space of the circuit. Infants with BPD and significantly increased dead space, as well as overly distensible airways and alveoli, may also need slightly larger V_T.
- Although pressure-limited and volume-limited modes can deliver fairly consistent V_T, changes in patient status including lung compliance and ETT leak can significantly influence delivered V_T. For this reason, volume-targeted modes seem to be the most efficient way to achieve consistent V_T delivery.
- mPaw should be optimized to avoid atelectasis and over-distension, both of which can be evaluated with a combination of ventilator graphics, chest radiograph, and blood gases. Adequate PEEP is an important component of optimizing mPaw and minimizing atelectasis.
- Ventilator support should be adjusted to maximize synchrony with the patient. This includes the timing of the onset of the ventilator breath and the duration of the ventilator breath.
- Modes which support all spontaneous breaths are preferable to modes that support only some breaths. This assures consistent V_T, with the assumption that consistent V_T minimizes atelectasis and over-distension, is more comfortable for the patient and is simpler to adjust. Although this can be approximated with SIMV/PSV, it is more efficient to use an A/C mode.
- Patient work of breathing should be "just enough." A patient who is not doing any respiratory work is at risk of losing respiratory muscle strength, while a patient who is struggling on the ventilator is probably inadequately (or inappropriately) supported. Unfortunately, there is no clear measure of acceptable or ideal work of breathing, and the judgement of "just enough work of breathing" varies by institution and caregiver.
- Provide adequate inspiratory flow so the ventilator can always meet the patient's inspiratory flow demand and avoid "flow starvation." This is particularly important in large, vigorous infants who can "demand" very high flows during early inspiration. Inadequate inspiratory flow decreases ventilator efficiency, increases work of breathing, and is uncomfortable.
- All breaths should be monitored with graphics that give information about pressure, volume, and flow.
- Staff should be well versed in the ventilator mode(s) used. It is better to use a few modes which are understood by all than to use multiple modes which are poorly understood.

High-Frequency Ventilation

High-frequency ventilation (HFV) accomplishes gas exchange by fundamentally different mechanisms than conventional ventilation. While conventional ventilation depends on the bulk flow of gas moving into and out of the alveoli, HFV depends on a highly efficient mixing of gas between the upper airway and alveoli. Because of this, HFV can provide oxygenation and ventilation without ever allowing the lung to collapse to FRC or expand to the volume of a full tidal breath. Avoiding the large pressure and volume swings that are seen with conventional tidal ventilation potentially protects the fragile lung from damage secondary to both atelectasis and over-distension.

The fundamental relationships between HFV settings and gas exchange can be summarized as follows:

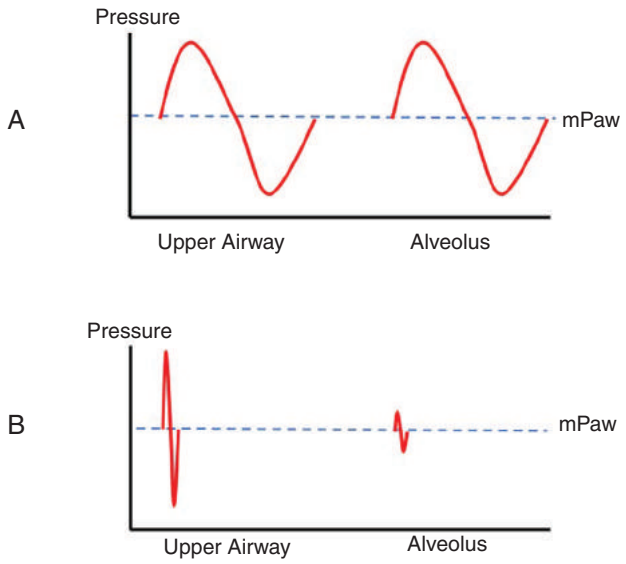
- Oxygenation is proportional to mean airway pressure (mPaw), similar to conventional ventilation.
- Ventilation (CO_2 exchange) is proportional to $F \times V_T^2$. The "V_T" in HFV is far smaller than the V_T in conventional ventilation and is typically less than dead space volume, so technically not a true tidal volume. With some HFV devices, "V_T" is directly measured, while with others it is assumed to be proportional to pressure amplitude or "delta pressure."
- This can be simplified into two simple rules:
  - Adjusting mPaw changes lung inflation and oxygenation
  - Adjusting amplitude changes "chest wiggle" and ventilation

It is important to note that changes in HFV "V_T" or amplitude have an exponential effect on CO_2 exchange ($F \times V_T^2$), compared to conventional ventilation where ventilation and V_T have a linear relationship ($F \times V_T$).

Another important difference between HFV and conventional ventilation is the fact that the airways act as a *low-pass filter* that attenuates high-frequency signals. The pressure transmission to the distal airways is greatly influenced by the resistance of the ETT and the airway, so only a small fraction of high-frequency delta pressure generated by the ventilator is transmitted to the terminal air spaces. In an infant with RDS, a pressure wave at a relatively low frequency of 1 Hz (i.e., rate of 60 breaths/min) is typically transmitted to the alveoli without attenuation (i.e., low frequency is *passed* through the airways filter). In this case, the alveolar pressures are the same as the set PEEP and peak inspiratory pressure. However, at a high frequency of 10 Hz (600 breaths/min) the pressure wave will be markedly attenuated (i.e., filtered, or not passed), with the alveolar pressure changes being much smaller than the pressures delivered to the upper airway. For this reason, the amplitude measured by the ventilator during HFV represents a relative value rather than the real pressure excursion in the distal airways. Although the amplitude of the HFV pressure wave is attenuated between the upper airway and the lower airways, the mPaw at the distal airway is essentially the same as at the upper airway (Fig. 40.2).

One consequence of this low-pass filtering effect with HFV is that changes in frequency have a seemingly paradoxical effect on CO_2 elimination. At high frequencies, decreasing F leads to less attenuation of the pressure wave, and increased amplitude ("V_T") at the alveolar level. And because the effect of V_T on ventilation is exponential compared to the linear association of F and ventilation ($F \times V_T^2$) the decreased F, and resultant increased effective V_T, will lead to increased CO_2 elimination and chest movement.

During conventional ventilation, gas exchange occurs by the filling and emptying of the distal air spaces and alveoli with fresh



• **Fig. 40.2** Low-Pass Filter Effect. (A) At low frequencies, the pressure changes are fully transmitted ("passed") between the upper airway and the alveoli, so pressures measured in the distal lung are the same as those measured in the upper airway. (B) At high frequencies, the upper airways act as a filter so the pressures do not have time to equalize between the upper airway and the distal lung and the pressure wave is attenuated. Note that mPaw measured at the upper airway is the same as mPaw measured at the alveolar level regardless of filtering.

gas with each breath. In contrast, during HFV, the volume of fresh gas delivered by each cycle is very small and does not reach the most distal portions of the lung. The gas exchange which occurs is best thought of as highly efficient mixing and is due to a combination of different mechanisms.[110] These include bulk flow into the more proximal portions of the lung, enhanced mixing of gas within the conducting airways, and out-of-phase movement between different regions of the lung that have different time constants. There is also enhanced diffusion of gas in large and medium-sized airways due to asymmetric velocity profiles during inspiration and expiration. Finally, there is molecular diffusion in the more distal air spaces that moves the different gas molecules from areas of higher concentration to areas of lower concentration.

Because of the effectiveness of HFV in enhancing alveolar ventilation, it is easy to quickly drive P_aCO_2 to very low values. For this reason, it is important to monitor P_aCO_2 values closely, especially when HFV is initiated or when settings are changed. Because of the very short inspiratory and expiratory times, it is extremely important to maintain the airway as patent as possible, ensuring a correct position of the ETT. Any obstruction will produce a decrease in pressure transmission and in V_T and can lead to gas trapping.

As with conventional ventilation, it is critical to ensure proper humidification of the inspired gas to prevent airway damage that can be produced by the high velocity of gas injected into the airway.

Types of High-Frequency Ventilation

There are multiple ways to generate high-frequency "breaths" in the upper airway. High-frequency ventilators are generally divided into HFOV, high-frequency jet ventilators (HFJV), and other devices such as high-frequency flow percussive ventilators (HFPV). While they share many physiologic similarities, they also have important design differences which impact the way they accomplish gas exchange.

High-Frequency Oscillatory Ventilation

High-frequency oscillatory ventilators deliver a sinusoidal pressure wave to the upper airway. The key factor differentiating HFOV from other high-frequency devices is the active push-pull effect on the pressure in the airways, giving the pressure wave its typical sinusoidal form. Some ventilators combine HFOV options with tidal ventilator modes, and some include HFV "tidal volume" monitoring targeting, allowing automatic adjustment of amplitude.

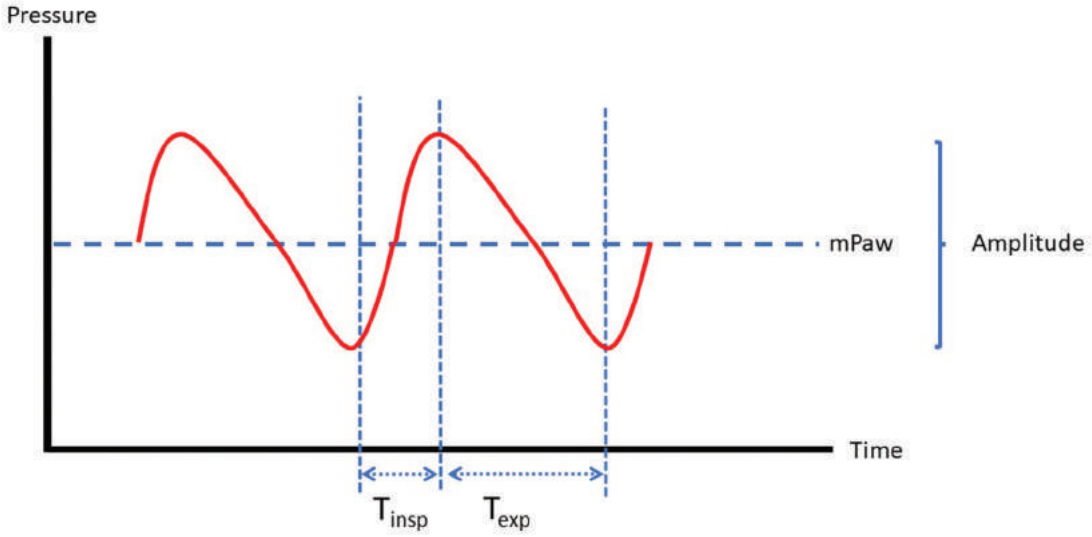
The sinusoidal pressure wave of HFOV generally has four main adjustable parameters:

- mPaw is directly adjustable, and is proportional to lung inflation, and therefore proportional to oxygenation. With sicker, less compliant lungs, a higher mPaw is used. Titration of mPaw is based on chest radiographic estimates of lung inflation as well as on patient oxygenation.
- Amplitude or "delta pressure" is the magnitude of the sinusoidal pressure wave and is an adjustable parameter. Because of the exponential relationship between amplitude and CO_2 exchange, small adjustments in delta pressure can have a marked effect on ventilation. The visible correlate of amplitude is the movement of the infant's chest wall, sometimes termed "chest wiggle." With increasing delta pressure, the amplitude of the chest movement increases.
- Frequency is directly set. Because of the low-pass filter effect of the upper airways, *decreasing frequency* will *increase the alveolar amplitude* (and "chest wiggle"), and will *increase CO_2 elimination*. Although F can be as high as 15 Hz (900 breaths/min), this may cause air trapping in all but the smallest and least compliant infants. More typically, an F of 10 to 12 Hz (600 to 720 breaths/min) is used in preterm infants with RDS. An F as low as 4 to 6 Hz may be used in larger infants or infants with diseases characterized by longer time constants (eg term infants with meconium aspiration). In most cases, an F is set for an infant, and is rarely adjusted; instead, amplitude (delta pressure) is used as the sole parameter adjusted to change ventilation.
- Inspiratory:Expiratory ratio. I:E ratio is typically not a pure sine wave, but instead has an "expiratory" phase longer than the "inspiratory" phase. This minimizes the gas trapping which can occur if the "expiratory" phase is too short. In most cases, I:E ratio is never adjusted (Fig. 40.3).

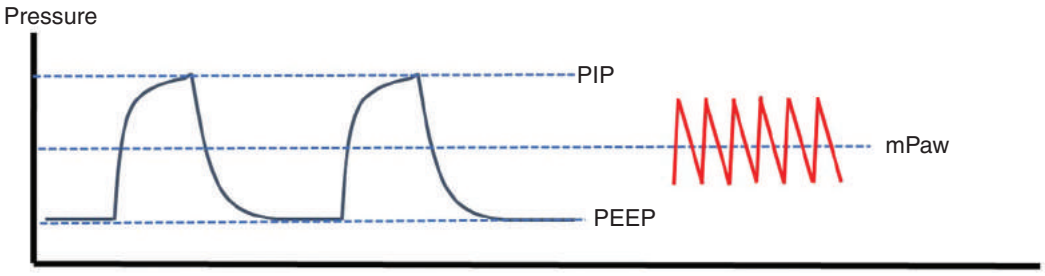
High-Frequency Jet Ventilation

Like HFOV, HFJV works by causing a highly efficient mixing of gas between the upper airway and the alveoli. Unlike the "push/pull" of HFOV oscillations, HFJV generates high-velocity gas pulses that are injected through a small-diameter opening into the airway connector. The high velocity of the gas injected into the airway produces a Venturi effect that pulls additional gas from the ventilator circuit, an effect termed *gas entrainment*. Expiration is passive, with the mixed gas diffusing back from the alveoli during the relatively longer "expiratory" phase. Like HFOV, HFJV is able to maintain a relatively high mPaw without the large alveolar pressure swings seen with conventional tidal ventilation (Fig. 40.4). Characteristics of HFJV include:

- Mean airway pressure is measured, not set directly. As with conventional ventilation, mPaw is primarily affected by PIP, PEEP, and rate.



• **Fig. 40.3** High-frequency oscillatory ventilation *(HFOV)* waveform produced by Sensormedics/Vyaire 3100. The diaphragm generates a sinusoidal push/pull, with a 1:2 inspiratory:expiratory time. mPaw and Amplitude (delta P) are adjusted independently to give separate control over oxygenation and ventilation. The frequency (breaths/s or Hz) and inspiratory:expiratory ratio can also be adjusted, although are usually left fixed for a given patient.



• **Fig. 40.4** Simplified comparison of pressure delivered to the upper airway with conventional tidal ventilation and high-frequency jet ventilators *(HFJV)*. HFJV can maintain a high mPaw without the large pressure swings between peak end-expiratory pressure *(PEEP)* and peak inspiratory pressure *(PIP)* that are seen with conventional tidal ventilation. As with high-frequency oscillatory ventilation *(HFOV)*, the HFJV pressure amplitude is attenuated at the lower airway and alveolar level while mPaw is maintained.

- Amplitude (delta pressure) is measured and, as with conventional ventilation, is the difference between PEEP and PIP.
- F is adjusted directly and is typically 7 Hz (420 breaths/min) for most small infants. For larger infants with longer time constants, or in infants with air leak, F is sometimes decreased to 4 to 6 Hz (240 to 360 breaths/min).
- T_{insp} is usually 0.02 sec and rarely adjusted for most small infants. With larger infants, T_{insp} may be increased as F is decreased.
- Conventional, tidal volume breaths can be used in conjunction with HFJV. The main role of these tidal breaths is as "sigh" breaths that help to treat atelectasis, usually at a rate of only 2 to 5/min. Compared to the HFJV breaths, these conventional breaths provide some alveolar recruitment but are a relatively small part of the ventilation. Most babies can be managed on HFJV without tidal breaths.

High-Frequency Percussive Ventilation

HFPV is a pressure-limited, time-cycled high-frequency mode that uses a flow-interruption device to deliver percussive pulses to the upper airway. It has been studied in multiple patient populations ranging from preterm infants to adults and is used by some US centers to provide HFV on transport because of its small size and simple operation.

Clinical Use of High-Frequency Ventilation

The indications for HFV differ widely between centers. One of the reasons for this variation is that most of the key HFV clinical trials were carried out before modern synchronized and volume-targeted ventilation strategies were developed. It is difficult to extrapolate from these studies to current care and draw firm conclusions about the superiority or inferiority of HFV versus conventional ventilation. Some studies of HFOV versus tidal ventilation have shown better outcomes, such as increased survival with no BPD.[111,112] However, others have not shown differences.[113,114]

A recent meta-analysis of 19 HFOV trials concluded that there may be a small reduction in the risk of chronic lung disease with HFOV, but that the evidence is relatively weak.[115] A recent

meta-analysis of HFJV trials found only one which met their criteria for inclusion in the review.[116] In this cross-over design trial, the secondary analysis showed that rescue treatment with HFJV was associated with improved survival.[117]

Most centers that use HFV use it as a rescue when conventional ventilation has failed, or for patients at high risk of lung damage from conventional ventilation where HFV offers theoretical advantages. This includes patients with severe restrictive lung disease where a high mPaw can be delivered without the high PIP seen with conventional ventilation. In patients with air leak syndromes (PIE, bronchopulmonary fistula), the short T_{insp} and long T_{exp} of HFJV "breaths" lead to less air leak from compliant airways than is seen with the longer T_{insp} of conventional tidal ventilation.[118] Because HFV is effective at providing ventilation with minimal tidal volumes, it also appears to offer some advantages over conventional ventilation in infants with hypoplastic lungs secondary to congenital diaphragmatic hernia or other prenatal conditions.[119] In addition, some centers use HFV as the initial and primary mode of ventilation in very low birth weight infants with a high risk for BPD because of the theoretical advantages of avoiding both atelectasis and over-distension in these infants.

Animal studies, human trials, and clinical experience suggest several broad generalities about HFV:

- HFV can safely and effectively support a wide range of patients. While there is limited data to support the superiority of HFV over conventional tidal ventilation, there is no doubt that it works well if used correctly.
- HFV is very effective in severe lung disease where decreased compliance leads to short regional time constants. As lungs improve, and time constants become longer, HFV has fewer theoretical advantages over conventional ventilation.
- There is a large overlap between HFOV and HFJV in the ability to support patients. While some centers use both types of HFV, others have found that they can manage patients successfully with only one type of HFV. Careful application of the correct ventilator strategy is usually more important than the device used.
- HFOV works particularly well for patients with uniform, non-compliant lungs, such as those with severe RDS. With HFOV, patients can be supported with a high mPaw to reverse or prevent atelectasis, without the high PIP that may be needed with conventional ventilation, and thus minimize the damage associated with over-distension.
- HFJV works particularly well for patients where air-trapping is a problem, such as treatment of PIE or broncho-pleural fistula. The ability to achieve adequate ventilation with very short HFJV Ti_{nsp} and long T_{exp} (I:E ratio of 1:12 at a rate of 240 breaths/min) leads to less air leak than with some forms of conventional ventilation and leads to better resolution of air leak that is already present. HFJV also works well with non-homogeneous severe lung disease, such as meconium aspiration, where the addition of sigh breaths can support patients without causing over-distension of long time-constant regions of the lung.
- The shorter the patient's time constant ($R \times C_{dyn}$), the higher the rate that can be used. For this reason, larger infants and infants with non-homogeneous lung disease are treated with lower F than smaller infants with more homogenous disease restrictive disease (where both R and C_{dyn} are low).

When to Initiate Invasive Mechanical Ventilation

Invasive mechanical ventilation, i.e., ventilation through an endotracheal tube, is typically reserved for patients with one or more of the following:

- Inadequate respiratory drive. Intractable apnea, sedation or analgesia, and underlying neuro-muscular compromise can all make infants incapable of sustaining spontaneous ventilation, or of being supported with noninvasive ventilation.
- Inadequate oxygenation. Atelectasis and ventilation/perfusion mismatch may be so severe that they cannot be reversed or prevented with CPAP or noninvasive ventilation. However, the degree of impaired oxygenation which can be tolerated and managed with noninvasive support varies widely depending on the infant's condition and center-specific guidelines.
- Inadequate ventilation. Atelectasis or other pulmonary conditions may compromise minute ventilation and effective CO_2 exchange despite noninvasive support. As with oxygenation, there is a wide range of what is considered acceptable, although most centers appear to agree that an infant with respiratory acidosis and a pH less than 7.20 to 7.25 will likely benefit from increased support. Note that the P_aCO_2 which is associated with this pH range is dependent upon whether the infant has acute or chronic, compensated hypercarbia.
- Unacceptable work of breathing. This is hard to characterize or quantitate, but most experienced clinicians have a sense of what they would consider unacceptable work of breathing, suggesting impending respiratory decompensation and failure. This is usually a complex mixture of the patient's gestational age, disease trajectory, degree of retractions and tachypnea, and degree of apnea or periodic breathing.

Weaning From Mechanical Ventilation

Although sometimes unavoidable, prolonged invasive mechanical ventilation is associated with significant complications, including laryngeal/tracheal injury leading to stenosis, over-distension, lung injury, loss of respiratory muscle strength and endurance, and BPD. For these reasons, consideration of weaning ventilatory support begins almost immediately after the initiation of invasive ventilation.

Weaning From High-Frequency Ventilation

In some centers, HFV is used for only the sickest infants, who are transitioned from HFV to conventional tidal ventilation when they are judged stable enough to be managed on "reasonable" conventional ventilation settings. In this case, the transition strategy usually entails approximately matching the HFV mPaw with the conventional mPaw and delivering an appropriate V_T, typically around 5 mL/kg.

However, many infants with resolving lung disease can be extubated directly from HFV to noninvasive support. Weaning infants from HFV, whether HFJV or HFOV, is essentially a process of decreasing the HFV amplitude until the infant is breathing spontaneously above the HFV. F_IO_2 should be weaned to approximately 0.4 or lower and mPaw should be weaned to 1 to 2 cmH_2O above the approximate level of CPAP or NIV that the infant will receive after extubation. If the amplitude has been weaned so that the infant is breathing spontaneously with reasonable estimated V_T, HFV can be thought of as providing "vibratory CPAP" rather

than real ventilation. At this point, patients can be extubated to NCPAP or NIV.

Weaning From Conventional Ventilation

Weaning from conventional mechanical ventilation is essentially the same as optimizing mechanical ventilation, and can be approached with several simple questions:

- Can the level of support (PIP in pressured-limited modes) be decreased without compromising delivered V_T, ventilation, oxygenation, or work of breathing?
- If in a SIMV mode, can the F be decreased without compromising ventilation or work of breathing?
- What is the expected time course of the need for support? Some babies can be weaned aggressively off ventilation in a period of hours to a few days, while others may require several weeks of ventilation, and not tolerate weaning attempts more than once a day, or even once every several days.
- With close observation of the baby's work of breathing, respiratory pattern, and V_T, as well as continuous S_pO_2 (and possibly $P_{TC}CO_2$) monitoring, weaning can frequently be done safely without blood gas measurements following each change.

There are no clear guidelines for the optimal time to extubate infants from invasive ventilation to noninvasive support. While extubation readiness tests have been described, a recent meta-analysis suggests that the evidence supporting their utility is not strong.[120] A number of investigators have looked at predicting successful extubation and found that, as expected, higher gestational age, lower level of support, and lower PCO_2 all predict successful extubation.[121–123] There is an online model for predicting extubation success of infants with less than 1250g birth weight based on data from 312 infants in a single NICU (www.extubation.net).[123]

There are several general principles for maximizing success at extubation:

- Most small preterm infants should be extubated to some form of noninvasive support, at least for a brief transition and observation period. There is some evidence that synchronized NIV is superior to NCPAP or HFNC, particularly in smaller infants.
- Caffeine is useful in preventing extubation failure in smaller infants.
- Larger infants or infants without lung disease (e.g., postoperative patients) can often be extubated directly to NC or to room air without support.
- A conservative approach is to wean infants to ventilator settings with a mPaw similar to that which can be provided with noninvasive support. Larger and less sick infants usually tolerate a decrease in mPaw following extubation.

Extubation Failure

Regardless of the strategy employed to optimize extubation, there will be some infants who fail and require re-intubation. In fact, there is a strong argument that if all infants are tolerating extubation, the criteria for extubation are too stringent. There are no published data on the optimal failure rate which balances aggressive extubation with minimizing the adverse effects of re-intubation.

There are several reasons an infant may fail extubation, and require re-institution of invasive support:

- Inadequate respiratory drive, resulting in intractable apnea. This usually results from a combination of respiratory center immaturity and increased work of breathing due to atelectasis or upper airway compromise.
- Atelectasis and ventilation/perfusion mismatch leading to increased work of breathing and increased need for supplemental oxygen.
- Upper airway compromise, either edema or stricture, from prolonged ETT presence, or tracheomalacia.

Inspired Gas Conditioning

During normal breathing, inspired gas is heated and humidified in the nasal passages and upper airways. In contrast to ambient air, medical gases are typically cold (≤15°C) and dry (<2% relative humidity). Exposure to dry and cold-inspired gas can produce inflammation of the airway epithelium, cause cold stress in preterm infants, increase the risk of airway damage, as well as adversely affect the mucociliary transport system. Even adults report discomfort from the drying effect of unconditioned NC gas.

Because of the relationship between temperature and the water carrying capacity of gasses, simply bubbling medical gas through unheated water will not adequately humidify the gas. The standard method for conditioning the inspired gases consists of a combined heater/humidifier device and heated breathing circuits.[124] Dry and cold medical gases are heated in the heater/humidifier chamber to 37°C and 100% relative humidity. The gas travels through the ventilator circuit, where it is heated to 39°C to prevent condensation. The gas temperature decreases as the gas travels through the ETT and gas is delivered at approximately 37°C and near 100% saturated at the distal end. Gas conditioning is recommended during HFNC, NCPAP, or nasal ventilation, as well as during invasive mechanical ventilation.

Other Respiratory Gases

In addition to supplemental O_2, several other respiratory gases can be used in the NICU.

Nitric Oxide

The discovery of iNO revolutionized the care of infants with pulmonary hypertension.[125,126] Nitric oxide is a potent endogenous signaling molecule with multiple actions, the most important of which, therapeutically, is its action as a pulmonary vasodilator. When administered as a respiratory gas, it crosses the alveolar/capillary space to work directly on the pulmonary vasculature. By stimulating the endothelial cGMP system, it causes Ca^{2+} mediated pulmonary arterial dilation. Because it is quickly and irreversibly bound to hemoglobin, iNO acts only at the pulmonary vascular level, with minimal systemic effects.

iNO is typically used in doses of 1 to 20 ppm. Delivery of iNO safely requires complex dedicated equipment, both because of the need for accurate delivery and monitoring of minute doses of iNO, and because it is a very reactive molecule that can rapidly combine with O_2 to form toxic radicals.

Nitrogen

Supplemental N_2 has been sometimes used to decrease the F_IO_2 to less than 0.21 for infants with congenital heart disease who

benefit from hypoxia-mediated pulmonary vasoconstriction. In these infants with single ventricle physiology (for example, hypoplastic left heart syndrome), it is important to maintain relatively high pulmonary vascular resistance to decrease the amount of blood that is shunted from the systemic to the pulmonary circulation.

Supplemental N_2 is mixed with room air to give an F_IO_2 of less than 0.21, typically in the range of 0.18 to 0.20. It is essential to have accurate monitoring of N_2 delivery rate and mixing, as well as the measurement of resultant F_IO_2 because an error in N_2 delivery could be disastrous.

Heliox

Although more commonly used in pediatric and adult ICUs, a mixture of helium and oxygen (Heliox) can be a useful tool for some infants with severe increased airway resistance, such as with severe BPD.[127] Helium is of lower density than N_2 so when mixed with O_2 it flows through narrow passages more readily because it has less turbulent flow than N_2 at the same concentration and flow rate. This will increase maximal gas flow and decrease the work of breathing. Heliox is usually an 80:20 mixture of He_2 and O_2 which can be blended with pure O_2, similar to how room air and O_2 are typically blended. However, the beneficial effects of Heliox are dependent on a relatively high concentration of He_2, so this therapy is ineffective in infants who require a high F_IO_2. This is one of the reasons this technique is not widely used in NICUs.

Pulmonary Drug Delivery

Direct pulmonary drug delivery is an important part of modern NICU care, including the delivery of liquid surfactant and the delivery of aerosolized drugs.

Surfactant

Surfactant replacement therapy is the best example of direct pulmonary delivery of a drug that is not a gas. Delivered below the vocal cords in one or more large boluses, it spreads rapidly and evenly over the alveolar surface. Interestingly, slow "drip" administration of surfactant leads to less even surfactant deposition.[128,129]

For many years, surfactant instillation was done by passing a thin catheter through an endotracheal tube so that it was positioned slightly above the carina. While highly effective, this technique requires the placement of an endotracheal tube that was typically left in for continued mechanical ventilation. This was followed by the development of the INSURE (INtubate, SURfactant, Extubate) technique that allowed effective surfactant delivery followed by rapid removal of the endotracheal tube. More recently, there has been interest in other techniques of surfactant instillation that can be accomplished without an endotracheal tube.[130,131] The use of a thin catheter, without placement of an endotracheal tube, is usually referred to as LISA (Less Invasive Surfactant Administration) and appears to be an effective approach to surfactant delivery. A recent meta-analysis suggests that LISA, compared to INSURE, is associated with a significantly lower rate of mechanical ventilation.[132] One significant limitation of both INSURE and LISA is that it must be performed in infants who are awake and vigorous so they do not require ventilatory support after completion of surfactant instillation. This makes administration of adequate analgesia prior to laryngoscopy impossible, a significant concern given recommendations to provide analgesia prior to laryngoscopy and intubation.[97,98]

Another approach to surfactant administration is through a laryngeal mask airway (LMA).[133] While the LMA approach is appealing in that it does not require laryngoscopy, it is limited by the size of commercially available LMA devices that do not extend to VLBW babies. LMA application of surfactant does not appear to be superior to INSURE in terms of neonatal outcomes.[132]

The twin desires of neonatologists to provide adequate surfactant and to avoid the potential complications of intubation have led to multiple attempts to aerosolize surfactant. Several studies have suggested that surfactant can be aerosolized and safely delivered to preterm infants.[134–137] However, the data on the efficacy of these techniques are limited. Further trials with more sophisticated aerosol delivery techniques are in progress.

There have been attempts to use surfactant as a vehicle for the administration of steroids, particularly budesonide, directly to the lung.[138] Because liquid surfactant spreads so well throughout the lungs, this is a theoretically good approach to achieving uniform, reliable pulmonary drug delivery. This approach is currently under evaluation in several large clinical trials and should still be considered experimental.

Aerosol Drug Delivery

Direct delivery of aerosolized drugs to the lungs is an attractive concept and is widely used in adult and pediatric intensive care units. However, the practical application of this technique for infants has been limited because the infant upper airway is superbly designed to act as a filter. If an aerosol is composed of particles that are too large, they are filtered out in the upper airway. And if aerosol particles are too small, they remain suspended in gas, do not condense at the alveolar level, and are exhaled. It appears that aerosols which contain particles of approximately 2 to 3 μm are most likely to be deposited at the alveolar surface.[139]

The amount of aerosolized drug which is deposited beyond the upper airway is significantly influenced by the route of administration. For drugs that are delivered to an infant on mechanical ventilation, the drug is often deposited in the ventilator circuit and/or endotracheal tube before it reaches the infant. Depending on where the aerosol is injected into the circuit, and the relationship between bias and inspiratory flow, much of the drug may be lost in the bias flow. For infants who are spontaneously breathing there is variation in the amount of drug which is actually entrained by the infant's inspiratory flow, as opposed to being lost to the surrounding room. Even with good delivery of the aerosol to the upper airway, there is likely to be variation in how much drug is deposited in the upper airway as opposed to reaching the small airways.

There are three commonly used techniques for generating aerosols in the NICU: jet nebulizers, vibrating mesh nebulizers, and metered-dose inhalers (MDIs):

- Jet nebulizers inject a high-pressure air source through a liquid reservoir, then through small holes which produce the aerosol. Jet nebulizers are simple and a good source for "blow-by" aerosol drug delivery for spontaneously breathing infants. However, the volume of gas needed to generate the aerosol makes mixing jet nebulization into the inspiratory limb of ventilator circuits problematic.
- Mesh nebulizers vibrate a thin mesh with holes of a specified diameter at a high rate, turning liquid into an aerosol. They can be designed to generate a specific particle size and have the advantage of not adding additional gas to a ventilator circuit.

- Metered dose inhalers use a small amount of propellant to generate a brief "puff" of aerosol. The amount of propellant is small, so it can easily be injected into a ventilator circuit. However, the fact that the "puff" is brief can cause issues for the synchronization of the aerosol delivery with inspiration.

Effective aerosol delivery to the distal air spaces is dependent on multiple factors, including:

- Particle size. Large particles (>3 μm) are more likely to be deposited in the upper airway.
- Where the aerosol is introduced to the respiratory gas. Options include "blow-by" to the spontaneously breathing infant in room air, and to the ventilator circuit of an infant on support. For ventilated infants, options further include the inspiratory limb of the circuit before the heater, after the heater, and close to the patient interface. The location is an important determinant of (1) whether there is adequate mixing of injected aerosol and bias flow gas, and (2) deposition of aerosol particles in the circuit before reaching the patient.
- For ventilated patients, the flow of bias gas through the circuit and the patient's inspiratory flow. If the infant's inspiratory flow is low compared to the bias gas flow, most of the aerosol may be lost in the bias flow.

Many drugs can be successfully aerosolized. However, the number of drugs for which aerosolization is an advantage in infants is limited. Most neonatal experience with aerosolized drugs is limited to bronchodilators, steroids, and mucolytics. Other categories such as antibiotics and prostacyclines, which have been used in older patients, should be considered investigational in infants.

Bronchodilators

Because severe BPD is characterized by airway obstruction, inhaled bronchodilators have long been used for the treatment of infants with BPD. Both beta agonist agents and anticholinergics have been studied and shown to improve lung mechanics in infants with BPD and significant airway obstruction.[140] Whether treatment with inhaled bronchodilators improves outcomes in BPD, as opposed to transient symptomatic improvement, is unclear.[141] There is no evidence that early administration of bronchodilators prevents BPD.[142] The role of inhaled bronchodilators in the management of infants with BPD appears to increase as infants mature from early preterm patients to older BPD patients.

Steroids

Steroids have a long history of use for the treatment of infants with evolving or established BPD. Because of the side effects of systemic steroids, there have been multiple attempts to find effective and safe steroids which could be delivered directly to the lungs. Inhaled steroids, similar to those used to treat asthma and other obstructive lung diseases in older patients, have been used with some success. Unfortunately, inhaled steroids have the same problems as inhaled bronchodilators in terms of variable deposition within the ventilator circuit and/or upper airway before reaching the distal airways. Like bronchodilators, there is likely some systemic effect from inhaled steroids which are either deposited in the upper airway or the distal smaller airways. Despite these variables, the studies of inhaled corticosteroids suggest that inhaled steroids have fewer systemic side effects than systemic corticosteroids, and can improve outcomes compared to placebo in some infants.[141] Like inhaled bronchodilators, the role of inhaled corticosteroids seems to increase as the infant with severe BPD matures into early childhood.

Mucolytics

Drugs that change the composition or structure of pulmonary mucous such as acetylcysteine or DNAase are attractive candidates for aerosolization. The theory is that they can facilitate the break-up of mucous plugs and reduce plug-related atelectasis. However, there are several problems with this in practice. Aerosolized drugs are preferentially distributed to open lung spaces, so are not concentrated where plugging occurs. Also, mucous which is too thin is, at least theoretically, also difficult for the airway cilia to transport, raising the question of how much mucolysis is too much. Finally, the degree to which improvement in atelectasis is due to mucolytic therapy vs changes in individual lung recruitment strategy vs disease progression is difficult to quantitate. Although there is some limited clinical experience with DNAse in infants, the data supporting its use are not compelling.[143,144] For these reasons, mucolytic therapy remains of limited use in most NICUs.

Extracorporeal Membrane Oxygenation

Extracorporeal membrane oxygenation (ECMO) is a technique for providing support to infants with severe parenchymal lung disease, severe pulmonary hypertension, or severe myocardial dysfunction.[145,146] It is essentially the adaptation of intra-operative cardio-pulmonary bypass technology to provide long-term support to patients in an ICU environment. When initially introduced into routine neonatal practice in the late 1980s and early 1990s, its primary role was in the management of infants with a high predicted mortality rate from severe meconium aspiration syndrome and pulmonary hypertension. With changing obstetric practices, and far fewer post-term deliveries, the rate of severe meconium aspiration syndrome and associated pulmonary hypertension has significantly declined, leading to a decrease in the number of neonates needing ECMO. However, there continue to be enough infants with severe pulmonary hypertension or parenchymal disease (including infants with a congenital diaphragmatic hernia) that ECMO is an important part of the care of infants in quaternary medical centers. Despite advances in ECMO pump and circuit design, it remains a complex form of support that should only be administered in select regional centers with rigorous programs, typically in conjunction with programs for ECMO support of pediatric and/or adult patients.

One of the long-standing criteria for ECMO therapy in infants is a high predicted mortality rate, usually defined by oxygenation index (OI). OI is a measure of the amount of respiratory support required to achieve adequate oxygenation, and is calculated as:

$$OI = [(F_IO_2) \times 100 \times (mPaw)] / (P_aO_2)$$

Since F_IO_2 is almost always 1.0 in these patients, the calculation is simple to perform knowing just mPaw and P_aO_2. For example, if $F_IO_2 = 1.0$, mPaw = 20 cmH_2O, and $P_aO_2 = 50$, the OI is 40. In general, an OI less than 20 has a good prognosis, an OI above 40 has a high predicted mortality, and an OI between 20 and 40 has an intermediate risk which warrants consideration of possible ECMO.

Types of Extracorporeal Membrane Oxygenation

Extracorporeal support is categorized by whether blood is both drained and returned to the venous side of the circulation

(veno-venous or VV) or drained from the venous circulation and returned to the arterial side (veno-arterial or VA). There are multiple ways cannulae can be placed to achieve VA or VV support, including trans-thoracic (especially for post-operative cardiac patients), via the femoral vessels (mainly used in pediatric and adult patients), and via the neck vessels (primarily used in neonates).

Regardless of the type of ECMO, there are significant risks that limit its application to patients with severe disease who are treated in dedicated ECMO centers. Among these risks are:

- Bleeding. ECMO involves the circulation of blood through a series of plastic tubes, a membrane oxygenator, and a heater, all of which can promote clotting. For this reason, most patients on ECMO are on continuous anti-coagulation, with the attendant risks of bleeding internally or from cannula insertion sites or surgical sites. In addition, platelet consumption during ECMO is usually significant, requiring multiple platelet transfusions. Of particular concern is the risk for potentially devastating intra-cranial hemorrhage. Close monitoring of coagulation status is essential in ECMO patients.
- Clotting. To avoid bleeding, the anti-coagulation of ECMO patients is tightly controlled, so that clotting of the circuit and thrombosis is always a concern. While small clots in an ECMO circuit are common, large clots that can obstruct the circuit and/or embolize are a potentially devastating complication.
- Ligation of vessels. In the neonate, the vessels which are used to provide canula access are ligated. Early attempts to repair these vessels led to strictures at the anastomosis sites with resultant risk for clots. Since vessels accessed for ECMO include the carotid artery and internal jugular, there are concerns about the long-term implications of the loss of these vessels. Fortunately, the cerebral circulation of the neonate appears to adjust well to the loss of these vessels in early life.

Veno-Arterial Extracorporeal Membrane Oxygenation

Veno-arterial ECMO was the first form of ECMO successfully used in infants and remains an important form of support for infants with combined respiratory and cardiac compromise. In infants, VA ECMO is usually accomplished by passing a large catheter through the right internal jugular vein into the right atrium and another catheter through the right common carotid artery to the arch of the aorta. Venous blood is drained from the right atrium, then run through the ECMO circuit where gas exchange (CO_2 removal and O_2 addition) is accomplished in the membrane oxygenator. The blood, which has cooled slightly in the passage through the ECMO circuit, is warmed and then returned to the arterial circulation through the carotid artery catheter at the arch of the aorta. With adequate size catheters and good catheter placement, most of the infant's cardiac output is replaced with circulation through the ECMO circuit. Thus, a baby can be supported with minimal to no native lung function or cardiac function. As the infant's pulmonary and/or cardiac status improves, the ECMO flow is gradually decreased, allowing the infant's native circulation to take over. When ECMO flow has been weaned to a relatively small amount, usually around 100 mL/min, the infant can be de-cannulated.

Veno-Venous Extracorporeal Membrane Oxygenation

Veno-venous ECMO offers a significant advantage in the infant who needs ECMO but has adequate cardiac output because it does not involve the carotid artery. Instead, a double-lumen catheter is placed through the right internal jugular vein into the right atrium, positioned so venous blood is drawn from the right atrium into the venous lumen and arterialized blood returning from the ECMO circuit is injected into the right atrium and directed across the tricuspid valve. This is technically more difficult than VA ECMO because it requires very accurate placement of the catheter to avoid recirculation of blood within the right atrium. Unlike VA ECMO, VV ECMO requires cardiac function which can provide full right and left cardiac output. In addition to the advantage of not sacrificing the carotid artery, VA ECMO offers the theoretical advantage of sending fully oxygenated blood to the pulmonary circulation, possibly promoting the resolution of pulmonary hypertension. Also, unlike VA ECMO where arterialized blood is injected into the arch of the aorta, VV ECMO provides oxygenated blood leaving the left ventricle to the coronary arteries and may facilitate cardiac function by optimizing myocardial oxygenation.

Liquid Ventilation

In the 1960s it was shown that some liquid perfluorocarbons, such as perfluorooctylbromide (PFOB), could contain enough dissolved O_2 and CO_2 that they could support an animal that was spontaneously breathing PFOB. This led to the concept of liquid ventilation where a patient was ventilated with an oxygenated liquid, typically PFOB.[147,148] The rationale for this was that ventilating a lung with liquid removed the air-liquid interface and thus avoided the problems associated with excess surface tension in a surfactant deficient lung. In addition, the weight of the liquid PFOB tended to open dependent regions of the atelectatic lung, an effect sometimes termed "liquid PEEP." The liquid also acts as a lung lavage, removing particulate material (e.g., meconium) and inflammatory cells and cytokines. PFOB and other perfluorocarbons have the advantage of being almost totally inert, with minimal systemic absorption.

Liquid ventilation was initially designed to totally replace the gas in the patient's lungs, a technique termed total or tidal liquid ventilation. This was followed with a simpler technique, generally termed partial liquid ventilation (PLV) in which the lung was filled to approximately FRC with liquid perfluorocarbon, then was ventilated with a normal gas ventilator. Because perfluorocarbons are volatile, the instilled perfluorocarbon was gradually exhaled and needed to be regularly replaced with more instilled liquid.

Liquid ventilation, both tidal and partial, has been used successfully in a number of animal models and in a variety of clinical settings, including infants, children, and adults with severe lung disease. Although technically feasible and physiologically intriguing, it has not found a clinical niche in which it is both clinically affordable and feasible, and clearly superior to more routine therapies. It remains an interesting avenue for potential research but is not in routine clinical use.

Monitoring Respiratory Status

One of the most important aspects of newborn intensive care is continuous monitoring of cardiorespiratory status. This is particularly true for the infant at risk of respiratory decompensation, or the infant who is on some level of respiratory support.

Physical Exam

Although monitoring devices play a key role in the modern NICU, the role of direct physical examination of the infant cannot be over-emphasized. It is important to remember that the neurologically intact infant has finely tuned chemoreceptors for O_2 and CO_2 levels and pH, as well as chest wall stretch receptors.

Together, these systems are designed to find the best respiratory strategy for simultaneously optimizing oxygenation, ventilation, and work of breathing. In most infants, simple observation can provide a wealth of information:

- The triad of "grunting, flaring, retracting," and associated tachypnea, are the hallmarks of neonatal respiratory distress in the non-ventilated infant. Of these, grunting is probably the most sensitive indicator of a restrictive/atelectatic disease such as RDS, because it represents the infant's attempt to counteract alveolar surface forces with end-expiratory pressure. Progressive increase in grunting is usually a clear indication of worsening disease.
- Tidal volume and respiratory rate in the spontaneously breathing infant are key indicators of respiratory status. With diseases characterized by atelectasis, infants will tend to decrease their V_T and increase their respiratory rate because this is the best strategy for minimizing the work of breathing in the setting of decreased compliance. Progressively decreasing tidal volume and increasing respiratory rate are usually ominous indicators of worsening work of breathing.
- Tidal volume in mechanically ventilated infants is an essential clue to the relative efficacy of the mechanical ventilation strategy. While bedside graphic ventilator monitoring is an important tool in assessing spontaneous and ventilator-supported breaths, direct observation of chest movement is also an important tool for assessing ventilator breaths.
- Work of breathing on mechanical ventilation is an important clue to the success of the ventilator strategy. An infant who is struggling as demonstrated by some combination of tachypnea, retractions, forced exhalation, or general discomfort is often one with a sub-optimal ventilatory strategy. With ventilated infants this is sometimes viewed as "fighting the ventilator," but usually represents a normal reaction to a sub-optimal ventilator strategy.
- Periodic breathing and apnea can represent impending respiratory failure, particularly in the more premature infant who is not able to sustain a significantly increased work of breathing.

Chest Radiograph

Chest radiographs are important tools to assess the status of the lung. However, the technique may vary, particularly in terms of whether the radiograph is obtained at full inspiration. In addition to giving information about diagnosis and ETT position, the chest radiograph can give some clues to whether the lungs are over- or under-inflated. Worrisome signs on chest radiograph include:

- Flat diaphragms, suggesting over-inflation.
- Small, narrow heart, suggesting over-inflation or low circulating blood volume (or both).
- Bulging of inter-costal pleura, suggesting over-inflation.
- Obvious regional over-distension or cystic areas or PIE.
- Dense or opaque "white-out" lungs suggesting under-inflation.
- Regional atelectasis.

Respiratory and Heart Rate Monitors

Transthoracic impedance is the standard method used to monitor neonatal respiration. This technique is based on changes in the electrical impedance of the thorax, as measured by skin surface electrodes, caused by changes in lung volume during respiration. This technique is effective at detecting apneas of central origin but may miss obstructive apnea where chest wall movement despite an obstructed airway is interpreted as respiration. Accurate detection of central, obstructive, and mixed apneas generally requires other sensors such as the detection of gas exhalation by thermistors or CO_2 monitors. Transthoracic impedance monitors can also measure heart rate, and display heart rhythm.

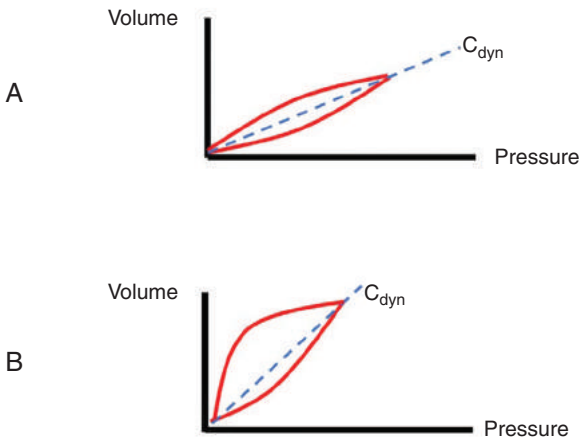
Graphics Monitoring During Conventional Ventilation

The integration of ventilator graphics into modern neonatal ventilators has markedly increased the amount of information available to clinicians for optimizing ventilatory support. This allows the monitoring of spontaneous breathing, assessment of respiratory system mechanics, and detection of excessive or insufficient V_T, hypoventilation, and gas trapping.[149] Different ventilators have different graphics options available, but most have basic similarities. The basic graphic data includes:

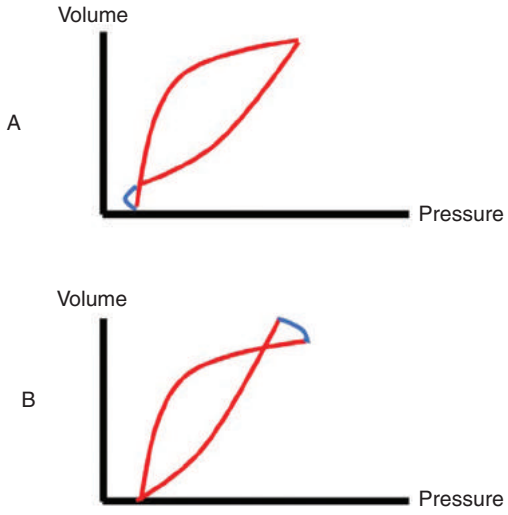
- Pressure. This is typically measured close to the hub of the endotracheal tube. In most cases, where T_{insp} and T_{exp} are adequate to allow pressure to be transmitted to and from the alveoli, the PEEP and PIP displayed are the same as at the alveolar level. If T_{insp} and/or T_{exp} are too short, relative to the lung time constant, then the alveolar pressure changes are smaller than the changes measured at the airway. This is usually seen with a high rate and inadequate T_{exp} where there is air trapping or "inadvertent PEEP."
- Flow. Flow sensors can be placed in the ventilator circuit close to the ETT, in which case they are referred to as mainstream or proximal flow sensors. Although mainstream flow sensors are usually small and typically have a dead space volume of less than 1 mL, the additional dead space can be a concern in small infants. Alternatively, flow sensors can be built into the ventilator. In small infants, mainstream flow sensors have better accuracy than those built into the ventilator because of the compressible gas volume and high bias gas flow in the ventilator circuit that may lead to inaccurate estimates of flow (and volume) delivered to the infant.
- Volume. Volume is calculated electronically by integrating the flow signal. Given the importance of optimizing V_T in the ventilated infant, it is probably the most important parameter to track.

The pressure, flow, and volume information can be displayed in multiple ways. The simplest way is scalar or "strip chart" display of each over time. This is particularly useful for looking at changes in breathing patterns over short periods of time, including breath-to-breath variation. The other way to display the same data is through Pressure-Volume (compliance) loops and Flow-Volume loops. With this approach, each successive breath is represented as a loop, frequently with the most recent loop overlaid on the last several loops. These Pressure-Volume and Flow-Volume loops contain information that is extremely useful in optimizing ventilatory support. Some of the most important information in these ventilator graphics are simple patterns that are easily recognized. These include:

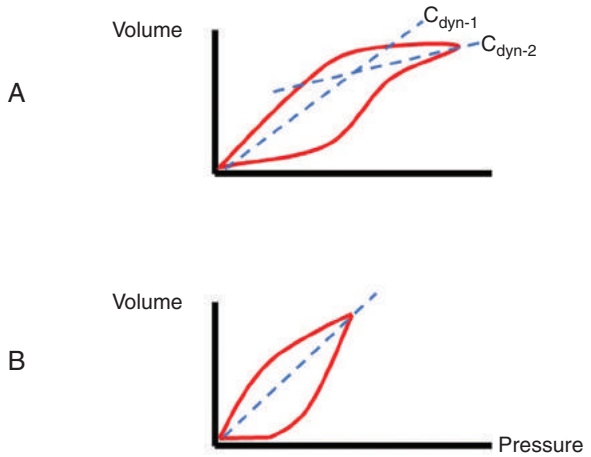
- Compliance and hysteresis, seen on Pressure-Volume loops (Fig. 40.5)
- Over-distension and inadequate PEEP, seen on Pressure-Volume loops (Fig. 40.6)
- Inadequate flow ("flow starvation") and active exhalation, seen on Pressure-Volume loops (Fig. 40.7)
- Endotracheal tube leak, seen on Flow-Volume loops (Fig. 40.8)
- Inadequate T_{exp}, seen on Flow-Time tracing (Fig. 40.9)



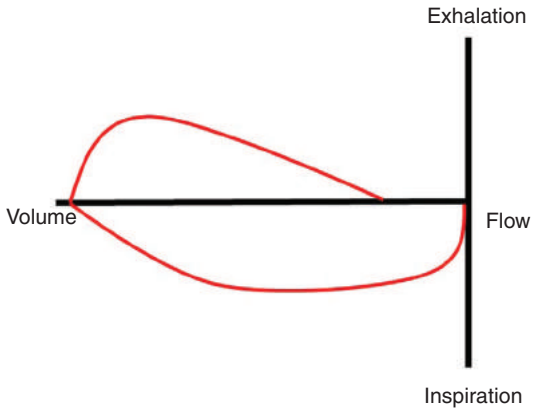
• **Fig. 40.5** Compliance and Hysteresis. (A) shows a simplified Pressure-Volume or compliance loop of a breath with low dynamic compliance (C_{dyn}). There is also low hysteresis, meaning the deflation (upper) part of the curve is close to the inflation (lower) part of the curve, typically indicating surfactant deficiency. (B) shows a Pressure-Volume loop of a breath with greater compliance. The slope of the C_{dyn} line is steeper, indicating more volume per pressure. There is also more hysteresis or deflation stability, with the flatter upper portion of the deflation limb showing that volume is maintained as pressure drops. This is typically seen with good surfactant function.



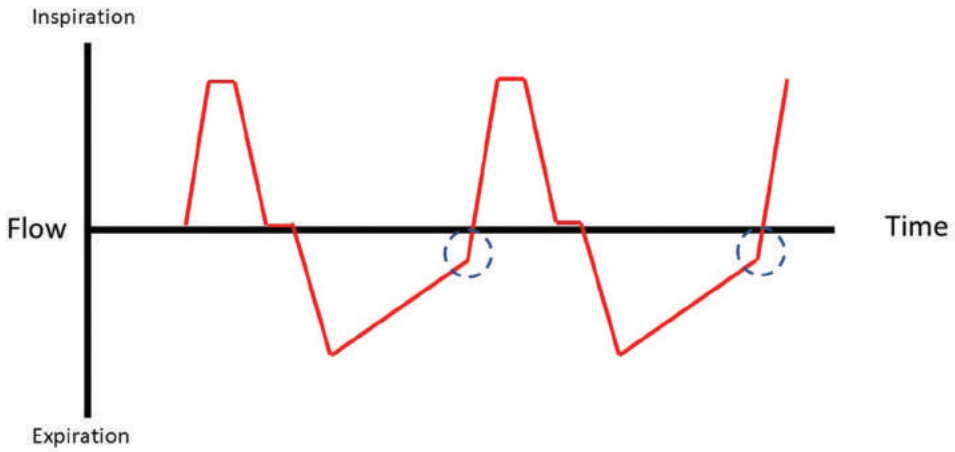
• **Fig. 40.7** Two Abnormal Pressure-Volume Loop Patterns. Part A shows a small "tail" or figure-8 (in *blue*) at the bottom of the pressure-volume loop. During early inspiration, pressure decreases to below peak end-expiratory pressure *(PEEP)* as volume is increasing. This occurs when the patient is drawing in a breath and there is inadequate flow to maintain PEEP, sometimes referred to as "flow starvation." Part B shows a figure-8 at the top of the compliance loop (in *blue*), as the patient actively exhales causing increased pressure and decreased volume. This occurs when the ventilator T_{insp} is too long or the V_T is too large and the infant actively tries to exhale during the ventilator inspiratory phase.



• **Fig. 40.6** Two Abnormal Pressure-Volume Curves. (A) shows a lung that is over-inflated. This pattern, sometimes described as "penguin leaning into the wind," results from having two C_{dyn} components. The C_{dyn-1} line shows the steep, good compliance in early- and mid-inflation, while the C_{dyn-2} line represents the decreased compliance that occurs when the lung is over-inflated. The point at which the curve "leans over" is the upper inflection point. (B) shows a compliance curve that is flat during early inspiration. This indicates pressure during early inspiration without associated volume increase, meaning peak end-expiratory pressure *(PEEP)* is inadequate and the lung is below its opening pressure at end exhalation.



• **Fig. 40.8** Endotracheal Tube Leak. This Flow-Volume loop shows a greater volume on inspiration (lower part of loop) than on exhalation (upper part of loop). The difference in volume between inspiration and expiration is due to a leak around the endotracheal tube. Since much of the leak probably occurs during inspiration, the exhaled V_T is likely a more accurate measurement of what is delivered to the lungs than the inspired V_T. Note that Flow-Volume loops are sometimes drawn as the mirror image of this.



• **Fig. 40.9** Inadequate T_{exp}. Simplified scalar plot showing flow over time. At end-expiration, the flow has not returned to zero before the next breath begins (dotted circle). This means the patient has not exhaled down to set peak end-expiratory pressure *(PEEP)*, so the short T_{exp} is causing air trapping, sometimes termed "inadvertent PEEP."

Blood Gas Measurement

Measurement or estimation of arterial blood gas status is key to the management of respiratory failure and lung disease in the neonate. Measurements of arterial oxygen tension (P_aO_2) and arterial carbon dioxide tension (P_aCO_2) are considered the reference standards for the assessment of respiratory status and adequacy of respiratory support. In addition to direct measurement of arterial blood gas values, there are indirect and noninvasive estimations.

Arterial Catheters

Repeated arterial blood sampling requires the placement of an arterial catheter. Typically, umbilical artery catheters (UACs) are used during the acute phase of respiratory failure. Samples obtained from a UAC are reliable, assuming proper sample handling and laboratory procedures are followed, and easily obtained without disturbing the infant. In addition to providing access for blood sampling, UACs can serve an important role in continuous direct blood pressure measurement.

In most cases, UACs can be placed relatively easily by skilled personnel. However, they cannot be placed in all infants and, even under the best of circumstances, are not without risks. Particularly important considerations in placing and maintaining a UAC are the risks of thrombi and emboli, vasospasm, and infection. Interference with blood flow to the lower extremities is a particular concern. Blue discoloration of the toes and/or foot are relatively common, and usually not a concern. However, blanching of any part of the lower extremity in any infant with a UAC in place indicates severe vascular compromise and is an indication for immediate removal of the UAC. UAC lines with the tip above the celiac plexus are associated with fewer complications than those with the tip below the renal or mesenteric artery.[150]

Although UACs can sometimes be maintained in place for up to several weeks, this is usually not done except in the sickest infants where there are no other options for vascular access. Patency of UACs is usually maintained by infusion of a solution of heparinized saline. UACs should not be used for the administration of medications unless there are no other alternatives. Vasoactive agents and hypertonic medications should never be infused through a UAC.

Percutaneously placed peripheral artery lines are frequently used in infants where a UAC cannot be placed, or for management of older infants in whom the UAC has been removed. The most common sites for peripheral artery cannulation are the radial, ulnar, posterior tibial, and dorsalis pedis arteries. Placement of these lines can be technically challenging and quite uncomfortable if local and/or systemic analgesia is not used. As with UACs, peripheral artery lines must be carefully observed for any evidence that they are causing distal vascular compromise, in which case they should be removed. Like UACs, peripheral artery line patency is maintained by a low-rate infusion of heparinized saline. Peripheral artery catheters should not be used for the administration of medications.

Arterial Puncture

Although it is technically possible to perform intermittent arterial punctures to obtain blood gases (and other labs), this is not ideal. Arterial puncture is usually more technically difficult and more painful than venous puncture. Because of the pain associated with arterial puncture, infants frequently respond with agitation, hyperventilation or apnea, and desaturation. In addition, an arterial blood gas gives a value that is only representative of a single point in time. Thus, the value of a blood gas obtained by arterial puncture is limited. In general, arterial punctures should be avoided in favor of less traumatic estimations of arterial blood gas status.

Capillary Blood Gas

Arterialized capillary blood is frequently used as an approximation of arterial blood for blood gas analysis. It is typically obtained by sampling from the medial or lateral plantar heel surface after warming the heel to increase capillary flow. The purpose of warming is to "arterialize" the blood by increasing flow and decreasing the time for O_2 and CO_2 exchange to occur in the capillary bed. Although there is a general rough correlation with P_aCO, individual capillary values may be significantly different than arterial values. In addition, capillary blood gases are painful and frequently lead to agitation and changes in respiratory status similar to those

seen with arterial puncture. Thus, capillary blood gas values are of limited value as a routine way of tracking respiratory status and should always be interpreted cautiously.[151] Capillary blood gases are probably most useful for following trends in pH, rather than for measuring exact PCO_2 values, and they are not at all useful for estimating P_aO_2.

Venous Blood Gas

For patients with an umbilical venous catheter or other central venous catheters, the measurement of venous blood gas values is technically simple. However, systemic venous gases are of limited value. The amount of O_2 which has been extracted, and CO_2 which has been added, to the blood at the level of the capillary bed varies widely and is impacted by multiple factors such as cardiac output and regional perfusion. In practical terms, the only certainty with systemic venous blood gases is that the venous PO_2 is lower than P_aO_2, and venous PCO_2 is higher than P_aCO_2.

Mixed Venous Saturation

Mixed venous saturation (S_VO_2) is the saturation of blood in the main pulmonary artery, where venous blood from the superior vena cava and inferior vena cava have been thoroughly mixed. In infants, S_VO_2 is usually approximated with blood sampled from the right atrium. This is particularly useful in the management of the infant on ECMO, where blood is continuously drained from the right atrium, and can be analyzed with continuous noninvasive monitoring as well as intermittent blood gases.

With normal lungs and pulmonary blood flow, blood leaving the lungs is approximately 100% saturated. With typical systemic oxygen demand and adequate systemic blood flow, approximately 25% of the O_2 carried by hemoglobin is removed by peripheral tissues, leaving blood returning to the heart approximately 75% saturated. Thus, S_VO_2 of 75% suggests adequate delivery of oxygen to peripheral tissues. In patients on ECMO, S_VO_2 significantly below 70% to 75% suggests inadequate tissue oxygenation and is typically dealt with by increasing ECMO flow to deliver more 100% saturated blood from the ECMO circuit to the patient.

Errors in Blood Gas Measurement

Errors in blood gas measurement are usually technique related, including the delay between obtaining the sample and analysis, contamination of the sample by air bubbles, or contamination of the sample by fluid used to maintain catheter patency. Common errors include:

- Delay in analysis, particularly if the blood is not chilled, results in continued metabolism by the red blood cells, with resulting consumption of O_2 and generation of CO_2.
- Contamination of the blood sample with an air bubble or with fluid allows the O_2 and CO_2 to equilibrate between the sample and the contaminant.
- If the contaminant is fluid or room air, PCO_2 in the blood will typically decrease, and the buffering capacity of the blood will compensate to maintain pH, resulting in what looks like a respiratory alkalosis and metabolic acidosis.
- Contamination with fluid will typically lead to a decrease in sample PO_2, while contamination with air will tend to move the blood PO_2 closer to atmospheric PO_2.

Noninvasive Estimation of Blood Gases

Pulse oximetry is a reliable continuous measurement of arterial oxygen saturation. Estimation of P_aCO_2 is more problematic. While there is a general correlation between P_aCO_2 and noninvasive estimates of PCO_2, the correlation can vary significantly in individual measurements.[152]

Pulse Oximetry

Pulse oximetry provides continuous real-time monitoring of oxygenation status and is such an essential component of modern critical care that many consider it the "other vital sign." Measurement of the oxygen saturation in arterial blood (S_aO_2) by pulse oximetry (S_pO_2) is based on the difference in the light absorption between oxygenated hemoglobin (Hb) and deoxygenated Hb in the red and infrared regions of the light spectrum. Since deoxygenated Hb absorbs more red light and less infrared light than oxygenated Hb, as S_aO_2 increases, the ratio of the absorption of red light to that of infrared light decreases. It is assumed that in circulation, changes in this ratio can be produced only by pulsating arterial blood. The amount of light absorbed by pulsatile blood is only a small fraction of the light absorbed by tissue and venous blood, so careful application of the S_pO_2 probe is essential. Pulse oximetry probes may be quite susceptible to movement artifact which can interfere with accurate measurement in the active infant. Different models of pulse oximeters use slightly different algorithms for calculating S_pO_2 so there is not always perfect agreement between different pulse oximeters, or between S_pO_2 and measured S_aO_2.[153,154]

The interpretation of S_pO_2 must be done in the context of the sigmoid-shaped relationship between P_aO_2 and S_pO_2. This is particularly true of S_pO_2 approaching 100% which may reflect a P_aO_2 of less than 80 or well above 100 mmHg. On the other hand, most S_pO_2 values below 80% are associated with P_aO_2 below 40 mmHg.

Transcutaneous Blood Gas Monitoring

Transcutaneous estimate of PO_2 ($P_{TC}O_2$) and PCO_2 ($P_{TC}CO_2$) is possible because of the diffusion of gas from the tissue bed to the skin surface. Particularly in preterm infants with thin skin and good perfusion, this can provide a reliable tool for following trends in blood gas values. Transcutaneous monitors combine O_2 and CO_2 sensors with a heating element that warms the skin under the sensors, causing an increase in local blood flow. With this increase in blood flow, the blood directly under the transcutaneous monitor probe is partially "arterialized" and the tissue bed has less opportunity to exchange O_2 and CO_2 with blood in the capillary. While this increases the accuracy of the transcutaneous estimate of PO_2 and PCO_2, the warming can cause local burns. For this reason, transcutaneous probes are not left in one position for more than several hours. Fortunately, there is data to suggest that lower temperatures can be used, resulting in less risk of burns.[155]

Multiple factors impact the blood flow under a transcutaneous sensor and therefore the accuracy of transcutaneous measurement. These factors include gestational age, sensor position on the body, systemic vasoconstriction, and any other factor which may impact systemic perfusion. In addition, if the sensor becomes partially dislodged from the skin, the sensor will read values for PO_2 and PCO_2 which are influenced by the air between the skin and the sensor. For these reasons, transcutaneous monitors are best thought of as devices for following trends in PCO_2, rather than for absolute values. Correlation of transcutaneous measurements with blood gas measurements may be helpful, but if capillary blood gases are used to correlate with the transcutaneous monitor, the accuracy of the capillary blood gas values are not necessarily reliable as discussed above. In general, $P_{TC}CO_2$ is most useful for following infants who are labile or require significant ventilator adjustments, such as immediately after beginning mechanical ventilation or when

changing ventilator modes. With simple and reliable S_pO_2 monitoring readily available, $P_{TC}O_2$ is of little use for most patients.

End-Tidal Carbon Dioxide Monitoring

In some circumstances, monitoring the partial pressure of CO_2 at the end of exhalation (end-tidal, $P_{ET}CO_2$) is a convenient way to obtain an estimation of alveolar PCO_2 (P_ACO_2), which is usually a close approximation of P_aCO_2.[156,157] This has the advantage of being a simple noninvasive way to continuously monitor PCO_2. However, particularly in infants with significant lung disease, the correlation between P_aCO_2 and $P_{ET}CO_2$ is low. Accurate assessment of P_aCO_2 by $P_{ET}CO_2$ requires an exhalation phase that is slow enough to obtain a measurement of true exhaled alveolar gas ("alveolar plateau") that is not mixed with dead-space gas. In general, $P_{ET}CO_2$ is most useful in large patients with relatively little lung disease who have a low respiratory rate and reproducible alveolar plateau to their exhaled CO_2 level. It is least accurate in small babies with small tidal volumes, high respiratory rates, and severe lung disease. Despite this, it can be a useful trending tool and can decrease unwanted hypercapnia and wide swings in PCO_2.[158,159]

One area where $P_{ET}CO_2$ detection can be extremely useful, even if it is not an accurate estimation of P_aCO_2 is confirmation of endotracheal tube placement. Small chemical CO_2 detectors are useful for confirming the placement of an endotracheal tube in the trachea immediately after intubation. In addition, continuous monitoring of $P_{ET}CO_2$ provides a near-instantaneous indicator of whether an endotracheal tube has become dislodged.

Near-Infrared Spectroscopy

Near-infrared spectroscopy (NIRS) is a technique for assessing regional oxygenation.[160,161] Initially used to evaluate cerebral oxygenation, the technique has been used to assess regional oxygenation in other vascular beds including the kidney and intestine.[162,163] It is a theoretically attractive adjunct to other assessments of adequate oxygen delivery, particularly in patients with potentially altered systemic oxygen delivery such as sepsis or other forms of shock. It is also an intriguing option for monitoring patients with potentially impaired cerebral oxygenation, such as patients with HIE. However, it is not yet part of routine clinical monitoring in many NICUs.

Acknowledgment

Drs. Durand and Courtney wish to thank Drs. Eduardo Bancalari, Nelson Claure, and Deepak Jain for their expertise in this area and to acknowledge their significant contributions to previous versions of this chapter.

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41

Control of Breathing

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KEY POINTS

- Apnea of prematurity is universal in preterm infants and a manifestation of greater inhibitory (rather than excitatory) influences on the central respiratory network.
- In premature infants, excitation of peripheral arterial chemoreceptors by hypoxia predisposes to periodic breathing and the hypoxic ventilatory response is not sustained.
- The protective upper airway reflex (laryngeal chemoreflex) prevents aspiration, but in premature infants, profound bradycardia, apnea, and oxygen desaturation can occur in addition to laryngeal constriction.
- The excessively compliant chest wall and low lung volume in premature infants predispose them to intermittent hypoxia as a consequence of apnea.
- Caffeine is the mainstay of therapy for apnea of prematurity, although the optimal duration and dosing of therapy remain uncertain.
- By identifying the genetic mutations that are associated with marked abnormalities in respiratory control, we can obtain a better understanding of the key role of several neuromodulator systems.

How the respiratory network matures is of interest not only to physiologists, but also to clinicians who care for infants, newborns, and children with disorders of respiratory control. Neonatologists, in particular, are aware of the challenges of treating infants who are born prematurely. While breathing movements can be detected in the human fetus as early as 10 to 12 weeks' gestation, the purpose of fetal breathing is not gas exchange, but instead, the lung stretch that occurs during breathing is essential for lung development. The transition from fetal to neonatal life requires a rapid conversion from intermittent fetal respiratory activity not associated with gas exchange to continuous breathing on which gas exchange is dependent. Breathing in the most premature infants is akin to fetal breathing, which is episodic, punctuated by periods of disturbingly long apneic pauses interspersed with frequent periods of hyperventilation and sighs (augmented breaths). For the infant who is born prematurely, frequent apneas and periods of hypoventilation associated with oxygen desaturations and bradycardia are of significant concern. With maturation, breathing becomes more stable. Thus, the premature infant provides a unique opportunity to observe how the respiratory system matures in humans.

Even though breathing is more stable in term infants than in premature infants, the respiratory system at term gestation is still undergoing significant maturation and can become unstable in response to stressors such as infection or hyperthermia. In some term infants who appear well, subtle developmental abnormalities in the anatomy and neurochemistry of the respiratory system can lead to profound disorders of breathing. Careful epidemiologic, genetic, neurochemical, and neuroanatomic studies in human infants with disorders of respiratoryss control have allowed for a better understanding of genes that regulate the development of the respiratory system and how environmental factors in fetal and early neonatal life may adversely affect the normal development of systems that control breathing.

The purpose of this chapter is to better understand how infants breathe, why premature infants have apnea of prematurity, why term infants have apnea of infancy, and how environmental exposures modify mechanisms that control breathing during fetal and early neonatal life.

Animal Models of Control of Breathing

Much of our understanding of the basic mechanisms that lead to stable breathing comes from studies performed in newborn and adult animals. Earlier studies in newborn pigs, dogs, and cats, as well as the fetal and newborn sheep, characterized the developmental physiology, and much has been gained from these models. However, a more detailed understanding of the neuroanatomy, neurocircuitry, and neurochemistry has been obtained with in vitro models from fetal and newborn rats and mice. Of particular relevance, the stage of respiratory development of the rat born at term is similar to that of the human born at 25 to 29 weeks' gestation. Two different reduced in vitro preparations from rodents have been used to better delineate components of the respiratory network in the brainstem: (1) brainstem slices that include the area that contains the "pacemaker cells mediating rhythmogenesis," and (2) isolated brainstem spinal cord preparations from fetal and newborn rodents. Because the stability and viability of these in vitro preparations are best when tissues are used from the late embryonic stage or within the first week of postnatal life, data from these in vitro preparations are relevant only to respiratory control during very early development. Using these reduced models, specific regions of the brain involved in respiration could be identified and characterized. We now know that the groups of neurons that control the different phases of respiration have a genetic signature, allowing the use of genetic tools to manipulate the activity of the different regions and observe the physiologic responses in intact animals. Specifically, optogenetics and pharmacologic techniques are often combined to explore the functionality of the specific brain regions related to breathing in unanesthetized adult animals. Thus, what we know about how the respiratory network is integrated and functions is based on a significant amount of information obtained from well-designed in vitro and in vivo animal experiments using immunohistochemistry,

electrophysiology, fluorescent imaging, genetic manipulation, pharmacology, computational modeling[1,2] and more recently optogenetics in rodents.[3,4]

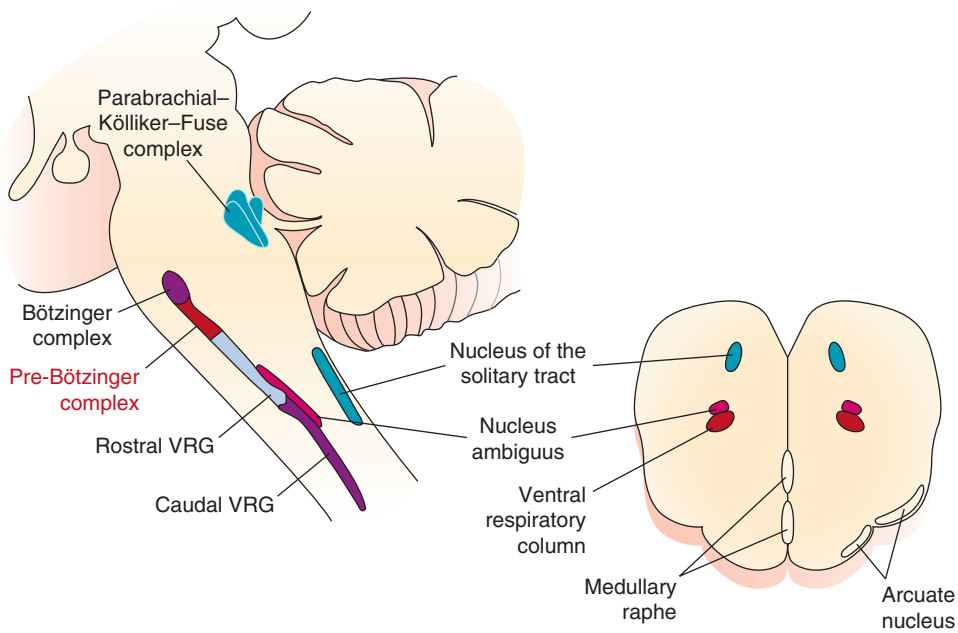
Respiratory Muscles

The diaphragm is the major muscle of respiration, but other muscles of respiration are also essential for unobstructed breathing at rest and augmented breathing during exercise and stress. Thus, the muscles of respiration include the pump muscles (diaphragm, intercostal muscles, and abdominal muscles) and muscles of the upper airway (alae nares, pharyngeal muscles, and laryngeal muscles). The diaphragm is innervated by the phrenic nerve, which originates in the spinal column (C3 to C5). The upper airway muscles are innervated by motoneurons originating in the brainstem, specifically, the nucleus ambiguus, the dorsal motor nucleus of the vagus, and the hypoglossal nucleus.[5,6] Different muscles are activated during different phases of the respiratory cycle: inspiration followed by post-inspiration and expiration. Upper airway muscles are particularly important in modulating the rate of inspiratory and expiratory airflow. During inspiration, the diaphragm, external intercostal muscles (in infants), and posterior cricoarytenoid (laryngeal dilator) contract.

During post-inspiration the diaphragm and the thyroarytenoid (laryngeal constrictor) contract. Diaphragmatic and thyroarytenoid post-inspiratory activity are common in newborns, both human[7] and animal.[8] Because the chest wall of newborns, particularly premature newborns, is highly compliant, diaphragmatic and laryngeal post-inspiratory activity of upper airway muscles is often audible, known as *grunting*, and is heard in infants with low lung volume disease states, such as surfactant deficiency and atelectasis to preserve lung volume. It is essential that the pump muscles, particularly the diaphragm and the upper airway muscles, contract in such a way that unobstructed breathing occurs. Because of lower airway tone or active closure of the glottis, premature infants may have an obstructed component during apnea. The resultant obstructed inspiratory efforts may prolong central apnea, resulting in so-called mixed apnea.

Brainstem Rhythmogenesis

As shown in the anatomic illustration in Fig. 41.1, the respiratory-related neurons are located in three main areas in the brainstem: (1) the dorsal respiratory group within the nucleus tractus solitarii (nTS); (2) the ventral respiratory column (VRC), which extends from the facial nucleus to the ventrolateral medulla at the spinal-medullary junction; and (3) the pontine respiratory group within the dorsolateral pons.[2,9] The VRC should not be confused with the ventral respiratory group (VRG). The VRC can be subdivided into a rostral part—involved in rhythmogenesis—and a caudal part, involved in pattern formation. Bulbospinal neurons are neurons that originate in the medulla (bulbo) and synapse with motoneurons in the spinal column such as the phrenic motoneurons. The rostral VRC contains both the rostral VRG, consisting of a large proportion of bulbospinal inspiratory neurons that project directly to the phrenic and external intercostal motoneurons



• **Fig. 41.1** **The anatomic relationship between the regions in the human brainstem that constitute the respiratory network.** These regions include specialized neurons in the dorsolateral pons (parabrachial and Kölliker-Fuse nuclei), nucleus of the solitary tract, and ventral respiratory column. The ventral respiratory column is organized rostrocaudally extending from the level just below the facial nucleus to the C1 level of the cervical cord. The ventral respiratory column consists of the Bötzinger complex, the pre-Bötzinger complex, and the rostral and caudal ventral respiratory groups *(VRGs)*. Vagal motor neurons of the nucleus ambiguus innervate the laryngeal muscles. The medullary raphé, arcuate nucleus, located just underneath the ventral medullary surface, contains neurons that depolarize in response to hypercapnia and hypoxia. The retrotrapezoid nucleus (not shown) is a CO_2/H^+ chemosensor and is located rostrally below the facial nucleus on the ventral medullary surface. (Modified from Benarroch EE. Brainstem respiratory control: substrates of respiratory failure of multiple system atrophy. *Mov Disord*. 2007;22:155–161.)

and the caudal VRG, containing bulbospinal expiratory neurons that project to abdominal and internal intercostal motoneurons. Propriobulbar neurons are neurons originating in the brainstem that send projections to other neurons in the brainstem.

Within the rostral VRC are two areas that are essential to the formation of respiratory rhythm: the pre-Bötzinger (PBC) and the Bötzinger complex. As outlined already, the PBC contains a core group of synaptically coupled excitatory neurons that have pacemaker properties, similar to the pacemaker cells in the atrioventricular node of the heart. These pacemaker cells are rostral to the nucleus ambiguus, have both intrinsic inspiratory and expiratory bursting properties, and are essential to maintaining respiratory rhythm.[10] Progressive destruction of the PBC disrupts rhythmogenesis, leading to death in animals.[11] Using immunocytochemistry to identify NK1 and somatostatin positive neurons and anatomical approaches, a similar area has been identified in the human brain. In individuals who had died of neurogenerative disease with central respiratory deficits, reduced numbers of neurons were identified in the area of the putative PBC as compared with the brains of individuals with neurodegenerative disease without central respiratory deficits.[12] Moreover, Bright et al. found that premature infants who died of sudden infant death syndrome (SIDS) had significantly lower levels of NK1 receptor binding (NK1R) in the nTS, and paragigantocellularis lateralis (PGCL) nucleus, the homologue of the PBC, than in premature infants who did not die of SIDS, and male sex was associated with less NK1R binding in the inferior olivary-cerebellar complex in preterm and term infants who died of SIDS vs control infants.[13] Both male sex and premature birth increase the risk of SIDS. In newborn rats, destruction of NK1R expressing neurons within the preBötC causes abnormal respiratory pattern, and, the more immature the circuit is at the time of destruction, the more permanent the change in the pattern of respiration.[14]

The post-inspiratory complex (PiCo) is another area, recently discovered, with autonomous rhythm-generating properties that controls post-inspiratory activity.[4] The PiCo is medial to the nucleus ambiguus and caudal to the nucleus of cranial nerve VII. The Bötzinger complex contains propriobulbar expiratory neurons that provide strong inhibitory inputs to inspiratory and expiratory bulbospinal neurons in the VRC.

Another important group of neurons are those within the retrotrapezoid nucleus (RTN) located along the ventral medullary surface beneath the facial nucleus. These neurons have chemosensitive properties and depolarize in response to increasing carbon dioxide (CO_2) concentration and decreasing pH and synapse with rhythm- and pattern-generating neurons in the VRC.[15] All these neuronal groups and networks that contribute to rhythmogenesis are present in newborn animals born at term (e.g., sheep, cats, and pigs) or born prematurely (e.g., rodents) in which rhythmogenesis is well established before birth. Episodic spontaneous fetal breathing movements occur in human fetuses as early as 10 weeks' gestation.[16] In rodents, respiratory rhythmogenesis is first detected at embryonic day 15 in rats and embryonic day 17 in mice.[17] The emergence of this respiratory-related activity in rats is coincident with the characteristic expression of NK1 receptors of the PBC.[17]

In summary, respiratory rhythm and inspiratory-expiratory patterns emerge from dynamic interactions between (1) excitatory neuron populations in the PBC and rostral VRG, which are active during inspiration and form the inspiratory motor output; (2) excitatory neurons in the PiCo, which are active during post-inspiration; (3) inhibitory neurons in the PBC that provide inspiratory inhibition within the network; and (4) inhibitory neurons in the Bötzinger complex, which are active during expiration and provide inhibitory inputs to inspiratory and expiratory neurons within the network and to phrenic motor neurons. Because of the limitations in performing mechanistic experiments in humans, much of what we know about how we breathe is extrapolated from animal models, but many similarities exist between animals and humans regarding the respiratory network. Harper et al.[18] have described the relationship between damage to specific brain regions observed on brain imaging and specific disorders of respiratory control in humans.[18]

Neurochemical Control of Respiration

Glutamate is the major neurotransmitter mediating excitatory synaptic input to brainstem respiratory neurons and respiratory premotor and motor neurons. Gamma aminobutyric acid (GABA) and glycine are the two major inhibitory neurotransmitters mediating inhibitory synaptic input in the respiratory network; they have a key role in pattern generation and termination of inspiratory activity.[19] GABA (via $GABA_A$ receptors) and glycine (via glycine receptors) mediate fast synaptic inhibition via activation of chloride channels.[20] Throughout development, glutamate always functions as an excitatory neurotransmitter; however, it is not the case that GABA and glycine are always inhibitory neurotransmitters. In early development, GABA and glycine mediate *excitatory neurotransmission* in many neuronal networks, including the respiratory network.[21] GABA and glycine signaling modify the level of chloride in the cell. Activation of the sodium (Na^+)-potassium (K^+)-chloride (Cl) cotransporter (NKCC1) and the potassium-chloride transporter (KCC2) on the cell modulates intracellular ion concentrations. Specifically, NKCC1 brings Na^+, K^+, and $2Cl^-$ into the cell, while activation of KCC2 moves K^+ and Cl^- outside the cell. Low expression of KCC2 during early development, resulting in a high NKCC1/KCC2 ratio, causes *high* intracellular chloride concentrations in immature neurons. When GABA then binds to $GABA_A$ receptors, a net outward movement of Cl^- ions occurs, leading to membrane depolarization. With maturation, KCC2 expression increases, reversing the NKCC1/KCC2 ratio and lowering intracellular Cl^- ions. Now when GABA binds to $GABA_A$ receptors, more Cl^- ions come into the cells, leading to hyperpolarization.[22,23] With brain injury, NKCC1 expression increases, making the GABAergic system less inhibitory. Moreover, myoclonic jerks are associated with midazolam exposure ($GABA_A$ receptor agonist) in premature infants.[24] $GABA_B$ receptors, which are metabotropic G protein-coupled receptors, also have a greater role in inhibiting respiratory rhythm in adult animals as compared with newborn animals.[25] Finally, the baseline excitatory and inhibitory influences mediated by glutamate and GABA-glycine, respectively, on major neuronal networks are further altered by many endogenously released neuromodulators that shape and fine-tune respiratory pattern and rhythm throughout development, as outlined in Table 41.1.

Genetic Mutations Affecting Respiratory Control

Some neuromodulators may be more critical in supporting respiratory rhythmogenesis than others. By identifying the genetic mutations that are associated with marked abnormalities in respiratory control, we can obtain a better understanding of the key

TABLE 41.1 Neurotransmitters and Neuromodulators That Mediate Respiratory Rhythm

Neurotransmitter/ Neuromodulator	Receptor Subtype	Source of the Endogenous Ligand	Excitatory or Inhibitory on Respiratory Rhythm	Comment
Glutamate	NMDA, AMPA, GluR	Ubiquitously expressed	Excitatory	Major excitatory neurotransmitter
Ach	M3	PAG, LC, X	Excitatory	
NE	α1-Adrenergic	LC	Excitatory	
Serotonin	5-HT2A2B, 5-HT3, 5-HT4	Raphé	Excitatory	
Dopamine	Likely D_1	PVN, hypothalamus	Excitatory	
ATP	P2X2	Ventral medulla; CO_2/H^{+-} sensitive cells in the RTN	Excitatory	
Adenosine	P2Y1	Ventral medulla	Excitatory	
Substance P	NK1	nTS, NA	Excitatory	
CCK	CCK1	nTS, raphé	Excitatory	
TRH	TRH-R (1 and 2)	Raphé	Excitatory	
GABA	$GABA_A$, $GABA_B$	Ubiquitously expressed	Inhibitory	Major inhibitory neurotransmitter (can be excitatory during fetal life)
Glycine	GlyR		Inhibitory	Can be excitatory during fetal life
NE	α2-Adrenergic	Pons	Inhibitory	
Dopamine	D4	PVN, hypothalamus		
Adenosine	A_1, A_2	Ubiquitous from metabolism of ATP that increases during hypoxia	Inhibitory	Contributes to respiratory depression at the baseline (A_1), and mediates HVD
Opioid	μ, δ, κ	nTS, PBN, PVN, raphé	Inhibitory	Prominent inhibitory effect during early development
PDGF	PDGF-β	nTS	Inhibitory	Contributes to HVD

Ach, Acetylcholine; *AMPA*, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate; *ATP*, adenosine triphosphate; *CCK*, cholecystokinin; *GABA*, γ-aminobutyric acid; *GluR*, glutamate receptor; *GlyR*, glycine receptor; *HVD*, hypoxic ventilatory depression; *LC*, locus ceruleus; *NA*, nucleus ambiguus; *NE*, norepinephrine; *NMDA*, *N*-methyl-D-aspartate; *nTS*, nucleus tractus solitarii; *PAG*, periaqueductal gray; *PBN*, parabrachial nucleus; *PDGF*, platelet-derived growth factor; *PVN*, paraventricular nucleus; *RTN*, retrotrapezoid nucleus; *TRH*, thyrotropin-releasing hormone; *TRH-R*, thyrotropin-releasing hormone receptor; *X*, vagal nucleus.

Data from Doi A, Ramirez JM. Neuromodulation and the orchestration of the respiratory rhythm. *Respir Physiol Neurobiol*. 2008;164:96; and Simakajornboon N, Kuptanon T. Maturational changes in neuromodulation of central pathways underlying hypoxic ventilatory response. *Respir Physiol Neurobiol*. 2005;149:273.

role of several neuromodulator systems. For example, serotonergic neurons in the caudal medullary raphé nuclei have extensive projections to phrenic and hypoglossal motoneurons, the nTS, the RTN, and the PBC.[26] The serotonergic system has a significant influence on the modulation and integration of diverse homeostatic functions, including cardiorespiratory responses and thermogenesis.[27] Individuals with Prader-Willi syndrome, who may exhibit breathing abnormalities at birth,[28] have mutations in the necdin gene (*NDN*) on chromosome 15 leading to abnormalities in the brainstem serotonergic system.[29] Mice lacking the necdin gene also have abnormal brainstem serotonergic neurochemistry.[30] Medullary serotonergic neurons are also CO_2 sensitive.[31] In genetically modified mice that do not develop medullary serotonergic neurons, CO_2 sensitivity is reduced by 50%.[32]

In some infants who have died of SIDs/sudden unexplained infant death (SUID), neuropathologic studies have shown disruptions of the brainstem serotonergic system.[27,33] While specific single-gene mutations that regulate serotonin production and function have not been identified in infants who have died of SIDS, some studies have shown a higher proportion of specific polymorphisms in the 5′ regulatory region of the *SLC6A4* gene, which encodes serotonin transporter, which regulates the reuptake of serotonin from the extracellular space. Specifically, infants who have died of SIDS have an increased frequency of the long allele variant and the variable number 12-tandem repeat in intron 2 polymorphisms[34] in the promoter. The long allele occurs more frequently in African-Americans; African-Americans also have a 2.0-fold greater incidence of SIDS/SUID than whites. The higher risk of SIDS/SUID in non-Hispanic Blacks when compared to non-Hispanic whites has remained consistent even though the overall incidence of SIDS/SUID has decreased in both groups.[35–37] Effectively, the polymorphisms result in increased activity of serotonin transporter, thereby decreasing the time that serotonin stays in the synapse, leading to a relative serotonin deficiency causing dysregulation of the cardiorespiratory system.

Rett syndrome is an X-linked disorder with mutations in several genes, but the most common genetic defect (90%) is in the methyl CpG binding protein 2 gene (*MECP2*). Affected individuals are

normal at birth and then experience progressive deterioration leading to severe motor, cognitive, and autistic behaviors. They also have characteristic severe respiratory disturbances with prolonged apnea and hyperventilation that can be fatal. Genetically modified mice that lack the *MECP2* gene have reduced levels of norepinephrine and serotonin in the medulla and have breathing patterns similar to those of humans with Rett syndrome.[38] Pharmacologic treatment to increase brain norepinephrine and serotonin levels stabilizes breathing and prolongs the life of these mice.[39] A case report demonstrating the efficacy of fluoxetine and buspirone in reducing breathing dysregulation in a patient with Rett syndrome has been published.[40] Altered GABA neurotransmission might also be causative as suggested by experiments using stem cells from a patient with Rett syndrome that demonstrated that the functional switch of GABA neurotransmission from excitation to inhibition was impaired.[41]

Congenital central hypoventilation syndrome (CCHS), also known as *Ondine's curse*, is another rare autosomal dominant genetic disorder, occurring in 1 in 200,000 live births. Affected individuals characteristically have adequate ventilation during wakefulness but profound hypoventilation during sleep as well as impaired ventilatory responses to CO_2 and hypoxia during sleep and wakefulness.[42] Although the disorder most commonly presents during infancy, milder forms may present later in childhood or even during adulthood. More than 90% of individuals with CCHS have mutations in the *PHOX2B* gene.[43–45] *PHOX2B* is a homeobox gene located on chromosome 4 that is specifically expressed in limited types of neurons involved in autonomic processes.[46] Its expression is required for the development of the carotid body, nTS, and catecholaminergic neurons. It is also expressed in chemosensitive glutamatergic neurons in the RTN that receive polysynaptic inputs from peripheral arterial chemoreceptors.[15] *Neurons in the nTS that express PHOX2B also appear to be chemosensitive to CO_2 in preclinical models*. Thus, mutations in the *PHOX2B* gene alter the development of key structures that regulate the chemical control of breathing.

The *PHOX2B* gene has a stretch of 20 alanine repeats in exon 3. Most affected individuals have the classic mutation that adds additional alanine repeats to the 20-alanine repeat, resulting in a stretch of 25 to 35 alanine repeats instead of 20. This mutation is classified as polyalanine repeat mutations (PARMs). The severity of the disease is correlated with the number of extra alanines. Patients with $PHOX2B^{20/25}$ have 25 versus 20 alanines; they have the mildest disease, may never need 24-hour ventilatory support, and may present only after infection or exposure to agents that inhibit respiration. On the other hand, patients with 28 to 32 alanine repeats in the gene ($PHOX2B^{20/28\text{-}32}$) often need continuous ventilatory support. Fewer affected individuals with CCHS have non-polyalanine repeat mutations (NPARMs) resulting in deletions in exon 3 that cause frameshift mutations. Depending on the mutation, some patients require tracheostomy and long-term ventilation and/or diaphragmatic pacing.[47] While respiratory stimulants are not effective in increasing respiratory drive, drugs that cause respiratory depression may be harmful.[48]

From genetically modified mouse models with mutations in the *PHOX2B* gene, we know that its expression is essential for the development of the RTN in the brainstem and catecholaminergic and cholinergic traits in the autonomic nervous system.[49] The RTN contains putative central chemoreceptors that have intrinsic pH sensitivity and release the excitatory neurotransmitter glutamate, thereby stimulating breathing during hypercapnia.[50] However, abnormalities throughout the autonomic nervous system are often seen in patients with CCHS. Specifically, patients can present with Hirschsprung disease (Haddad syndrome) and neuroblastomas (neuroblastoma-Hirschsprung disease-CCHS syndrome).[51] As reviewed by Moreira et al.[49] Hirschsprung disease occurs in 80% of patients with NPARM and 10% of patients with PARM. Similarly, neural crest tumors are found in 1% of PARM patients and in 41% of NPARM patients.

Bronchopulmonary Reflexes That Modulate the Central Respiratory Network

The nTS in the brainstem is where sensory information from vagally mediated reflexes and chemical signals from the blood (arterial chemoreceptors) and cerebrospinal fluid (central chemoreceptors) and information from higher brain regions are integrated; neurons from the nTS synapse onto respiratory-related neurons, thereby augmenting or attenuating minute ventilation.

Essentially all bronchopulmonary reflexes that modify the depth and duration of inspiration and expiration are mediated through the vagus nerve. The vagus nerve has both myelinated and unmyelinated fibers. Myelinated vagal afferent fibers are activated via (1) slowly adapting stretch receptors (SARs), which are activated by volume and stretch of the lung (mediating the Breuer-Hering reflex); or (2) rapidly adapting receptors (RARs) that are activated in response to inhaled irritants (e.g., ammonia, cigarette smoke) and large inflations or deflations of the lung.[52] Activation of SARs changes the duration of inspiration and expiration, whereas activation of RARs causes sighs (i.e., augmented breaths) and cough. Unmyelinated vagal afferents, specifically C-fibers in the airway, are activated by a multitude of chemical stimuli, including CO_2 and capsaicin, in addition to lung edema and elevated temperature. Activation of C-fibers in the lung causes rapid shallow breathing and apnea. Table 41.2 lists bronchopulmonary and upper airway reflexes and their physiologic responses.

Slowly Adapting Stretch Receptors: Major Modulators of Respiratory Timing

The duration of inspiratory or expiratory effort is greatly influenced by mechanoreceptors that are activated by changes in lung volume. The most well-characterized vagally mediated bronchopulmonary reflex is the pulmonary stretch reflex mediated through SARs, discovered by Josef Breuer in 1868. In adult cats, Breuer showed that expansion of the lungs reflexively inhibits inspiration and promotes expiration and that deflation of the lungs promotes inspiration and inhibits expiration.[53] In humans the contribution of the Breuer-Hering reflex to tidal breathing is determined by occlusion of the airway at the end of expiration. The following occluded inspiratory effort is prolonged, and expiratory effort is shortened. Alternatively, the occlusion can be performed at end of an inspiratory effort; then the following occluded expiratory effort is prolonged and inspiratory effort is shortened.[54] With this technique, the Breuer-Hering reflex significantly contributes to tidal breathing in infants. The reflex is strongest at birth and then decreases during the first year of life.[55] When lung volume is increased on CPAP the resultant prolongation of expiration and slowing of respiratory rate is a manifestation of the Breuer-Hering reflex.[56]

TABLE 41.2 Airway Receptors and Reflex Responses

Receptor	Characteristics	Stimulant	Responses	Comment
Slowly adapting stretch receptors	1. Mechanoreceptors 2. Mediated by fast-conducting, myelinated vagal fibers 3. Located in lung parenchyma	Lung volume and transmural pressure	1. Breuer-Hering reflex 2. Termination of inspiration and prolongation of expiration 3. Bronchodilation 4. Tachycardia	Breuer-Hering reflex more active in infants than in adults
Rapidly adapting receptors	1. Mechanoreceptors 2. Irritant receptors 3. Located throughout the airways 4. Mediated by fast-conducting, myelinated vagal fibers	1. Inhaled irritants 2. Low lung volumes	1. Cough 2. Mucus production 3. Augmented breaths (sighs)	Responsible for inducing sighs in premature infants—restoring functional residual capacity
Bronchial and pulmonary C fibers	1. Located throughout the airway from the nose to alveoli 2. Stimulated by substances in the pulmonary circulation and inhaled 3. Slowly conducting, nonmyelinated vagal fibers	1. Capsaicin 2. Respiratory irritants 3. Lung edema 4. Inflammatory mediators	1. Rapid, shallow breathing 2. Apnea 3. Bronchoconstriction 4. Laryngoconstriction 5. Mucus secretion 6. Vasodilatation (pulmonary C fibers) 7. Bradycardia	J-receptors located in alveoli activated by lung edema
Laryngeal chemoreflex	1. Potent airway-protective reflex from aspiration 2. Receptors in laryngeal mucosa 3. Mediated by sensory fibers in the superior laryngeal nerve	1. Hypoosmolarity 2. Low chloride content	Response in newborns: 1. Hypoventilation/apnea 2. Laryngoconstriction 3. Swallowing 4. Bradycardia 5. Shunting of blood flow to brain, heart, adrenals Response in adults: 1. Cough 2. Arousal 3. Swallowing	May contribute to apnea and bradycardic events associated with oral feedings in premature infants. Immature responses are exacerbated during hypoxia

Modified from Kubin L, Alheid GF, Zuperku EJ, et al. Central pathways of pulmonary and lower airway vagal afferents. *J Appl Physiol (1985).* 2006;101:618–627; Thach BT. Maturation and transformation of reflexes that protect the laryngeal airway from liquid aspiration from fetal to adult life. *Am J Med.* 2001;111(suppl 8A):69S–77S; and Widdicombe J. Reflexes from the lungs and airways: historical perspective. *J Appl Physiol. (1985)* 2006;101:628–634.

Rapidly Adapting Receptors: Cough, Augmented Breaths

Lung deflation, mechanical stimulation, and chemical irritants also stimulate vagal afferents of RARs, causing augmented breaths, cough, and increased mucus production.[53] RARs are also activated at low lung volumes. Newborns, especially premature newborns, have excessive compliance of the chest wall predisposing them to low lung volumes during tidal breathing. Activation of RARs and the resulting augmented breath are particularly important in restoring lung inflation in premature and term infants. The frequency of augmented breaths is inversely related to gestational age, with premature infants having the greatest frequency[57] when compared with term infants and adults.

C-Fiber Receptors: Apnea, Bronchoconstriction, Rapid Shallow Breathing

Pulmonary and bronchial C-fiber receptors are unmyelinated vagal fibers located throughout the respiratory tract, extending from the nose to the lung parenchyma. Pulmonary C-fibers are accessible from the pulmonary circulation, whereas bronchial C-fibers are accessible from the bronchial circulation and have similar sensitivity to various stimuli.[58] C-fibers are activated by a variety of substances: inflammatory mediators, capsaicin, lobeline, and phenylbiguanidine. Capsaicin and phenylbiguanidine are used experimentally to identify vagal afferents as C-fibers and characterize stimulus-response profiles. C-fiber simulation induces central and local effects—cough, apnea, laryngospasm, and bronchoconstriction—followed by rapid shallow breathing, bradycardia, and hypotension. Juxtacapillary receptors (J receptors) are composed of C-fibers located in the alveolar walls. They are activated by lung edema and congestion and cause rapid shallow breathing. By far the most common respiratory response from C-fiber stimulation is reflex apnea characterized by prolongation of the expiratory time from excitation of post-inspiratory neurons and continuous firing of central expiratory neurons.[58]

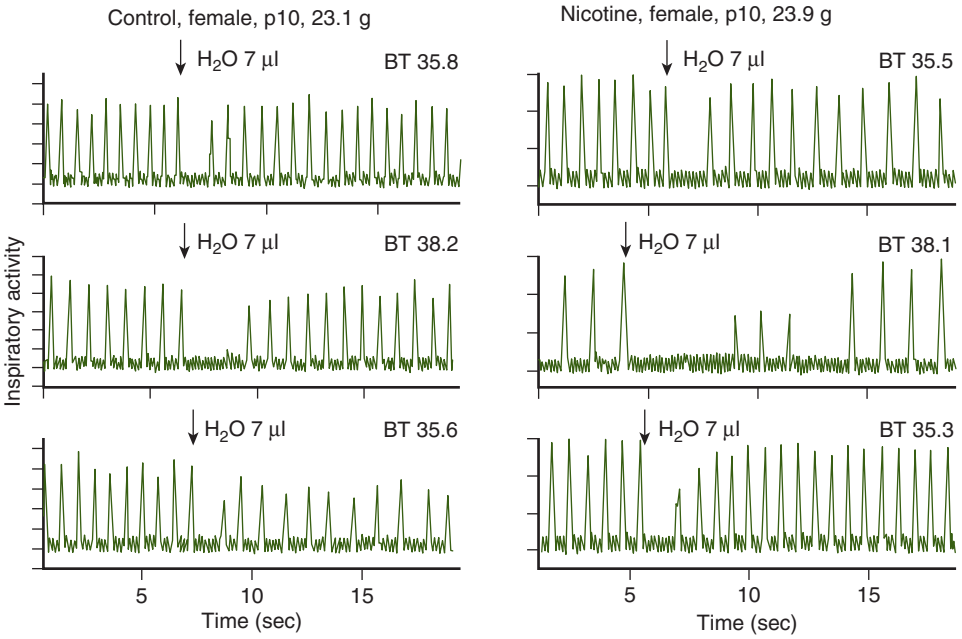
In newborns the stimulation of pulmonary C-fibers by chemical stimulants causes bronchoconstriction and apnea.[59] Capsaicin-induced apneic response and the sensitivity of the reflex were greatest in newborn rat pups younger than 10 postnatal days.[60] Bronchopulmonary C-fibers are also stimulated by acidosis, adenosine, reactive oxygen species, hyperosmotic solutions, and lung edema. Furthermore, inflammatory mediators in the local environment sensitize C fibers to other stimuli.[61]

Pulmonary C-fiber-mediated respiratory inhibition may cause persistent apnea beyond term gestation in infants born at the limit of viability who have lung inflammation from chronic lung disease.[7] Local C-fiber activation in the lung may also be

the mechanism accounting for the increase in apnea observed in infants with viral infections[62] and unlikely to be from direct viral invasion of the central nervous system.[63] Prenatal exposure to nicotine/tobacco smoke is a known risk factor for SIDS. In newborn rat pups, prenatal nicotine exposure sensitizes bronchopulmonary C fibers and prolongs apnea in responses to C-fiber stimulation. As outlined already, C-fibers are also activated by inflammation.[64] The epidemiologic association between infection, prenatal tobacco smoke exposure, and SIDS could be attributed to sensitization of bronchopulmonary C-fibers from tobacco smoke exposure, confounded by acute infection leading to prolonged apnea from which the infant does not recover.[65]

Laryngeal Reflexes

Receptors that respond to changes in upper airway pressure and chemicals are abundantly distributed throughout the laryngeal mucosa. These receptors can be slowly adapting, rapidly adapting irritant receptors, or C-fibers. Water receptors that are stimulated by hypo-osmolarity and low chloride content may also be involved. Stimulation of upper airway mechanoreceptors and chemoreceptors modifies the activity of upper airway muscles as well as the pattern and timing of diaphragmatic activity. The upper airway reflex that mediates significant cardiorespiratory effects that occur in newborns is the laryngeal chemoreflex (LCR). The LCR is one of the most potent defensive reflexes protecting the respiratory tract from inadvertent aspiration.[66] Laryngeal chemoreceptors are stimulated by liquid in the airway, which induces coughing, swallowing, and arousal in mature models. However, the response in immature models is apnea followed by hypoventilation, laryngeal constriction, and swallowing.[67] In addition to respiratory inhibition, bradycardia, peripheral vasoconstriction, and redistribution of blood flow also occur. The associated apnea and bradycardia can be life threatening in newborns,[67–70] and in newborns baseline hypoxemia enhances the severity of the apnea and bradycardia induced by the LCR.[71] Afferent fibers for this reflex travel in the superior laryngeal nerve, a branch of the vagus nerve. These afferents synapse with neurons in the nTS, which then send (1) excitatory projections to motoneurons of the recurrent laryngeal nerve in the nucleus ambiguus, causing constriction of the thyroarytenoid muscle (laryngeal constrictor), resulting in laryngospasm; (2) inhibitory projections to phrenic motoneurons in the cervical spinal cord, inhibiting diaphragmatic contraction, resulting in apnea; and (3) an excitatory pathway to cardiac vagal neurons in the nucleus ambiguus, causing bradycardia. The LCR occurs in the fetus and likely functions to prevent aspiration of amniotic fluid, which contains approximately half the chloride content of pulmonary fluid.[72,73] In premature infants the reflex may be involved in the apnea and bradycardic responses associated with feeds and gastroesophageal reflux (GER) that reaches the larynx or nasopharynx. Whether the immature response is still present in term infants or how the maturation of the reflex is affected by premature birth has not been determined. Studies in newborn animals show that the apnea associated with the LCR is prolonged during hyperthermia in newborn rats[74] and is further accentuated if the animal was exposed prenatally to nicotine.[75] This relationship is illustrated in Fig. 41.2.



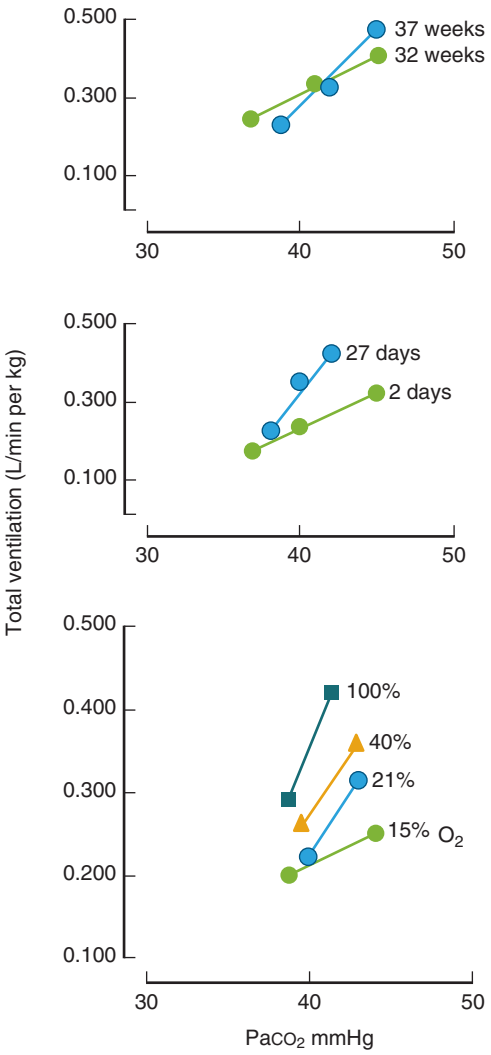
• **Fig. 41.2** Prenatal Exposure to Nicotine and Increased Body Temperature Prolongs the Laryngeal Chemoreflex in Rat Pups at Postnatal Day 10. Example of laryngeal chemoreflex from female postnatal day 10 rat pups during baseline conditions *(top panels)*, during mild hyperthermia *(middle panels)*, and during a final, recovery period *(bottom panels)*. The data displayed on the *left* were obtained from a rat pup born to a control dam, and the data displayed on the *right* were obtained from a rat pup born to a dam infused with nicotine during pregnancy. The *downward arrows* indicate the time at which small volumes of water were injected into the larynx, and the body temperature *(BT)* is listed to the right of each example. Note the prolonged apnea and respiratory disruption during hyperthermia compared with normothermic conditions, and note that maternal exposure to nicotine markedly prolonged the laryngeal chemoreflex in the rat pup shown on the right. (From Xia L, Leiter JC, Bartlett D Jr. Gestational nicotine exposure exaggerates hypothermic enhancement of laryngeal chemoreflex in rat pups. *Respir Physiol Neurobiol*. 2010;171:17–21.)

Because of profound inhibitory cardiorespiratory effects that are further accentuated by prenatal nicotine exposure, stimulation of the LCR may be an important reflex that is operative in some SIDS cases and infants with acute life-threatening events.[67,76–78] Overheating and prenatal exposure to nicotine are both risk factors highly associated with SIDS.[27]

Maturation of CO_2/H^+ Sensitivity of Central Chemoreceptors

In air-breathing animals, respiratory rhythmogenesis is primarily driven by the level of $Paco_2$ in the blood and cerebrospinal fluid and, to a lesser extent, by oxygen tension. For every 1 mmHg increase in $PaCo_2$, ventilation will increase by 20% to 30%. Specialized chemosensitive cells in the brainstem depolarize in response to changes in CO_2/H^+ concentration; they drive breathing through synaptic inputs to respiratory-related neurons.[79] Although peripheral arterial chemoreceptors in the carotid body also depolarize in response to increasing CO_2/H^+ concentration, these receptors are primarily responsible for modifying breathing in response to changes in oxygen tension (reviewed later). In fetal sheep, hypercapnia causes an increase in the depth of fetal breathing movements, with no change in respiratory rate. In humans, maternal exposure to CO_2 also increases fetal breathing.[80] Ventilatory responses to CO_2 are present immediately after birth in most mammalian species, and CO_2 sensitivity increases with maturation. However, in the newborn rat, CO_2 sensitivity is robust during the first several days of postnatal life; it declines markedly in the next 2 weeks of life and then gradually increases to reach adult levels by the end of the third week.[81] Premature and term infants tested at 2 days of postnatal age have modest ventilatory responses to 2% and 4% CO_2, although the strength of the ventilatory response is less in the more immature infants.[82] In premature and late preterm newborns, CO_2 sensitivity increases with postnatal age, reaching a mature response by four weeks of postnatal age (Fig. 41.3).[83,84] Similar to the response in the fetus, the increase in ventilation is predominantly due to an increase in tidal volume and not respiratory rate. If the increase in inspiratory effort against an occluded airway is used as an indicator of central respiratory drive, the increase in CO_2 sensitivity with postnatal development is due to an increase in central respiratory drive in human infants.[82] Premature infants with apnea of prematurity have reduced ventilatory responses to CO_2 compared with control infants of the same postconceptional age.[85,86] This finding suggests that infants with apnea of prematurity have reduced central respiratory drive to breathe when compared with infants who do not have apnea of prematurity at the same postconceptional age.

It is unknown whether the maturation of synaptic inputs from chemosensitive neurons to respiratory-related neurons in the brainstem or the maturation of intrinsic properties of chemosensitive neurons accounts for the increase in CO_2 sensitivity with early postnatal development. Although such studies of human infants are impossible, data from studies performed in neonatal rats show that intrinsic responses of chemosensitive neurons in the nTS and locus ceruleus are already mature at birth. It is less clear whether there is a developmental increase in the sensitivity of chemosensitive neurons in the medullary raphé, an increase in the number of chemosensitive neurons in the RTN, or both.[21] However, within the first several weeks of postnatal development in the rat, the size of brainstem neurons changes,



• **Fig. 41.3** The relationship between ventilatory responses to carbon dioxide and gestational age *(top panel)*, postnatal age *(middle panel)*, and the concentration of inspired oxygen *(bottom panel)*. $Paco_2$, Partial pressure of carbon dioxide. (From Rigatto H, Brady JP, de la Torre Verduzco R. Chemoreceptor reflexes in preterm infants: II. The effect of gestational and postnatal age on the ventilatory response to inhaled carbon dioxide. Pediatrics. 1975;55[5]:614–620; Rigatto H, De La Torre Verduzco R, Gates DB. Effects of O_2 on the ventilatory response to CO_2 in preterm infants. *J Appl Physiol*. 1975;39[6]:896–899.)

and both dendritic arborization in the nTS and astrocyte proliferation increase. Astrocytes contribute substantially to the pH of the extracellular milieu surrounding chemosensitive neurons.[21] It is likely that all these morphologic and neurochemical changes within and between neurons and astrocytes in the brainstem contribute to the maturation of CO_2 sensitivity in the early weeks of postnatal development.

Maturation of O_2 Sensitivity of Peripheral Arterial Chemoreceptors

The peripheral arterial chemoreceptors in the carotid body, located at the bifurcation of the carotid artery, are best known for reflex control of ventilation in response to changes in arterial oxygen tension.[87] However, specialized cells within the carotid

body also depolarize in response to changes in blood CO_2/H^+ concentration, reflexively increasing ventilation in response to acidosis and hypercapnia and decreasing ventilation in response to hypocapnia. Histologically, the carotid body chemoreceptors consist of (1) type 1 or glomus cells, similar to presynaptic neurons, which are chemosensitive and contain neurotransmitters and autoreceptors; (2) postsynaptic afferent nerve fibers from the carotid sinus nerve, which oppose glomus cells,[87,88] contain neurotransmitters and postsynaptic receptors, and have cell bodies in the petrosal ganglion;[87,88] (3) type 2 cells, similar to glial cells, which are not chemosensitive; (4) microganglion cells that express cholinergic traits;[89] and (5) blood vessels and sympathetic fibers innervating these vessels. In response to hypoxia or increased CO_2/H^+ concentration, type 1 cells depolarize. The major excitatory neurotransmitter in peripheral arterial chemoreceptors is ATP. ATP then binds to the rapid cation ligand-gated ion channel on postsynaptic receptors on the carotid sinus nerve. Neuromodulators, inhibitory (such as dopamine) and excitatory, further shape the response. Changes in ventilation during the first 30 seconds of hypoxia are used to assess the maturation of hypoxic chemosensitivity of the peripheral arterial chemoreceptors, while changes in ventilation in response to hyperoxia measure the contribution of peripheral arterial chemoreceptors on baseline breathing. Hypoxic and hyperoxic sensitivity do not follow the same developmental trajectory. Although oxygen tension less than 25 mmHg stimulates the carotid body in exteriorized fetal sheep, peripheral arterial chemoreceptors are not functional at any level of hypoxia for the first several days after birth in most mammalian species (for a historical overview, see Walker, 1984), including human infants (term and preterm). The rapid increase in arterial oxygen tension during the transition from fetal to neonatal life likely contributes to the resetting of the activation of the carotid body to higher oxygen tension. After birth, hypoxic chemosensitivity gradually *increases* with postnatal maturation and reaches adult levels by 2 to 3 weeks postnatally.[90,91] On the other hand, the contribution of peripheral arterial chemoreceptors to eupneic breathing determined by a drop in ventilation in response to hyperoxia gradually *decreases* with maturation; the drop in ventilation in response to hyperoxia is greatest in premature versus term infants and least in adults.[92,93] Moreover, premature infants who have a greater frequency of apnea and periodic breathing have a greater reduction in ventilation when exposed to fractional inspired oxygen of 100% (Dejours test). While activity from the peripheral arterial chemoreceptors is not essential for breathing to be established at birth, trophic factors from peripheral arterial chemoreceptors acting on central mechanisms that control breathing during early postnatal development are key in stabilizing rhythmogenesis so that breathing is maintained. The first two weeks of postnatal development appear to be the critical period for this trophic influence since sectioning of the carotid sinus nerve (the conduit for trophic support to the central respiratory network) results in the death of several mammalian species from respiratory failure several weeks after denervation.[94] Exposures during this critical period of development (reviewed in Gauda et al.[65]) that can lead to sustained alterations in chemoreceptor function include

- Environmental exposure to the extremes of oxygen tension (chronic or intermittent hypoxia and hyperoxia)[91,95,96]
- Nicotine exposure[97–99]
- Maternal separation—in male rats[100]
- Perinatal inflammation[101,102]

Hypoxic Ventilatory Depression: Consequences for the Neonate

Although the peripheral arterial chemoreceptors function to increase ventilation in response to hypoxia, minute ventilation significantly declines after 2 to 3 minutes of hypoxic exposure. This decline is commonly referred to as *hypoxic roll-off, hypoxic ventilatory decline*, or *hypoxic ventilatory depression*. Hypoxic ventilatory depression occurs in individuals of all ages, but it is most pronounced in the fetus and newborn.[103] Whereas the hypoxic ventilatory decline is usually still above baseline ventilation in mature models, the hypoventilatory response in newborns is usually below baseline ventilation and is often associated with apnea. This hypoxic roll-off is not because of a decline in activity of peripheral arterial chemoreceptors. Mechanisms accounting for hypoxic respiratory depression are most well characterized in the fetal animals in which the central brainstem nuclei mediating this response have been attributed to the pons. Transverse section of the upper pons results in a sustained hyperventilatory response to hypoxia in fetal and newborn sheep.[104] Hypoxia activates expiratory neurons in the ventrolateral pons, and chemical blockade of this area blocks the hypoxic respiratory depression in newborn rats.[105]

Several neuromodulators have been implicated in mediating hypoxic ventilatory decline, including norepinephrine, adenosine, GABA, serotonin, opioids, and platelet-derived growth factor, as shown in Table 41.1.[106] All these neuromodulators contribute to the ventilatory depression in newborns, but particular attention has been paid to adenosine. Degradation of intracellular and extracellular ATP is the main source of extracellular adenosine, which then mediates its cellular effects by binding to A_1, A_{2a}, A_{2b}, and A_3 adenosine receptors. In response to hypoxia, brain adenosine levels can increase 2.3-fold in fetal sheep[107] and 100-fold in rats in response to ischemia.[108] Non-specific adenosine receptor blockers, particularly caffeine and methylxanthines, are commonly administered to premature infants to increase central respiratory drive, and aminophylline inhibits hypoxic ventilatory depression in newborns.[109] A_1 adenosine inhibitory receptors are found on respiratory-related neurons.[103] Specific A_1 adenosine receptor agonists depress phrenic output in a reduced brainstem spinal cord preparation, whereas A_1 adenosine receptor blockers reverse this inhibitory effect.[110] In fetal sheep the hypoxic respiratory depression appears to be mediated by excitatory A_{2a} receptors because blockade of A_{2a} receptors eliminates hypoxic ventilatory roll-off in conscious newborn sheep.[107] Xanthines (e.g., caffeine, aminophylline) block both A_1 and A_{2a} adenosine receptors; therefore, their effectiveness in stabilizing ventilation and decreasing the frequency of apnea in premature infants may be by directly altering the ventilatory response to hypoxia as well as by nonspecifically increasing respiratory drive (see Apnea of Prematurity).

Effect of Sleep State on Breathing

Sleep state has a profound influence on breathing in the fetus and newborn, and most disorders of breathing that affect the young and old are worse during sleep. Active sleep (AS) and quiet sleep (QS) in infants are equivalent to rapid eye movement (REM) and non-REM sleep, respectively in older children and adults. Breathing during AS is mostly driven by inputs from the reticular activating system, with less influence from $Paco_2$, whereas breathing during QS is driven by chemical control. Similar to REM sleep in adults, AS is associated with paralysis of striated muscles. Although this

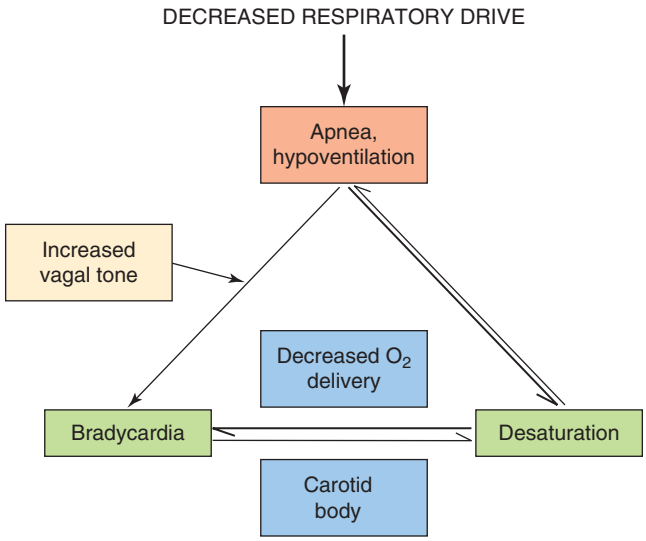
paralysis may be necessary to prevent the acting out of dreams, paralysis of striated muscles that are involved in breathing can be problematic for the newborn. Breathing becomes more irregular in AS and REM sleep because of inhibition of the intercostal muscles and upper airway dilating muscles. The discoordination between chest wall muscles and the diaphragm during AS causes paradoxical breathing: the chest wall moves inward during inspiration, with the abdomen moving outward. The more compliant the chest wall, the greater propensity for paradoxical breathing, which is common in the most immature infants. In addition, during inspiration, intrathoracic pressure becomes more negative, and this "suction pressure" causes narrowing or collapse of the compliant upper airway, particularly pharyngeal structures, leading to upper airway obstruction. Paradoxical breathing movements seen on physical examination or detected on inductive plethysmography are often interpreted as a sign of upper airway obstruction. During QS, breathing is characterized by smooth, regular breaths of consistent frequency and depth associated with tonic and phasic activity of the muscles of respiration that are in phase with each other. The chest wall and the abdomen move outward during inspiration, whereas they move inward during expiration. AS and QS can be reliably assessed at 30 to 32 weeks' gestation.[111] At this gestational stage, premature infants spend 80% of their sleep time in AS; the proportion decreases to 50% by term, and in adulthood, REM sleep accounts for only 20% of sleep time. Sleep state in normal infants also modifies the time to arousal and the ventilatory responses. The time to arousal on exposure to a hypoxic, somatosensory, or auditory stimulus is greater in AS than with QS in infants during the first 6 months of life.[112] The arousal latency in response to a hypoxic stimulus in AS is longer in preterm infants at 2 to 5 weeks' postnatal age than that of term infants at the same postnatal age.[113] The level of oxygen desaturation at the time hypoxic arousal occurs is similar in AS and QS.[114] Irregular respiratory patterns are more common during AS in both term and premature infants, however, periodic breathing and the ventilatory response to CO_2 are greater during QS.[115] Because of the complexity of the ventilatory response to hypoxia and the frequent occurrence of arousals induced by hypoxic exposure, assessment of the effect of sleep state on the ventilatory response to hypoxia in newborns is more difficult. Other than the clear difference in arousal in response to a hypoxic stimulus between the two sleep states, differences between sleep states in other respiratory parameters are more variable.[114] Recent data in preterm infants indicate that even long apneas or hypoxia fail to elicit arousals or any sign of sleep disruption.[116] Arousal may, therefore, not be the main mechanism for apnea termination in preterm infants.

Although most disorders of breathing, such as obstructive sleep apnea, become more severe during sleep, the breathing disorder that is most significantly influenced by sleep state in the newborn is CCHS. As noted previously, CCHS is characterized by abnormalities in chemical control of breathing; therefore, with maturation, as the frequency of QS increases, so does the severity of the disorder. During QS, CO_2 sensitivity is markedly impaired in affected individuals, and exposure to hypercapnia does not significantly increase minute ventilation.[117] Of infants who died of SIDS, their sleep state during their terminal event was unknown. However, adequate arousal mechanisms are key in preventing respiratory failure and death, and impaired arousal mechanisms are hypothesized to be causative in SIDS. Prone sleeping position increases the percentage of QS,[118] and QS is associated with increased time to hypoxic arousal in human infants.[114] A hypoxic microenvironment from rebreathing with defective arousal and auto-resuscitative mechanisms is hypothesized to have occurred in infants who have died of SIDS in the face-down (i.e., prone) sleeping position.[119] Therefore, sleep state can have a significant influence on the control of breathing during health and disease, especially in the newborn.

Thus far this chapter has outlined the neurocircuitry and neurochemistry of the respiratory network along with its synaptic inputs that undergo significant maturation during the newborn period. Because these pathways are less developed in premature infants, premature infants have apnea of prematurity, which often requires active therapeutic intervention and can delay hospital discharge. The components of the respiratory network, similarly to other developing organ systems, are plastic and uniquely vulnerable to pathologic processes in premature infants.[65] Therefore, the episodes of intermittent hypoxemia and bradycardia that accompany apnea of prematurity may be a cause of acute and chronic morbidities in this high-risk population.

Apnea of Prematurity

Respiratory pauses are universal features in infants born prematurely and are most prominent in the lowest gestational-aged infants. There is no consensus as to when a respiratory pause can be defined as an apneic episode. It has been proposed that apnea be defined by its duration (e.g., longer than 15 seconds) or accompanying bradycardia and desaturations. However, even the 5 to 10-second pauses that occur in periodic breathing can be associated with bradycardia or desaturation. It should be emphasized that periodic breathing (ventilatory cycles of 10 to 15 seconds with pauses of 5 to 10 seconds) is a normal breathing pattern that should not require therapeutic intervention. It is thought to be the result of dominant peripheral chemoreceptor activity responding to fluctuations in arterial oxygen tension. Episodic bradycardia and desaturation in preterm infants are almost invariably secondary to apnea or hypoventilation (Fig. 41.4).[120] The rapidity of the fall in oxygen saturation after a respiratory pause is directly proportional to baseline oxygenation, which is

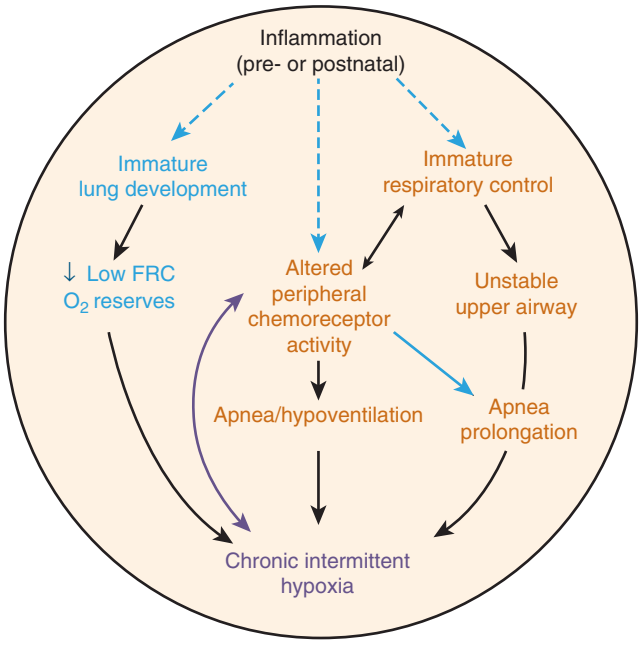


• **Fig. 41.4** The sequence of the events whereby apnea results in various combinations of desaturation and bradycardia. (From Martin RJ, Abu-Shaweesh JM. Control of breathing and neonatal apnea. *Biol Neonate*. 2005;87:288–295.)

related to lung volume and the severity of lung disease.[121] Prenatal and postnatal inflammation directly affect the developing lung and peripheral arterial chemoreceptors,[102] which further increases the frequency of oxygen desaturations during short respiratory pauses (Fig. 41.5).[121]

Apnea is classified traditionally into three categories on the basis of the absence or presence of upper airway obstruction: central, obstructive, and mixed. Central apnea is characterized by total cessation of inspiratory efforts with no evidence of obstruction. In obstructive apnea, the infant tries to breathe against an obstructed upper airway, resulting in chest wall motion without airflow through the entire apneic episode. Mixed apnea consists of obstructed respiratory efforts, usually following central pauses. The site of obstruction in the upper airways is primarily in the pharynx, although it also may occur in the larynx and possibly at both sites. It is assumed that there is an initial loss of central respiratory drive, and its recovery is accompanied by a delay in activation of upper airway muscles superimposed on a closed upper airway.[54] Mixed apnea typically accounts for more than 50% of long apneic episodes, followed in decreasing frequency by central apnea and obstructive apnea. Purely obstructive spontaneous apnea in the absence of a positional problem is probably uncommon. Because standard impedance monitoring of respiratory efforts via chest wall motion cannot recognize obstructed respiratory efforts, mixed versus obstructive apnea is frequently identified by the accompanying bradycardia or desaturation.[122,123]

Presentation of apnea can reflect a non-specific alteration in either the environment (e.g., thermal) or the general well-being of preterm infants. For example, neonatal sepsis can manifest itself as an increase in the frequency or severity of apnea, and the underlying cause must be treated. Studies using a rat pup model suggest that the systemically released cytokine interleukin-1β binds to its receptor on vascular endothelial cells at the blood-brain barrier.



• **Fig. 41.5** The consequences of the adverse effects of prenatal or postnatal inflammation on the developing lung and respiratory network leading to the emergence of chronic intermittent hypoxia in premature infants. *FRC*, Functional residual capacity. (From Di Fiore JM, Martin JM, Gauda EB. Apnea of prematurity—perfect storm. *Respir Physiol Neurobiol*. 2013;189:213–222.)

This binding induces the synthesis of prostaglandin E_2, which induces respiratory depression in the brainstem.[124] These studies provide insight into mechanisms whereby sepsis often manifests itself as apnea of prematurity. Anemia, presumably via decreased oxygen delivery, is also frequently implicated as a cause of apnea. Transfusion of packed red cells can provide some variable benefit for apnea of prematurity.[125] Furthermore, there may be some utility in reducing persistent intermittent desaturations in extremely low birth weight newborns younger than 7 days of age;[126] this is recently confirmed by Kovatis et al.[127]

Therapeutic Approaches

Continuous Positive Airway Pressure

Continuous positive airway pressure (CPAP) of 4 to 6 cmH_2O is a relatively safe and effective therapy for apnea of prematurity. Because longer episodes of apnea frequently involve an obstructive component, CPAP appears to be effective by splinting the upper airway with positive pressure and decreasing the risk of pharyngeal or laryngeal obstruction.[128] CPAP also has a beneficial effect in apnea by increasing functional residual capacity, thereby improving oxygenation status. At a higher functional residual capacity, the time from cessation of breathing to desaturation and resultant bradycardia is prolonged. Heated high-flow nasal cannula therapy has been suggested as an equivalent treatment modality to allow CPAP delivery while improving ease of care. Although this approach is used widely, its efficacy for the treatment of apnea of prematurity has not been studied in depth. For example, a recent small study in 10 premature infants with a crossover design suggested that work of breathing may be greater when infants are treated with heated high-flow nasal cannula therapy (HHFNC) versus CPAP therapy.[129] Non-invasive ventilatory strategies, using a nasal mask to deliver intermittent positive pressure, avoid the need for full ventilatory support in some infants and can be effective in reducing the need for reintubation for persistent apnea.[130] However, for severe or refractory episodes, endotracheal intubation and mechanical ventilation may be needed. Minimal ventilator settings should be used to allow for spontaneous ventilatory efforts and to minimize the risk of barotrauma.

Methylxanthines

Methylxanthines have been the mainstay of pharmacologic treatment of apnea of prematurity for several decades. Both theophylline and caffeine are used and have multiple physiologic and pharmacologic mechanisms of action. Xanthine therapy appears to increase minute ventilation, improve CO_2 sensitivity, decrease hypoxic depression of breathing, enhance diaphragmatic activity, and decrease periodic breathing. The likely major mechanism of action is through competitive antagonism of adenosine receptors. Adenosine acts as an inhibitory neuroregulator in the central nervous system via activation of adenosine A_1 receptors.[131] In addition, activation of adenosine A_{2a} receptors appears to excite GABAergic interneurons, and released GABA may contribute to the respiratory inhibition induced by adenosine.[132]

Methylxanthines have some well-documented short-term adverse effects. Toxic levels can produce tachycardia, cardiac dysrhythmias, feeding intolerance, and seizures (infrequently), although these effects are seen less commonly with caffeine at the usual therapeutic doses. Mild diuresis is caused by all methylxanthines. The observation that xanthine therapy causes an increase

in metabolic rate and oxygen consumption of approximately 20% suggests that caloric demands can be increased with this therapy at a time when nutritional intake is already compromised.[133]

A large, international, multicenter clinical trial was designed to test short-term and long-term safety of caffeine therapy for apnea of prematurity. In the neonatal period, caffeine treatment was associated with a significant reduction in the postmenstrual ages at which both supplemental oxygen and endotracheal intubation were needed.[134] Of even greater interest was the significant decrease in the incidence of cerebral palsy and cognitive delay in the caffeine-treated group, which was not sustained by 5 years of age,[135] but caffeine exposure was associated with a sustained beneficial effect on developmental coordination disorders at 5 years of age.[136] This finding raises interesting questions regarding possible mechanisms underlying this beneficial effect of caffeine on neurodevelopmental outcomes (Fig. 41.6). These beneficial effects include the observation in animal models that loss of the adenosine A_1 receptor gene is protective against hypoxia-induced loss of brain matter[137] and a potential benefit of caffeine on immune mechanisms that mediate lung and brain injury.[138] Recent data in rodent models also support a beneficial anti-inflammatory effect of xanthine therapy in immature lungs exposed to the proinflammatory effects of prenatal exposure to lipopolysaccharide, postnatal hyperoxia, or both.[139,140] The inhibitory effects of caffeine on adenosine receptor subtypes may differ depending on the xanthine concentrations; this should present a note of caution in the clinical situation when high doses of caffeine are considered. Data in an immature ovine model do not, however, demonstrate an adverse effect on developing white matter.[141] Interestingly, there was a reduced risk of motor impairment at 11 years of age in the multicentral trial cohort without clear academic benefit. The precise reasons for this selective benefit are not entirely clear.[142]

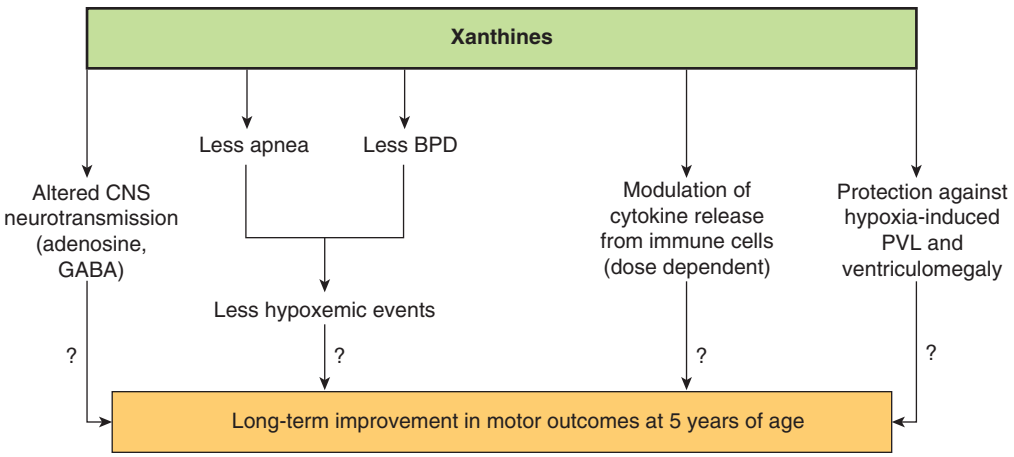
In recent years the practice of caffeine administration has expanded as a result of both increased use of non-invasive ventilation and the success of the earlier caffeine trial. Caffeine is now widely used in a prophylactic mode to prevent, rather than treat, clinically significant apnea; early onset of treatment appears beneficial.[143] Extended use beyond the apparent resolution of apnea has also been proposed to decrease intermittent hypoxic episodes.[144] As already suggested, increased dosing beyond the traditional caffeine citrate loading dose of 20mg/kg followed by a maintenance dose of 5 to 10 mg/kg every 24 hours should, however, proceed with caution pending further study. A recent small pilot randomized trial of high-dose caffeine therapy suggested an association between a high loading dose and cerebellar injury on magnetic resonance imaging in preterm infants.[145] Although measurement of serum caffeine levels is not widely practiced, these levels may not become subtherapeutic until 11 to 12 days after cessation of treatment.[146] Increased respiratory drive may increase within minutes after intravenous caffeine administration as measured by diaphragmatic activity in preterm infants.[147]

In conclusion, safety and efficacy have been clearly established for the timing and dosing of caffeine as employed in the international multicenter clinical trial cited earlier.[134] For infants with apnea and about to be extubated, clinicians can prescribe the drug with confidence.[148] However, in asymptomatic infants not on respiratory support xanthine therapy may not be warranted. It remains unclear whether earlier and longer duration therapy might confer additional benefit.

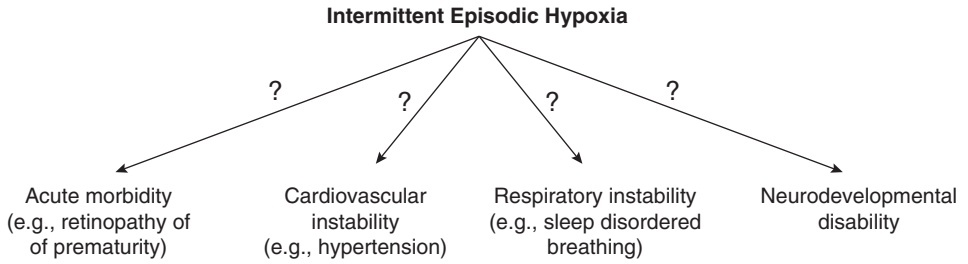
Doxapram has been proposed as an additional mode of pharmacotherapy in the face of apnea resistance to xanthine therapy. This is currently under study in Europe. However, until efficacy versus safety data become available, doxapram cannot be recommended.[149]

Gastroesophageal Reflux and Apnea of Prematurity

GER is often incriminated as a cause of neonatal apnea. Despite the frequent coexistence of apnea and GER in preterm infants, investigations of the timing of reflux in relation to apneic events indicate that they are rarely related temporally. When these events coincide, there is no evidence that GER prolongs the concurrent



• **Fig. 41.6** Multiple proposed mechanisms are demonstrated whereby xanthine therapy for apnea of prematurity may improve motor outcomes in former premature infants. These outcomes include functional changes in neurotransmitters in the brain, a decrease in hypoxemic episodes that accompany apnea, especially in the presence of bronchopulmonary dysplasia *(BPD)*, a proposed protective effect of adenosine receptor inhibition on hypoxia-induced white matter injury, and the beneficial effect of adenosine receptor blockade on inflammatory cytokine-mediated lung or brain injury. *CNS*, Central nervous system; *GABA*, γ-aminobutyric acid; *PVL*, periventricular leukomalacia. (Modified from Abu-Shaweesh JM, Martin RJ. Neonatal apnea: what's new? *Pediatr Pulmonol*. 2008;43:937–944.)



• **Fig. 41.7** Proposed acute and longer-term morbidities that might be a consequence of intermittent hypoxic episodes in early postnatal life. (Modified from Martin RJ, Wilson CG. What to do about apnea of prematurity. *J Appl Physiol*. 2009;107:1015–1016.)

apnea.[150] Although physiologic experiments in animal models reveal that reflux of gastric contents to the larynx induces reflex apnea, there is no clear evidence that treatment of reflux affects the frequency of apnea in most preterm infants. Therefore, pharmacologic management of reflux with agents that decrease gastric acidity or enhance gastrointestinal motility generally should be reserved for preterm infants who exhibit signs of emesis or regurgitation of feedings, regardless of whether apnea is present, and infants at specific risk (e.g., exhibiting neurodevelopmental delay or following gastrointestinal surgical repair). Extreme caution should be taken when histamine H_2 receptor blockers are used to change gastric pH since the use of these agents in hospitalized very low birth weight infants has been associated with an increased incidence of necrotizing enterocolitis and increased mortality.[151] Therapy for such infants should begin with non-pharmacologic approaches, such as thickened feeds because acid suppression therapy has been shown to alter the gastrointestinal microbiome and increase the risk of lower respiratory infection in infants[152] and necrotizing enterocolitis.[153] There remain considerable differences of opinion among neonatologists, pediatric gastroenterologists, and pediatric pulmonologists regarding the diagnosis and management of this problem.[154]

Resolution and Consequences of Neonatal Apnea

Apnea of prematurity generally resolves by 36 to 40 weeks' postconceptional age; however, apnea frequently persists beyond this time in more immature infants. Available data indicate that cardiorespiratory events in such infants return to the baseline normal level at 43 to 44 weeks' postconceptional age.[155] In other words, beyond 43 to 44 weeks' postconceptional age, the incidence of cardiorespiratory events in preterm infants does not significantly exceed that in term infants. The persistence of cardiorespiratory events may delay hospital discharge for a subset of infants. In these infants, apnea longer than 20 seconds is rare; rather, they exhibit frequent bradycardia to less than 70 to 80 bpm with short respiratory pauses.[156] The reason that some infants exhibit marked bradycardia with short pauses is unclear, but available data suggest a vagal phenomenon and benign outcome. For a few of these infants home cardiorespiratory monitoring, until 43 to 44 weeks' postconceptional age, is offered in the United States as an alternative to a prolonged hospital stay. The apparent lack of a relationship between persistent apnea of prematurity and SIDS has significantly decreased the practice of home monitoring, with no increase in the SIDS rate. Infants born prematurely experience multiple problems during their time spent in the neonatal intensive care unit, and many of these conditions can contribute to poor neurodevelopmental outcomes. For example, a history of hyperbilirubinemia has been associated with persistent apnea of prematurity in preterm infants and animal models.[157,158] The problem of correlating apnea with the outcome is compounded by the fact that nursing reports of apnea severity may be unreliable, and impedance monitoring techniques will fail to identify mixed and obstructive events. Despite these reservations, available data suggest a link between the number of days of assisted ventilation and the number of days of apnea after extubation with impaired neurodevelopmental outcome.[159] A relationship has also been shown between delay in resolution of apnea and bradycardia beyond 36 weeks' corrected age and a higher incidence of an unfavorable neurodevelopmental outcome.[160] Finally, a high number of cardiorespiratory events recorded after discharge via home cardiorespiratory monitoring appear to correlate with less favorable neurodevelopmental outcome.[161]

Recent studies have focused more on the incidence and severity of desaturation events because techniques for long-term collection of pulse oximetry data are now more advanced. Furthermore, it is likely that recurrent hypoxia is the detrimental feature of the breathing abnormalities exhibited by preterm infants. Recent data demonstrate that hypoxic episodes of at least 60 seconds' duration are associated with an unfavorable neonatal outcome in a large cohort of preterm infants.[162] Other clinically significant morbidities associated with intermittent hypoxic episodes include retinopathy of prematurity[163] and bronchopulmonary dysplasia.[164] Such associations between intermittent hypoxia and morbidity may well be causal. Fig. 41.7 summarizes proposed morbidities that might be attributable to intermittent hypoxic episodes in early life. Recurrent episodes of desaturation during early life and resultant effects on neuronal plasticity related to peripheral and central respiratory control mechanisms may serve as an important future direction for study.

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42

Acute Neonatal Respiratory Disorders

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KEY POINTS

- Marked hypoxemia in the newborn can be caused by parenchymal lung disease, pulmonary vascular disease, or congenital heart defects.
- Events occurring at delivery, as well as the response to supplemental oxygen and to continuous positive airway pressure, can provide important clues to the pathophysiology of hypoxemic respiratory failure in the newborn.
- The echocardiogram has become a vital tool in the clinical management of newborns with hypoxemic respiratory failure, primarily to assess for pulmonary hypertension and to interrogate shunts that may be contributing to hypoxemia.
- Although gestational age is a critical variable in the relative frequency with which different respiratory disorders affect newborns, a broad spectrum of potential causes must be considered in an individual baby of any gestational age.
- In extremely preterm newborns with respiratory distress, surfactant deficiency is the most common cause.
- Transient tachypnea of the newborn is one of the most common causes of respiratory distress in the term newborn.

The evaluation and management of respiratory distress in the newborn poses unique challenges and remains one of the most vexing problems facing neonatal caregivers. Although some of the pathophysiologic features of respiratory disorders in the preterm infant are similar to those in term newborns, the incidence of disorders varies by gestational age. Table 42.1 lists the most common causes of respiratory distress in newborns and demonstrates the relative frequency of each diagnosis. The traditional perspective of categorizing hypoxemia and respiratory failure in the newborn, due to cardiac, pulmonary vascular, or air space (lung) disease, is insufficient because of the inevitable complex interactions among the three. For example, the problem of persistent pulmonary hypertension of the newborn (PPHN) is defined by severe pulmonary vasoconstriction leading to suprasystemic pulmonary artery pressure with extrapulmonary right-to-left venoarterial admixture across the fetal channels of the foramen ovale and the ductus arteriosus. However, PPHN rarely occurs without concomitant parenchymal lung disease and disturbances in cardiac performance.

In this chapter, we present an algorithm for evaluation of the newborn with hypoxemia and respiratory distress. We then focus on common causes of neonatal respiratory failure, progressing from those most frequently encountered in preterm infants to those more commonly diagnosed in near-term and term infants.

Evaluation of the Newborn With Hypoxemia/Respiratory Distress

One of the most anxiety-provoking experiences for many clinicians (particularly those in training) is the initial evaluation and management of a newborn with hypoxemia and respiratory distress. Traditional textbooks provide a wealth of information about individual conditions once they have been identified. However, there are few sources designed to guide the clinician in an orderly fashion through a comprehensive diagnostic evaluation. In this section, we propose an approach to the evaluation of the hypoxemic newborn that may be useful in clarifying the cause of hypoxemia/respiratory distress and in determining the proper sequence of diagnostic and therapeutic interventions.

History

Marked hypoxemia in the newborn can be caused by parenchymal lung disease with ventilation–perfusion (V/Q) mismatch or intrapulmonary shunting, pulmonary vascular disease causing extrapulmonary right-to-left shunting (i.e., PPHN), or anatomic right-to-left shunting associated with congenital heart disease. Evaluation should begin with a history that includes assessment of risk factors for hypoxemic respiratory failure. A relevant history may include the results of prenatal ultrasound studies. Lesions such as congenital diaphragmatic hernia (CDH) and congenital cystic adenomatoid malformation are diagnosed prenatally with increasing frequency. Although many anatomic congenital heart defects can be diagnosed prenatally, vascular abnormalities (e.g., coarctation of the aorta, total anomalous pulmonary venous return) are more difficult to diagnose with prenatal ultrasonography. A history of a structurally normal heart by fetal ultrasonography should be confirmed by echocardiography in the newborn with cyanosis (see section on Echocardiography later).

Other historical information that may be important in the evaluation of the cyanotic newborn includes a history of severe and prolonged oligohydramnios causing pulmonary hypoplasia. Also important is a history of prolonged fetal bradyarrhythmia and/or tachyarrhythmia and marked anemia (caused by hemolysis, twin–twin transfusion, or chronic hemorrhage) that may cause congestive heart failure, pulmonary edema, and respiratory distress. Maternal illness (e.g., diabetes mellitus), medication use (e.g., aspirin or medications containing nonsteroidal anti-inflammatory drugs causing premature constriction of the

TABLE 42.1 Causes of Respiratory Distress

Cause	Frequency (%)
Respiratory distress syndrome	46
Transient tachypnea of newborn	37
Pneumonia/sepsis	5
Meconium aspiration syndrome	2
Congenital cardiac malformation	2
Chromosomal disorder/multiple congenital anomalies	1.4
Spontaneous pneumothorax	1.2
Perinatal asphyxia	1.1
Pulmonary hemorrhage	1.0
Persistent pulmonary hypertension	0.8
Diaphragmatic hernia	0.8
Apnea of prematurity	0.6
Pulmonary hypoplasia	0.3
Pulmonary dysplasia	0.2
Hydrothorax	0.2
Postsurgical diaphragmatic palsy	0.2

Data from Rubaltelli FF, Dani C, Reali MF, et al. Acute neonatal respiratory distress in Italy: a one-year prospective study. *Acta Paediatr*. 1998;87:1261–1268.

• BOX 42.1 Neonatal Respiratory Failure: History and Risk Factor Assessment

Prenatal

Prenatal ultrasound study results
History of oligohydramnios and duration
History of fetal brady/tachyarrhythmia
Maternal illnesses, drugs, medications
History of fetal distress or nonreassuring fetal heart tones
Risk factors for infection
Use of antenatal steroids

Delivery

History of positive pressure ventilation in delivery room
Meconium-stained amniotic fluid
Hemorrhage (acute fetomaternal or fetoplacental hemorrhage; abruption)
Birth trauma
Low Apgar score

• BOX 42.2 Physical Examination

Respiratory Distress (Retractions, Grunting, Nasal Flaring)

Suggests lung parenchymal disease (compliance), upper airway disease, or metabolic acidemia

No Significant Respiratory Distress (Tachypnea Alone)

Suggests hypoxemia caused by cyanotic heart disease without lung disease

ductus arteriosus, association of Ebstein malformation with maternal lithium use), and illicit drug use may contribute to acute cardiopulmonary distress in the newborn. Risk factors for infection that cause sepsis/pneumonia should be considered, including premature or prolonged rupture of membranes, fetal tachycardia, maternal leukocytosis, uterine tenderness, and other signs of intraamniotic infection. Additionally, the use of antenatal steroids should be ascertained in the event of a preterm delivery.

Events at delivery may provide clues to the cause of hypoxemic respiratory failure in the newborn. For example, if positive pressure ventilation is required in the delivery room, the risk of pneumothorax increases. A history of meconium-stained amniotic fluid is the sine qua non of meconium aspiration syndrome (MAS). Birth trauma (e.g., clavicular fracture, phrenic nerve injury) or acute fetomaternal or fetoplacental hemorrhage may cause respiratory distress in the newborn (Box 42.1).

Physical Examination

The initial physical examination provides important clues to the cause of cyanosis. Marked respiratory distress in the newborn (retractions, grunting, nasal flaring) suggests the presence of pulmonary parenchymal disease with decreased lung compliance. However, it is important to recognize that upper airway obstruction (e.g., Pierre Robin sequence or choanal atresia) and metabolic acidemia can also cause severe respiratory distress. In contrast, the newborn with cyanosis alone or cyanosis plus tachypnea (i.e., nondistressed tachypnea) typically has cyanotic congenital heart disease, most commonly transposition of the great vessels. Although pulmonary edema is a common complication of some forms of congenital heart disease and can lead to respiratory distress, this would be an unlikely presenting sign immediately after delivery when pulmonary vascular resistance (PVR) is still elevated.

The presence of a heart murmur audible in the first few hours after birth is an important sign in the newborn with cyanosis or respiratory distress. In this setting, it is unusual for the common left-to-right shunt lesions (patent ductus arteriosus, atrial septal defect, ventricular septal defect) to produce an audible murmur because PVR remains high and little turbulence is created across the defect. A murmur that sounds like a ventricular septal defect in the first few hours of life is most commonly caused by tricuspid regurgitation (associated with PPHN or an ischemic myocardium) (Box 42.2).

Response to Supplemental Oxygen

A marked increase (to 100%) in arterial oxygen saturation (Sao_2) with supplemental oxygen (by hood, mask, or endotracheal tube) suggests the presence of intrapulmonary shunt or V/Q mismatch caused by lung disease or reactive PPHN. The response to continuous positive airway pressure is also a useful discriminator between severe lung disease and other causes of hypoxemia. Most patients with PPHN have at least a transient increase in oxygenation in response to interventions such as high inspired oxygen concentration and/or initiation of mechanical ventilation. But if preductal Sao_2 never reaches 100%, the likelihood of cyanotic heart disease is high (Box 42.3).

• BOX 42.3 Short-Term Response to Supplemental Oxygen (High Fio_2 by Hood, Mask)

Minimal or Transient Change in Sao_2

Cyanotic heart disease, persistent pulmonary hypertension of the newborn

Marked Increase in Sao_2

Parenchymal lung disease, congenital diaphragmatic hernia with ductal-dependent systemic blood flow

Fio_2, Fraction of inspired oxygen; *Sao_2*, arterial oxygen saturation.

Interpretation of Pulse Oximetry Measurements

The interpretation of preductal (right hand) and postductal (lower extremity) arterial oxygen saturation, measured by pulse oximetry, provides important clues to the cause of hypoxemia in the newborn. Right-to-left shunting across the ductus arteriosus (but not the patent foramen ovale) causes postductal desaturation (with a >5% preductal–postductal saturation difference). However, it is important to recognize that variability in oximetry readings may be related to differences in available devices and may be affected by local perfusion.

If the measurements of preductal and postductal arterial oxygen saturation are equivalent, this suggests either that the ductus arteriosus is patent and PVR is subsystemic (i.e., the hypoxemia is caused by parenchymal lung disease with intrapulmonary shunting or cyanotic heart disease with ductal-dependent pulmonary blood flow) or that the ductus arteriosus is closed (precluding any interpretation of pulmonary artery pressure without echocardiography). It is uncommon for the ductus arteriosus to close in the first few hours after birth in the presence of systemic or suprasystemic pulmonary artery pressures.

The most common cause of preductal–postductal gradients in oxygenation is suprasystemic PVR in PPHN causing right-to-left shunting across the ductus arteriosus (associated with MAS, surfactant deficiency/dysfunction, CDH, non-CDH pulmonary hypoplasia, or idiopathic pulmonary hypertension [without accompanying pulmonary parenchymal disease]). However, ductal-dependent systemic blood flow lesions (hypoplastic left-sided heart syndrome, critical aortic stenosis, interrupted aortic arch, coarctation) may also present with postductal desaturation. Moreover, anatomic pulmonary vascular disease (alveolar capillary dysplasia, pulmonary venous stenosis, anomalous venous return with obstruction) can cause suprasystemic PVR with right-to-left shunting across the ductus arteriosus and postductal desaturation.

Finally, the unusual occurrence of markedly lower preductal Sao_2 compared with postductal Sao_2 suggests one of two diagnoses: transposition of the great vessels (TGV) with pulmonary hypertension (PH) or TGV with coarctation of the aorta (Table 42.2).

TABLE 42.2 Role of Pulse Oximetry in Evaluation of Neonatal Hypoxemic Respiratory Failure

Preductal Sao_2 = postductal Sao_2	Intrapulmonary shunt: PVR < SVR Cyanotic congenital heart disease with left-to-right shunting across the PDA Ductal-dependent pulmonary blood flow: pulmonary atresia/stenosis, tricuspid atresia, Ebstein anomaly PPHN: right-to-left shunt at PFO, PVR > SVR, ductus closed
Preductal Sao_2 > postductal Sao_2	PVR > SVR with right-to-left shunting across the PDA: PPHN: MAS, RDS, CDH Ductal-dependent systemic blood flow: HLHS, IAA, coarctation Anatomic pulmonary vascular disease: alveolar capillary dysplasia, pulmonary vein stenosis, TAPVR with obstruction
Preductal Sao_2 ≤ postductal Sao_2	TGV with pulmonary hypertension TGV with coarctation of the aorta

CDH, Congenital diaphragmatic hernia; *HLHS*, hypoplastic left-sided heart syndrome; *IAA*, interrupted aortic arch; *MAS*, meconium aspiration syndrome; *PDA*, patent ductus arteriosus; *PFO*, patent foramen ovale; *PPHN*, persistent pulmonary hypertension of the newborn; *PVR*, pulmonary vascular resistance; *RDS*, respiratory distress syndrome; *Sao_2*, arterial oxygen saturation; *SVR*, systemic vascular resistance; *TAPVR*, total anomalous pulmonary venous return; *TGV*, transposition of the great vessels.

• BOX 42.4 Chest Radiograph and Laboratory Evaluation

Chest Radiograph

Hypoxemia out of proportion to radiographic changes suggests congenital heart disease with ductal-dependent pulmonary blood flow or extrapulmonary right-to-left shunting with persistent pulmonary hypertension of the newborn.

Arterial Blood Gas, Complete Blood Count, Blood Pressure

Arterial blood gas: assess respiratory and metabolic acidemia
Complete blood count: for evidence of infection
Blood pressure: ductal-dependent systemic blood flow and closing of patent ductus arteriosus (e.g., coarctation)

Laboratory and Radiologic Evaluation

One of the most important tests to perform in the evaluation of the newborn with cyanosis is the chest radiograph (CXR). The CXR can demonstrate the classic findings of respiratory distress syndrome (RDS) (air bronchograms, diffuse granularity, underinflation), pneumonia (diffuse parenchymal lung disease), MAS (heterogenous parenchymal lung disease, often with adjacent areas of air trapping and atelectasis), or CDH. Perhaps the most important question to ask when one is viewing the CXR is whether the severity of hypoxemia is out of proportion to the radiographic changes. In other words, marked hypoxemia despite supplemental oxygen in the absence of severe pulmonary parenchymal disease radiographically suggests the presence of an extrapulmonary right-to-left shunt (idiopathic PPHN or cyanotic heart disease; Box 42.4).

Other essential measurements include measurement of arterial blood gas to determine the blood gas tensions and pH, a complete blood count to evaluate the newborn for signs of infection or evidence of symptomatic polycythemia, blood glucose to evaluate for symptomatic hypoglycemia, and blood pressure measurements in the right arm and a lower extremity to identify aortic obstruction (interrupted aortic arch, coarctation).[1]

Echocardiography

Echocardiography has become a vital tool in the clinical management of newborns with hypoxemic respiratory failure (Box 42.5). The initial echocardiographic evaluation is important to rule out structural heart disease causing hypoxemia (e.g., ductal dependent pulmonary blood flow lesions, anomalous pulmonary venous return). Moreover, it is critically important to diagnose congenital heart lesions for which inhaled nitric oxide (iNO) treatment would be contraindicated. In addition to the lesions mentioned earlier, congenital heart lesions that can present with hypoxemia unresponsive to high inspired oxygen concentrations (i.e., dependent on right-to-left shunting across the ductus arteriosus) include critical aortic stenosis, interrupted aortic arch, and hypoplastic left-sided heart syndrome. Decreasing PVR with iNO treatment in these conditions could lead to systemic hypoperfusion, worsening the clinical course and delaying definitive diagnosis.

Echocardiographic evaluation is an essential component in the initial evaluation and ongoing management of the hypoxemic newborn. As noted earlier, hypoxemia can be caused by intrapulmonary right-to-left shunting or V/Q disturbances associated with severe lung disease. In unusual circumstances, right-to-left shunting can occur across pulmonary-to-systemic collaterals. However, extrapulmonary right-to-left shunting at the foramen ovale and/or ductus arteriosus (PPHN) also complicates hypoxemic respiratory failure and must be assessed to determine initial treatments and evaluate the response to those therapies.

PPHN is defined by the echocardiographic determination of extrapulmonary venoarterial admixture (right-to-left shunting at the foramen ovale and/or ductus arteriosus), not simply evidence of increased PVR (i.e., elevated PVR without extrapulmonary shunting does not directly cause hypoxemia). Echocardiographic signs suggestive of PH (e.g., increased right ventricular systolic time intervals, septal flattening) are less definitive.

Doppler measurements of atrial-level and ductal-level shunts provide essential information when one is managing hypoxemic respiratory failure in a newborn. For example, left-to-right shunting at the foramen ovale and ductus arteriosus with marked hypoxemia suggests predominant intrapulmonary shunting, and interventions should be directed at optimizing lung inflation.

Finally, the measurements made with echocardiography can be used to predict or interpret the response or lack of response to various treatments. For example, in the presence of severe left-ventricular dysfunction with pulmonary hypertension, the left-ventricular dysfunction may contribute to pulmonary venous hypertension, such as occurs in congestive heart failure. Pulmonary vasodilation alone (without improvement in cardiac performance) may be ineffective in increasing oxygenation. In this setting, the echocardiographic findings include right-to-left ductal shunting (caused by suprasystemic PVR) and mitral insufficiency with left-to-right atrial shunting. Efforts to reduce PVR should be accompanied by targeted therapies to increase cardiac performance and decrease left-ventricular afterload. As such, careful echocardiographic assessment will provide invaluable information about the underlying pathophysiology and help guide the course of treatment. Furthermore, serial echocardiography is important to determine the response to interventions (e.g., pulmonary vasodilators) and to reevaluate cases where specific interventions have not resulted in improvement or with progressive clinical deterioration.

• BOX 42.5 Role of Echocardiography in Evaluation of Persistent Pulmonary Hypertension of the Newborn and the Use of Inhaled Nitric Oxide

Extrapulmonary Shunt

Right-to-left shunting at the arterial duct and/or foramen ovale is observed in infants with suprasystemic pulmonary hypertension.

If echocardiography demonstrates adequate left-ventricular performance, consider inhaled NO use after effective lung recruitment (see functional measurements below).

Anatomy

Inhaled NO use may be contraindicated in the presence of duct-dependent systemic blood flow (e.g., hypoplastic left heart syndrome).

Left-Ventricular Performance

Inhaled NO use may be contraindicated in the presence of left ventricular systolic/diastolic dysfunction (e.g., mitral insufficiency with left-to-right atrial shunting with right-to-left ductal shunting suggesting possible right ventricle–dependent systemic blood flow).

NO, Nitric oxide.

Persistent Pulmonary Hypertension of the Newborn

As described previously, PPHN is a syndrome associated with diverse neonatal cardiac and pulmonary disorders that are characterized by high PVR causing extrapulmonary right-to-left shunting of blood across the ductus arteriosus and/or foramen ovale. (The syndrome of PPHN and the role of iNO are discussed in more detail in Chapter 47.) However, because its relationship to respiratory failure in newborns is so vital to understanding the clinical pathophysiology and approaches to treatment, the current use of iNO and other approaches to PPHN treatment as they relate to specific respiratory diseases are discussed in the following sections.

Specific Pulmonary Conditions Causing Respiratory Distress in the Newborn

It is important to acknowledge that although gestational age is a critical variable in the relative frequency with which each of these disorders affect newborns, a broad spectrum of potential causes must be considered in an individual baby of any gestational age. We will start with RDS, the respiratory disease most commonly associated with prematurity, and then proceed to those more commonly encountered in newborns of progressively advancing gestational age.

Respiratory Distress Syndrome

More than half of extremely low birth weight newborns will have some type of respiratory distress (Fig. 42.1). In that population, RDS, historically known as *hyaline membrane disease* (HMD), is by far the most common diagnosis (51%), followed by transient tachypnea of the newborn (TTNB) (4%), and pneumonia/sepsis (2%). In preterm newborns at later gestational ages, the incidence

of any type of respiratory distress is much lower, and the proportion with TTNB increases. Compared with the incidences of the three diagnoses featured in Fig. 42.1, the incidence of other causes of respiratory distress (see Table 42.1) is very low in preterm infants, but the causes should be considered in those newborns with an atypical clinical course.

Risk Factors

The main risk factor for RDS, by far, is prematurity (see Fig. 42.1). Other factors that increase the risk of RDS include perinatal asphyxia, maternal diabetes, absence of labor, absence of prenatal steroid administration to the mother, male sex, and white race. The central feature of RDS is surfactant deficiency.

Pathophysiology

Because alveoli with insufficient (or dysfunctional) surfactant are unstable and tend to collapse, patients with RDS develop generalized atelectasis, ventilation–perfusion mismatching, and subsequent hypoxemia and respiratory acidosis. During breathing (either spontaneous or assisted), shear stress in the alveoli and terminal bronchioles occurs because of the repetitive reopening of collapsed alveoli and the overdistention of open alveoli.[2] These forces can quickly damage the fragile lung architecture, leading to leakage of proteinaceous debris into the airways (i.e., hyaline membranes). This debris (Fig. 42.2) may impair the function of what little surfactant is present, leading to a progression of respiratory symptoms and respiratory failure if not interrupted.

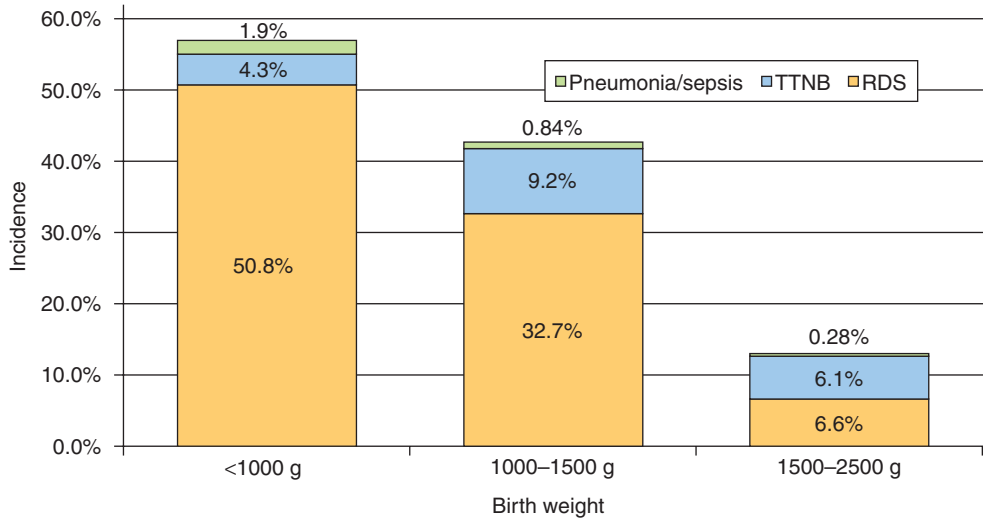
If supportive therapy is successful, the repair phase usually begins during the second day after birth with the appearance of macrophages and polymorphonuclear cells.[3] Debris is phagocytosed, and the damaged epithelium is regenerated. Interstitial fluid is mobilized into lymphatics, leading to the diuretic phase of RDS characterized by high urine output.

With uncomplicated RDS, the patient's condition improves by the end of the first week after birth. However, infants requiring high concentrations of oxygen and positive pressure ventilation for severe RDS may develop inflammation, progressive injury, and inappropriate repair of the growing lung, leading to bronchopulmonary dysplasia (BPD) (see Chapter 43).

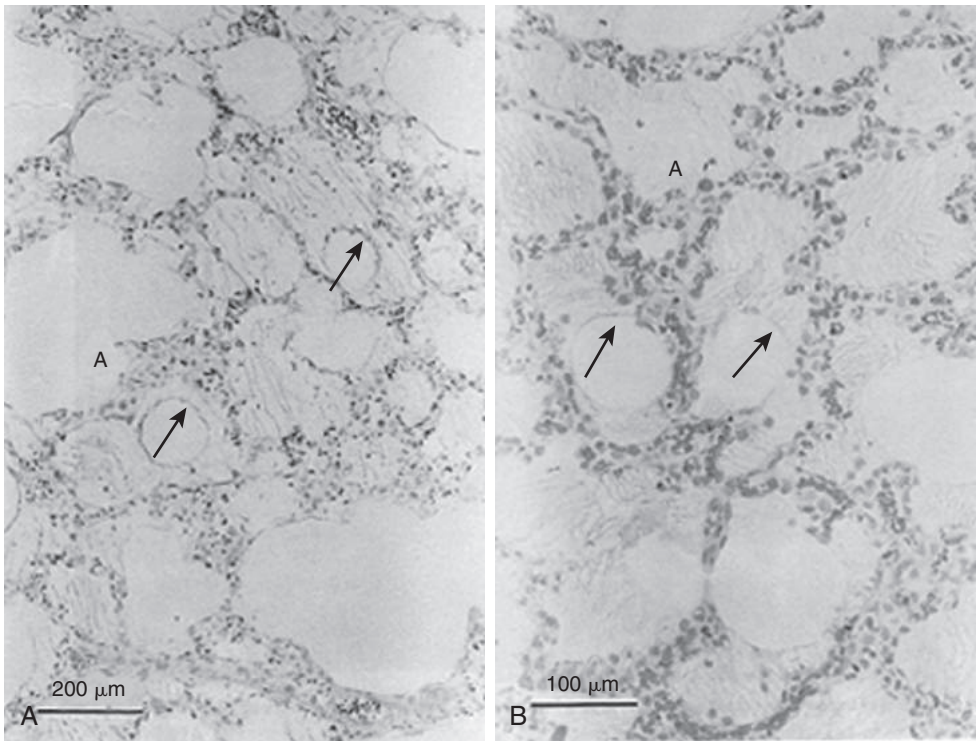
Surfactant Physiology

Surface tension is generated from molecular attractive forces within a liquid that oppose spreading; this is the reason that water "beads up" on a clean surface. According to Laplace's law, the pressure within a sphere is proportional to its surface tension and inversely proportional to its radius. Surfactants are surface-active materials that lower surface tension. Lung surfactant, composed of phospholipids and proteins, reduces surface tension as the radius of the alveolus decreases. In the absence of surfactant, there is high surface tension, so the pressure required to keep smaller alveoli open would exceed the pressure keeping open larger alveoli, resulting in collapse of the small alveoli into larger ones, as shown in Fig. 42.3. However, when lined with high-quality surfactant, the surface tension falls quickly as the alveolar radius gets smaller because the surfactant molecules become crowded during deflation (Fig. 42.4). When the radius is very small, the surface tension falls almost to zero, and the pressure required to keep smaller alveoli open is negligible, preventing collapse. During inflation, as the radius of each alveolus increases, surface tension increases even faster. This means that the pressure in the larger alveoli will be higher than that in smaller ones; this pressure difference will cause flow *to* the smaller alveoli, thereby improving gas distribution.

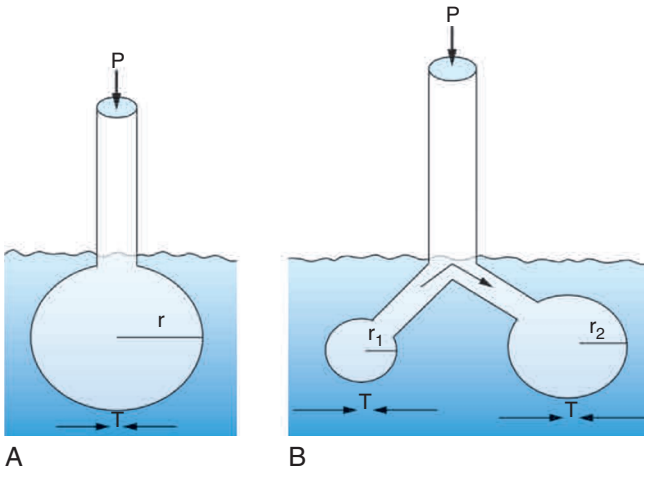
The cumulative result of surfactant sufficiency is a nonlinear pressure–volume relationship with maintenance of an adequate functional residual capacity (FRC) during expiration, compared with alveolar collapse in the surfactant deficient lung (Fig. 42.5A). Furthermore, during inflation, more pressure is required in the surfactant-deficient lung to achieve a similar tidal volume (see Fig. 42.5B), because of poor compliance ($\Delta V/\Delta P$) from having to reopen collapsed alveoli. For instance, to achieve a tidal volume of 5 mL/kg, an infant with RDS may require a pressure increase of 25 cmH_2O; dividing the volume change, ΔV, by the pressure change, ΔP, the calculated compliance is only 0.25 mL/kg per centimeter of water, approximately one-third of normal. For more information on surfactant, refer to Chapter 38.



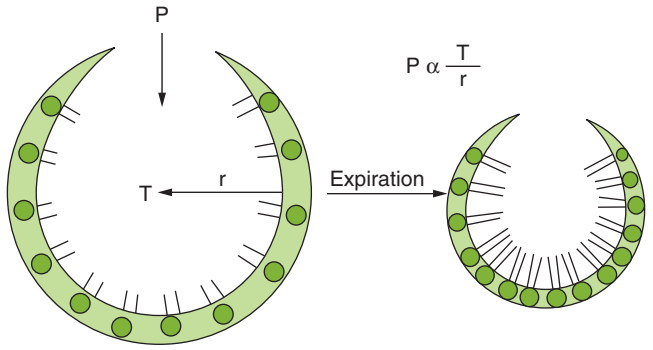
• **Fig. 42.1** Incidence of Respiratory Problems in Preterm Newborns. *RDS*, Respiratory distress syndrome; *TTNB*, transient tachypnea of the newborn. (Data from Rubaltelli FF, Dani C, Reali MF, et al. Acute neonatal respiratory distress in Italy: a one-year prospective study. *Acta Paediatr*. 1998;87:1261–1268.)



• **Fig. 42.2** Low power (A) and higher power (B) photomicrographs of lung tissue from an experimental animal with respiratory distress syndrome, flash frozen during inflation. Note the liquid–air interface (arrows). The alveolar debris forms hyaline membranes. (From the American Thoracic Society. Copyright 2017 American Thoracic Society. From Jackson JC, MacKenzie AP, Chi EY, et al: Mechanisms for reduced total lung capacity at birth during hyaline membrane disease in premature newborn monkeys, *Am Rev Respir Dis* 142:413–419, 1990.)



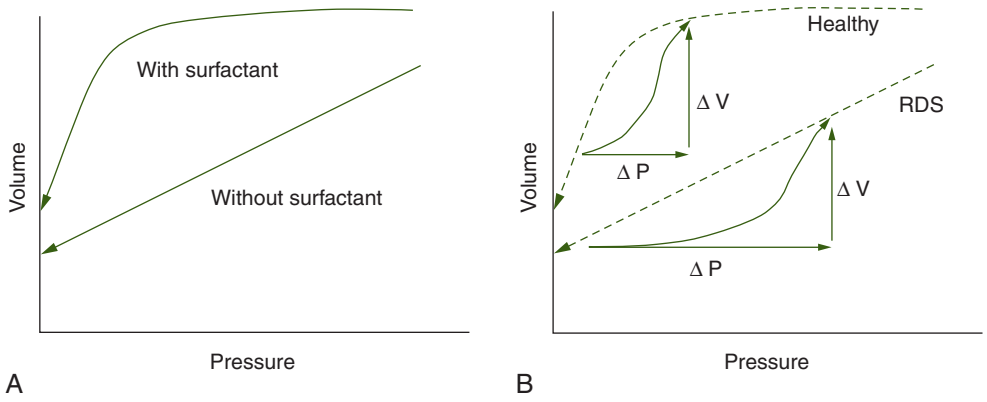
• **Fig. 42.3** Effect of Surface Forces Generated by Inflation of Bubbles Under Water. (A) A single bubble of radius *r* resists inflation and thus requires pressure *P* to overcome the surface tension *T*. (B) If the surface tension is the same in two bubbles of unequal size, the smaller one will collapse into the larger one, because of the Laplace relationship, $P = T/r$ (small *r* requires higher pressure for the bubble to stay inflated).



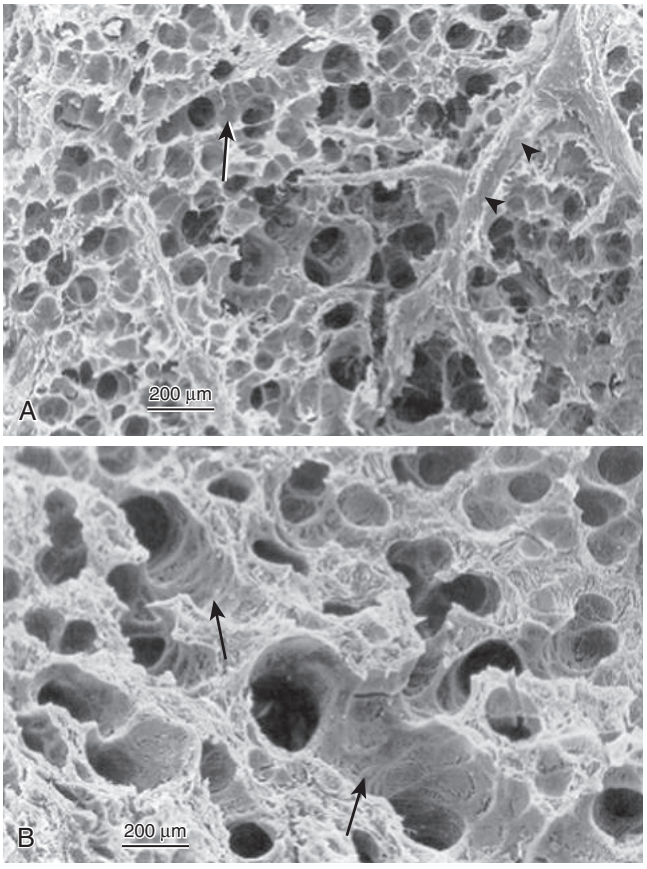
• **Fig. 42.4** Surfactant Molecules on Surface Crowding During Deflation. Less pressure (P) is needed to overcome the surface tension (T) as the radius (r) decreases due to surfactant crowding during deflation. (From Jackson JC. Respiratory distress syndrome. In: Osborn LM, DeWitt TG, First LR, Zenel JA, eds. Pediatrics. Philadelphia, PA: Mosby; 2005:1402–1404.)

Clinical Signs

In the absence of adequate functional surfactant in the newborn lung, there is inhomogeneous lung inflation with widespread alveolar collapse and overdistention of open alveoli (Fig. 42.6). Because reopening collapsed alveoli requires high pressure, the spontaneously breathing newborn with surfactant deficiency must generate highly negative intrathoracic pressure. Clinically, this is manifested by retractions of the chest wall and use of accessory muscles during inspiration. Because the rib cage in premature infants is so compliant, the sternum may deeply retract during inspiration. Newborns may also attempt to prevent alveolar collapse by "grunting." This partial closure of the glottis during expiration helps maintain an end-expiratory pressure, preserving FRC. The respiratory rate in infants with RDS is elevated in response to hypercarbia and hypoxemia. Furthermore, a consequence of widespread alveolar collapse is intrapulmonary shunting of blood



• **Fig. 42.5** Effect of Surface Forces on Pressure–Volume Relationships. During deflation (A) the lungs with surfactant retain gas even at very low pressures, because of falling surface tension as the alveoli get smaller. The alveoli without surfactant collapse as they get smaller. During inflation of respiratory distress syndrome *(RDS)* lungs (B), the starting lung volume (functional residual capacity) is lower, and much more pressure is required during inflation, compared with healthy lungs.



• **Fig. 42.6** Histology of Respiratory Distress Syndrome. Scanning electron micrograph of lung frozen during inflation with air in a healthy premature monkey (A) compared with one with respiratory distress syndrome (B). Lungs affected by respiratory distress syndrome have collapsed alveoli full of liquid and proteinaceous debris, with overdistended terminal airways. (From Jackson JC, Truog WE, Standaert TA, et al. Effect of high-frequency ventilation on the development of alveolar edema in premature monkeys at risk for hyaline membrane disease. Am Rev Respir Dis. 1991;143:865–871.)

past nonaerated lung tissue, limiting exchange of oxygen and carbon dioxide. Desaturation results from inadequate oxygenation, and pallor results from acidosis due to poor elimination of carbon dioxide. In addition, lungs that are poorly inflated have partially collapsed intrapulmonary vessels leading to pulmonary hypertension. The elevated pulmonary artery pressures lead to right-to-left shunting of unoxygenated blood across the patent ductus arteriosus to the descending aorta causing a differential between pre- and post- ductal oxygen saturations (see Chapter 47). The combination of increased work of breathing, oxygen desaturation, and acidosis causes lethargy, disinterest in feeding, and eventually apnea (Box 42.6).

On auscultation, there may be inadequate air entry from the fast inspiratory rate and low tidal volume, and fine inspiratory rales may be heard because of the reopening of collapsed, small airways. The onset of symptoms is always within hours after birth and, in severe cases, may occur almost immediately. In general, the respiratory distress from untreated RDS tends to worsen in the first 1 to 3 days after birth and then usually abates gradually thereafter (although the natural course may be interrupted by exogenous surfactant therapy or application of continuous positive airway pressure [CPAP]). This progressive worsening can be helpful in differentiating RDS from other newborn respiratory diseases.

• BOX 42.6 Clinical Signs of Respiratory Distress Syndrome

Tachypnea
Grunting
Increased work of breathing (nasal flaring, retractions)
Cyanosis
Pallor
Lethargy
Disinterest in feeding
Apnea

Laboratory Features

Initially, the arterial blood gases will show hypoxemia. $Paco_2$ is almost always elevated, but often to a lesser degree than anticipated because of tachypnea. As the infant tires, $Paco_2$ will rise further and cause respiratory acidosis. With imminent respiratory failure, there may be progressive metabolic acidosis due to inadequate oxygen delivery to tissues from poor peripheral perfusion.

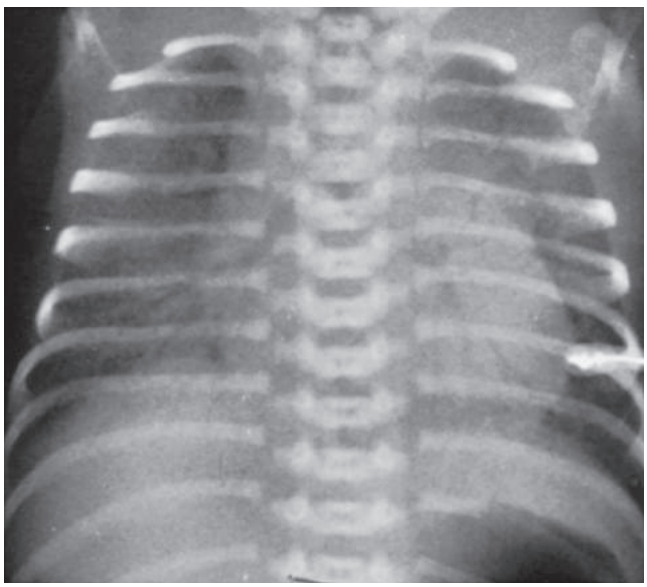
Radiographic Features

The classic radiographic findings of RDS include a reticulogranular (i.e., ground-glass) appearance, air bronchograms, and low lung volumes (in the absence of treatment with positive pressure) (Fig. 42.7). The lungs are diffusely and homogeneously dense because of widespread collapse of alveoli. The appearance is reticular (i.e., netlike) because the small airways are open (black) and surrounded by interstitial and alveolar fluid (white); in severe cases, the lungs may appear completely white on the radiograph. Air bronchograms are commonly seen because the large airways beyond the second or third generation are more visible than usual as a result of radiodensity from adjacent, engorged, peribronchial lymphatics and fluid-filled or collapsed alveoli. Low lung volume is due to widespread alveolar collapse and low FRC.

Treatment

Antenatal Steroids

Prenatal administration of steroids was shown in 1972 to be effective in reducing the risk of RDS.[4] A consensus panel convened by the National Institutes of Health in 1995 recommended that mothers at risk of preterm delivery between 24 to 34 weeks' gestation be given antenatal steroids.[5,6] A review of 27 published trials[7] concluded that prenatal administration of corticosteroids did not increase a mother's risk of death, chorioamnionitis, or endometritis, and treatment was associated with a reduction in neonatal death (RR 0.78, 95% CI 0.7 to 0.87), RDS (RR 0.71, 95% CI 0.65 to 0.78), and intraventricular hemorrhage (RR 0.58, 95% CI 0.45 to 0.75). Although the beneficial effects were maximized if delivery occurred between 24 hours and 7 days after treatment, there was also a benefit when delivery occurred within 24 hours.[8]



• **Fig. 42.7** Radiograph of a Patient With Respiratory Distress Syndrome. Note low lung volumes and a reticulogranular pattern. (From Welty S, Hansen TN, Corbet A. Respiratory distress in the preterm infant. In: Taeusch HW, Ballard RA, Gleason CA, eds. Avery's Diseases of the Newborn. 8th ed. Philadelphia: Elsevier; 2005:687–703.)

Repeat courses of antenatal steroids in the setting of persistently threatened preterm delivery are beneficial in reducing the occurrence and severity of RDS.[9] A study in 2007[10] found an association of repeat courses of antenatal steroids with cerebral palsy, but recent studies,[11,12] including a Cochrane review,[13] suggest that the evidence is inconclusive. However, repeat courses have been consistently associated with lower birth weight and smaller head circumference.[9,14] Additionally, although the most recent consensus panel in 2001[6] did not recommend repeated dosing of antenatal steroids, several studies in support of their use have been subsequently published.[9,14]

Recently, antenatal steroids have also been shown to be beneficial at periviable gestational ages (22 to 25 weeks)[15] and late preterm infants (34 to 37 weeks).[16,17] Further research on the use of antenatal steroids for threatened preterm delivery at periviable gestational ages is required, and their utilization at this time remains institutionally-dependent. Furthermore, although administration of antenatal steroids at 34 to 37 weeks' gestation reduced neonatal respiratory complications, it was associated with an increased risk of neonatal hypoglycemia and is not currently recommended.[18]

Continuous Positive Airway Pressure

In the early 1970s, Gregory et al.[19] first introduced CPAP therapy for newborns with RDS, primarily by an endotracheal tube. Since then, a variety of devices have been developed to deliver CPAP noninvasively.[20] Avery et al.[21] reported that centers that used early nasal CPAP therapy for RDS had a lower incidence of BPD, and, now, CPAP is a well-established therapy for infants with RDS across a range of initial severities. For more information on the principles and use of CPAP therapy and the risks and benefits of different modes of respiratory therapy, see Chapter 40.

The goals of CPAP therapy in preterm infants at risk of respiratory failure are to prevent end-expiratory alveolar collapse, reduce work of breathing, and better match ventilation to perfusion. If it is started in the delivery room, CPAP may help the newborn establish FRC, in addition to stabilizing the chest wall and reducing airway resistance. Furthermore, adequate expansion of the lungs at birth increases pulmonary blood flow. The prophylactic use of CPAP in the delivery room might make intubation for exogenous surfactant unnecessary,[22] and avoidance of intubation and mechanical ventilation may lower the risk of BPD. With advances in the use of CPAP since the first clinical trials of exogenous surfactant, several large trials (i.e., COIN,[23] SUPPORT,[24] VON-DRM[25]) have compared the use of early CPAP with prophylactic surfactant, and have found no difference in the primary outcome of death or BPD, suggesting that early CPAP use is at least comparable to intubation with prophylactic surfactant administration for the treatment of RDS. A meta-analysis[26] of studies enrolling babies less than 32 weeks gestation concluded that one additional infant could survive to 36 weeks without BPD for every 25 treated initially with nasal CPAP therapy in the delivery room rather than immediate intubation.

Others have compared the early use of CPAP to intubation for prophylactic surfactant followed by immediate extubation

(i.e., the InSurE technique) to limit complications from sustained endotracheal intubation and mechanical ventilation (including excessive tidal volumes and airway inflammation that may contribute to the development of BPD), and have additionally not found a benefit of InSurE over CPAP.[25,27–29] Additionally, there is a subset of infants who fail InSurE, requiring reintubation, leading some to recommend lung recruitment strategies after intubation but before surfactant administration (i.e., In-Rec-SurE technique) to decrease failure rates.[30] Further dilemma lies in the fact that, when compared to later surfactant administration, early surfactant (within 2 to 3 hours of birth) decreases the risk of pneumothorax, pulmonary interstitial emphysema, neonatal death, and BPD.[31] However, a subset of babies treated successfully with initial CPAP will later fail, eventually requiring intubation and surfactant administration, missing the advantages of early surfactant administration. As such, trials attempting to elucidate predictors of eventual CPAP failure have found that the most salient predictors are male sex, low birth weight, and, most importantly, Fio_2 requirements greater than 0.3 to 0.35.[32,33] Furthermore, research is ongoing regarding less invasive methodologies of surfactant administration that would avoid intubation and allow for early surfactant administration in combination with early CPAP use (see Exogenous Surfactant section below for more detail).[34–39]

The American Academy of Pediatrics Committee on the Fetus and Newborn supports the initial use of CPAP with ongoing assessment for surfactant need in the first 2 to 3 hours of life as an alternative to prophylactic surfactant.[40] Given the rapid evolution in published evidence contrasting and combining CPAP therapy and surfactant therapy, broad consensus regarding their use in preventing and treating respiratory distress in premature infants does not currently exist. However, most studies suggest that to maximize the benefit of either on survival and morbidity, they should both be started as soon as possible. Furthermore, the duration of mechanical ventilation should be kept to a minimum, and endotracheal intubation should be avoided, if possible.

Some centers report use of humidified high-flow nasal cannulas in infants with RDS to achieve the benefits of positive airway pressure with fewer of the perceived disadvantages of CPAP (e.g., challenges in keeping the nasal prongs in the nares, more difficult handling of the patient, and greater risk of pressure necrosis of the nasal septum).[41] However, the airway pressure generated from this therapy, although proven to produce a clinical effect, is variable, unpredictable, and unregulated, and the commercially available systems are not approved by the US Food and Drug Administration for this indication. It should be used only by practitioners aware of the balance of risks and benefits and those prepared to recognize and treat pneumothorax and respiratory failure. A 2015 review outlined the main advantages and disadvantages of the many types of CPAP delivery devices, as well as the implications for developing countries.[42]

Exogenous Surfactant

Historical Summary

The development of exogenous surfactant for treatment of RDS is one of the most important advances in the history of newborn medicine. This history is well told elsewhere.[43] The key milestones include von Neergaard's discovery in 1929 that surface tension contributes to lung recoil,[44] Gruenwald's demonstration in 1947 that lungs of stillborn infants have high surface tension,[45] Pattle's speculation in 1955 that absence of surfactant active material contributes to RDS,[46] Clements' description in 1957 of surfactant dysfunction in experimental animals,[47] and Avery and Mead's demonstration in 1959 that RDS in human infants is due to surfactant deficiency.[48] There was an increase in research interest and funding for RDS treatment after President John Kennedy's son, born at 34.5 weeks' gestation, died of RDS in 1963. In 1972, Enhörning and Robertson used natural surfactant to delay the progression of RDS in preterm rabbits,[49] and, in 1980, Fujiwara et al. demonstrated the first successful use of exogenous surfactant in human infants.[50] By 1990, exogenous surfactant was widely used throughout the developed world, and many large clinical trials have been conducted since then to refine and improve surfactant treatment and prevention of RDS. A meta-analysis of 13 randomized controlled trials suggests that animal-derived exogenous surfactant compared to standard therapy without surfactant reduces the risk of pneumothorax by 58%, the risk of pulmonary interstitial emphysema by 55%, the risk of death by 32%, and the risk of the combined outcome of BPD or death by 17%.[51] Notably, the "standard therapy" did not include use of CPAP started immediately after birth (see previous comments under Continuous Positive Airway Pressure).

Types of Surfactants Available for Clinical Use

A list of currently available surfactant preparations and sources in the United States can be found in Table 42.3. The first generation of commercially available artificial surfactants (e.g., colfosceril [Exosurf]), composed mainly of dipalmitoylphosphatidylcholine (DPPC) and lacking surfactant protein-B (SP-B) and SP-C, were eventually replaced by natural surfactants. A meta-analysis of 15 randomized controlled trials showed that natural surfactants were faster acting than artificial surfactants, with lower incidence of pneumothorax and death.[52] A new generation of synthetic surfactant products such as lucinactant and CHF5633 have synthetic peptides or proteins, such as KL4 in lucinactant, which mimics the actions of natural surfactant proteins.[53,54] Additional studies

TABLE 42.3 Commercially Available Surfactant Preparations in the United States and Their Sources

Brand Name	Generic Name	Constituents	Amount per Dose
Protein-Containing Animal Surfactants			
Curosurf	Poractant alfa	Porcine lung tissue	2.5 mL/kg (redose 1.25 mL/kg)
Infasurf	Calfactant CLSE	Bovine (calf) lung lavage	3 mL/kg
Survanta	Beractant	Bovine lung tissue	4 mL/kg
Alveofact	Bovactant	Bovine lung lavage	1.2 mL/kg
Peptide-Containing Synthetic Surfactants			
Surfaxin	Lucinactant	DPPC, POPG, PA, KL4	5.8 mL/kg

CLSE, Calf lung surfactant extract; *DPPC*, dipalmitoylphosphatidylcholine; *PA*, palmitic acid; *POPG*, palmitoyloleoylphosphatidylglycerol.
Modified from Walsh BK, Daigle B, DiBlasi RM, et al. AARC clinical practice guideline. Surfactant replacement therapy: 2013. *Respir Care*. 2013;58:367–375.

are underway comparing CHF5633 to poractant alfa, and evaluating the utility of using lucinactant as an aerosolized surfactant (Aerosurf).

Surfactant Selection

An important issue for clinical providers and managers is which surfactant to stock in their pharmacy because of the expense of the preparations and the need to standardize dosing guidelines. A 2015 meta-analysis of clinical trials[52] indicated that, compared with artificial surfactant, use of animal-derived surfactants leads to greater early improvement in ventilator support, fewer pneumothoraces, and fewer deaths, but may be associated with greater risk of necrotizing enterocolitis and grade 1 and grade 2 intraventricular hemorrhage. The most commonly used animal-derived surfactants that are commercially available include poractant alfa (from minced porcine lung; Curosurf) and beractant (from minced bovine lung; Survanta).[55] A recent meta-analysis of clinical trials comparing these two drugs for prevention or treatment of RDS in premature infants suggested that treatment with poractant alfa is associated with lower risk of death, BPD, and clinically significant patent ductus arteriosus and reduced need for repeated dosing.[56] However, other studies have found no difference.[57] Regardless, poractant alfa does have the advantage of being more concentrated than beractant, and the lower dosing volume may be advantageous.

Timing and Method of Surfactant Administration

In theory, surfactant would ideally be given with the first breath. This concept was evaluated by Kattwinkel et al.[58] by delivering the head of the infant, suctioning the nasopharynx, and then instilling calfactant into the airway before delivery of the shoulders. CPAP therapy was initiated immediately after delivery, but the trachea was not routinely intubated. The treatment appeared feasible and safe, but the sample size (23) was too small to prove that the approach was beneficial compared with surfactant instillation via an endotracheal tube during the first few minutes after delivery. Some have proposed administration of surfactant into the pharynx before the first breath in extremely preterm infants at high risk of RDS, but no clinical trials have yet been published.[59]

Animal studies suggest that surfactant is best distributed when administered as a bolus rather than by slow infusion over several minutes.[60] Additionally, a chest x-ray confirming satisfactory endotracheal tube position should be considered to avoid asymmetric surfactant administration. Although surfactant manufacturers recommend variable positioning of the infant during instillation to improve distribution of surfactant, there are no clinical trials supporting one particular method. Therefore, surfactant should be given as quickly as tolerated, with alveolar recruitment techniques, and with the least disruptive infant positioning.[61] Vigilance for endotracheal tube obstruction due to surfactant administration, particularly with smaller sized tubes, is recommended.

With persistent concerns about the risks of both endotracheal intubation and mechanical ventilation, even if brief, recent studies have sought to combine use of CPAP with less invasive, early surfactant delivery. There are ongoing studies evaluating the administration of surfactant through a small endotracheal catheter or feeding tube while CPAP is being provided (referred to as less invasive surfactant application [LISA], minimally invasive surfactant therapy [MIST], or surfactant without endotracheal tube intubation [SURE] technique), and the results have been promising.[34–36] Others have proposed administering surfactant via laryngeal mask airway to reduce the need for mechanical ventilation,[62] but there are obvious birth weight limitations to this approach. Preliminary clinical trials suggest that surfactant can be successfully aerosolized or nebulized,[37,38] but only one clinical trial comparing nebulized surfactant administration to CPAP alone has shown a decreased need for mechanical ventilation.[39] Furthermore, this finding was limited to a subgroup of more mature infants (32 to 33 weeks' gestation).

Number of Surfactant Doses and Dosing Intervals

A meta-analysis of two clinical trials that compared one dose versus multiple doses of animal-derived surfactant suggests a 49% reduction in the incidence of pneumothorax and a trend toward a 37% reduction in mortality when multiple doses are used.[63] Additionally, a repeat dose of surfactant improves oxygenation without significantly changing end-expiratory lung impedance or ventilation distribution.[64] There are no data to suggest that additional doses should be provided if the previous dose was ineffective or once the patient's ventilator and oxygen requirements are at minimal levels. The interval between doses is usually at least 6 hours, and most research protocols discontinue dosing after 48 hours. Late treatment with up to five doses of surfactant in ventilated premature infants receiving inhaled nitric oxide was well tolerated but did not improve survival without BPD.[65]

Clinical Care After Dosing

Because natural surfactants may work quickly, the clinician must be prepared after dosing to immediately lower Fio_2 while carefully monitoring the pulse oximeter. With improved lung compliance, the tidal volume and inspiratory pressures may change rapidly, resulting in the need to decrease respiratory support to avoid air leak syndromes and lung injury. Blood gases should be monitored by intermittent blood sampling. Positive end-expiratory pressure may be reduced if the starting levels are high, given that surfactant should help maintain FRC at a lower distending pressure.

A poor response to exogenous surfactant may occur if the patient does not have surfactant deficiency, but rather has lung hypoplasia, pneumonia, or congenital heart disease. Other causes for a lack of response may be poor distribution of the surfactant, such as administration down the right stem bronchus due to malposition of the endotracheal tube, plugging of the tube, or malposition of the tube in the esophagus. A less likely reason is an inadequate dose of surfactant.

Lastly, the rapid improvement in lung compliance after exogenous surfactant therapy may lead to excessive pulmonary blood flow from left-to-right shunting across a patent ductus arteriosus, increasing the risk of pulmonary hemorrhage.[66] (For more information, see the next section on Pulmonary Hemorrhage.)

Pulmonary Hemorrhage

Incidence and Clinical Signs

Pulmonary hemorrhage occurs in 3% to 5% of preterm infants needing respiratory support, usually at 1 to 3 days of age, with onset of pink or red frothy fluids in the endotracheal tube and sudden respiratory deterioration, cyanosis, pallor, hypotension, or

hypotonia.[67] The frequency is greater with increasing degree of prematurity and with intrauterine growth restriction.[68] The chest radiograph may show widespread "whiteout," unlike mucosal bleeding of the airway, which is usually accompanied by minimal clinical or radiographic changes. The hematocrit of the tracheal fluid after pulmonary hemorrhage is much lower than from a venous sample, indicating that the underlying problem is hemorrhagic pulmonary edema.

Etiology

Multiple factors likely contribute to neonatal pulmonary hemorrhage. There is a threefold increased risk after prophylactic synthetic surfactant,[69] leading to speculation that surfactant may have a direct cytotoxic effect that impairs membrane integrity in the alveolar capillary.[70] Alternatively, rapid improvement in lung compliance following exogenous surfactant treatment may cause volutrauma and subsequent pulmonary hemorrhage. Some have theorized that the decrease in pulmonary resistance associated with surfactant treatment of RDS facilitates a fall in PVR and left-to-right shunting through a patent ductus arteriosus, which increases the likelihood of pulmonary hemorrhage.[68] This theory is supported by evidence that infants who received early indomethacin therapy to close a large patent ductus arteriosus had a lower incidence of pulmonary hemorrhage.[71] Lastly, in some babies, left ventricular diastolic dysfunction may lead to pulmonary venous hypertension and increased wedge pressure, lowering the threshold for pulmonary hemorrhage.

Treatment

The mainstay of treatment includes careful suctioning of the trachea to prevent obstruction and increased ventilator pressure, particularly increased positive end-expiratory pressure, for pulmonary edema. High frequency ventilation could also be considered to provide higher mean airway pressures in cases of refractory pulmonary hemorrhage. Although a low platelet count and coagulopathy are rarely the cause, newborns should be checked for these and treated, if necessary. If anemia is severe, transfusion with packed red blood cells may be indicated, but this should be done very slowly to avoid compounding the pulmonary edema. Red blood cells and serum in the airspace impair surfactant function, causing a secondary surfactant inactivation. There is evidence that exogenous surfactant may help,[72,73] but no randomized controlled trials exist to prove benefit.[74]

Pulmonary Hypoplasia

Etiology and Incidence

Pulmonary hypoplasia and respiratory failure can develop in association with a number of conditions (Table 42.4). Most cause either restriction of normal fetal breathing motion or compression of the developing lung. Thus, among the most common causes of pulmonary hypoplasia are low amniotic fluid volumes, neuromuscular disorders, disease states causing pleural effusions/chylothoraces, and space-occupying lung lesions.

Inadequate amniotic fluid volume that limits normal fetal breathing can result from a number of circumstances. Because fetal urine is a primary contributor to amniotic fluid volume, any abnormality of renal development that limits fetal urine production or urine flow can result in pulmonary hypoplasia. In addition, preterm premature rupture of membranes (PPROM), particularly with persistent leak of amniotic fluid, can result in pulmonary hypoplasia.

One or both lungs of newborns with pulmonary hypoplasia are smaller than normal, including reduced numbers of lung cells, airways, blood vessels, and alveoli. Pulmonary hypoplasia is the cause of respiratory distress at birth in only 0.3% of newborns with respiratory symptoms,[75] but it is commonly fatal, especially in preterm infants. The incidence is about 1 per 10,000 live births, and 90% of cases are associated with congenital anomalies or pregnancy complications.[76] There is a continuum of severity of pulmonary hypoplasia—from negligible to severe—and all but the most severe cases are difficult to diagnose. This is especially true when one is attempting to diagnose pulmonary hypoplasia prenatally by imaging studies but is also true during clinical assessment of the newborn and even after postmortem examination of the lungs.

TABLE 42.4 Categories of Conditions Associated With Pulmonary Hypoplasia

Category	Representative Diagnoses
Restriction of thoracic space	Diaphragmatic hernia or eventration Intrathoracic mass Congenital cystic adenomatoid malformation Bronchogenic cyst Extralobar sequestration Thoracic neuroblastoma Pleural effusions Chylothorax Hydrothorax
Oligohydramnios	Renal Bilateral renal agenesis or dysplasia Bladder outlet obstruction (posterior urethral valves) Nonrenal Prolonged preterm rupture of membranes
Skeletal anomalies	Chondroectodermal dysplasia Osteogenesis imperfecta Thanatophoric dwarfism
Hydrops fetalis	Rhesus isoimmunization
Neuromuscular and central nervous system anomalies	Fetal akinesia Anencephaly Arnold–Chiari malformation
Cardiac anomalies	Hypoplastic right or left side of the heart Pulmonary stenosis Ebstein anomaly
Abdominal wall defects	Omphalocele Gastroschisis
Syndromes	Trisomy 13, 18, 21 Larsen syndrome Cerebrocostomandibular syndrome Jarcho–Levin syndrome Roberts syndrome Lethal multiple pterygium

Modified from Langston C. Pulmonary disorders in the neonate, infant, and child. In: Churg AM, Myers JL, Tazelaar H, Wright J, eds. *Thurlbeck's Pathology of the Lung.* 3rd ed. New York, NY: Thieme; 2005.

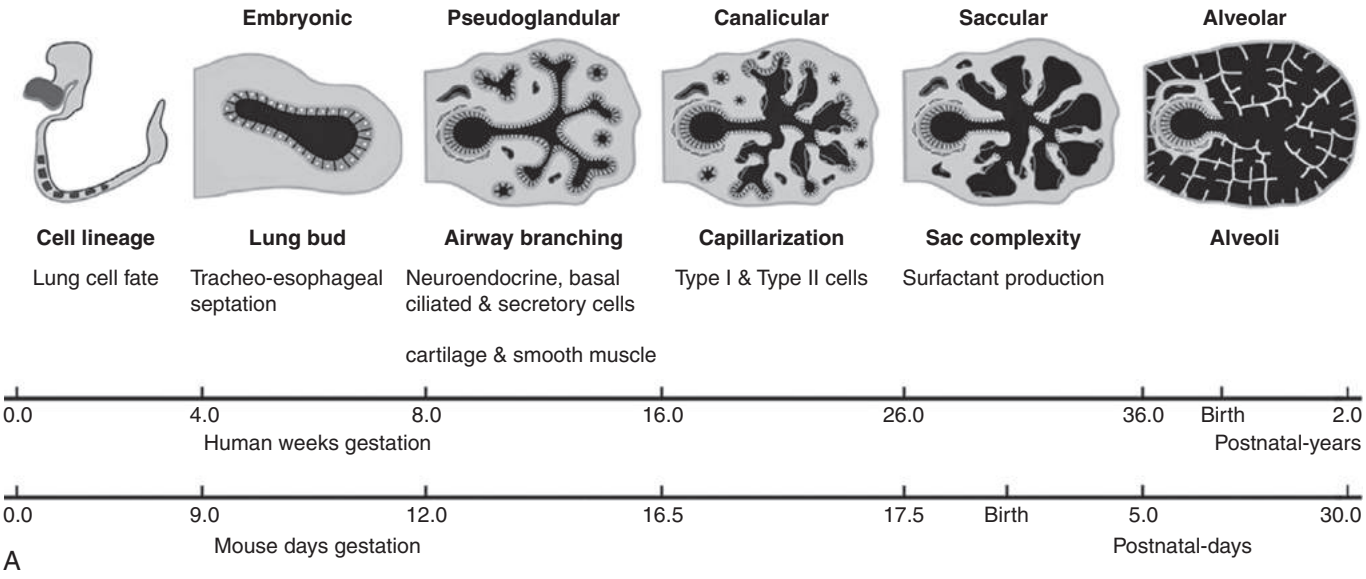
Prenatal Diagnosis

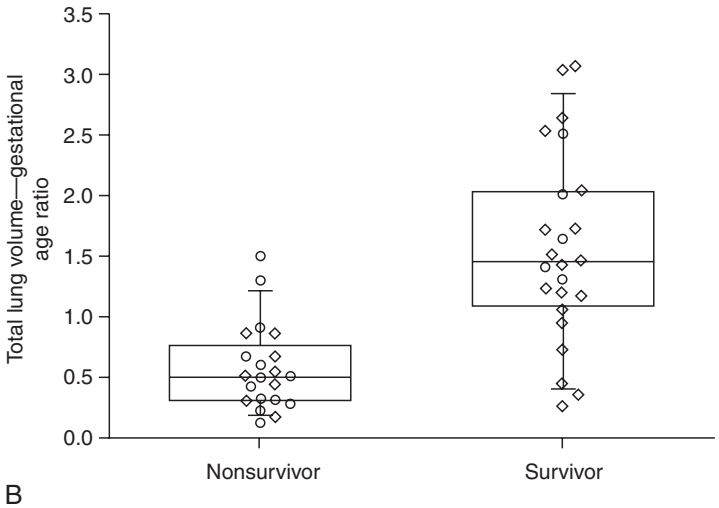
An accurate prenatal test predicting the degree of pulmonary hypoplasia would be important because it might affect the obstetric management. It would be particularly helpful to be able to discriminate lethal from nonlethal pulmonary hypoplasia, especially early in gestation, when termination of the pregnancy may be an option. However, quantifying the degree of fetal pulmonary hypoplasia is challenging even for the pathologist examining gross and microscopic lung sections. A recent review and meta-analysis of imaging parameters for prediction of fatal pulmonary hypoplasia found all to be poor to mediocre.[77,78]

Nonsurvivors with pulmonary hypoplasia due to fetal urinary anomalies were noted to have lower in utero lung volumes as determined by fetal magnetic resonance imaging (MRI), adjusted for gestational age,[79] but there was no clear separation between the survivors and nonsurvivors (Fig. 42.8B). Furthermore, there was considerable overlap of the confidence intervals before 26 weeks' gestation, which limits the usefulness of MRI assessment of fetal volume for prenatal counseling. Although there will continue to be advances in fetal imaging to assess lung volume, it will likely remain difficult to differentiate lethal from nonlethal pulmonary hypoplasia prior to birth given that there may not be a linear correlation between lung volume and lung function. Moreover, there may be additional underlying abnormalities that limit viability independent of lung size and function.

Prenatal Treatment

In cases where an infant is at risk for pulmonary hypoplasia due to PPROM, treatment of oligohydramnios with amnioinfusion with saline has been proposed to improve fetal survival by extending the latency period (the interval between premature rupture of membranes and delivery) and thus the gestational age at delivery.





• **Fig. 42.8** (A) Stages of lung development. (B) Measurement of fetal lung volume by magnetic resonance imaging and outcomes after delivery. (A from Kimura J, Deutsch GH. Key mechanisms of early lung development. Pediatr Dev Pathol. 2007;10:335–347. B from Zaretsky M, Ramus R, McIntire D, Magee K, Twickler DM. MRI calculation of lung volumes to predict outcome in fetuses with genitourinary abnormalities. Am J Radiol. 2005;185:1328–1334.)

A meta-analysis found that amnioinfusion reduced neonatal morbidity, neonatal sepsis, pulmonary hypoplasia, and puerperal sepsis and increased latency, but the authors recommended circumspection as the positive findings were mainly due to one trial with unclear allocation concealment.[80] When successful, the benefit may derive from restoration of back-pressure from the amniotic sac fluid to the lungs, stimulating fetal lung growth and development during the critical canalicular stage between 16- and 26-weeks' gestation (see Fig. 42.8A).

Pathology

A common method of defining pulmonary hypoplasia during postmortem examination is to calculate the ratio of lung weight to body weight. However, the lung weight to body weight ratio may be artificially elevated if the lungs are wetter than usual from edema, hemorrhage, inflammation, or lymphangiectasia, and this may lead to the false conclusion that the patient does not have pulmonary hypoplasia. Conversely, the ratio may be artificially low if the body is heavier than usual because of renal cystic disease, hydrops, ascites, tumors, hydrocephaly, and so forth. Therefore, a better method for postmortem diagnosis of pulmonary hypoplasia is to measure the lung volume by inflating the lung at physiologic pressure and then measuring the displacement of fluid when the lung is immersed.[76] This method facilitates comparison of postmortem estimates with in utero estimates of lung volume made during prenatal imaging. However, as previously noted, low lung volume does not necessarily correlate with abnormal lung structure and function. An alternative postmortem technique more physiologically relevant than lung volume is the radial alveolar count, which is proportional to alveolar surface complexity[76] and, thus, gas exchange surface area; however, this procedure is complex and time consuming.

Impairment of lung development before 16 weeks causes reduced airway branching, reduced cartilage development, reduced acinar complexity and maturation, delayed vascularization, and delayed thinning of the air–blood barrier (see Chapter 38). Impairment after 16 weeks typically causes reduced acinar complexity and maturation. These outcomes are predictable, given the time in gestation when these structures are developing (see Fig. 42.8A).

Because the growth of lung blood vessels parallels the development of the airways, pulmonary hypoplasia is invariably associated with decreased total size of the pulmonary vascular bed, decreased number of vessels per unit of lung tissue, and increased amount of pulmonary artery smooth muscle. This last phenomenon accounts for persistent pulmonary hypertension after birth.

Clinical and Radiographic Signs

The newborn with pulmonary hypoplasia often has immediate signs of respiratory distress and cyanosis indistinguishable from those in the newborn with severe RDS. However, respiratory failure from severe pulmonary hypoplasia often becomes maximally apparent within minutes of birth, whereas respiratory failure from RDS usually progresses over the first few hours after birth. The thorax may appear small or bell shaped, and, if oligohydramnios was severe, there may be flattening of the face and deformation (such as contractures of the extremities). Hypercarbia may be severe on the earliest blood gas measurement, despite aggressive mechanical ventilation. The hypoxemia from surfactant deficiency and lung immaturity may be compounded by right-to-left shunting of deoxygenated blood due to pulmonary hypertension, leading to severe desaturation. Early pneumothorax is common, in which case there may be asymmetry of breath sounds or malposition of heart sounds; in the setting of tension pneumothorax, there may be decreased cardiac output from impaired venous return to the thorax. Because lung immaturity and surfactant deficiency accompany pulmonary hypoplasia, particularly in preterm infants, the lungs may be radiographically dense with air bronchograms, as with RDS, but the lung volumes may be smaller and the diaphragms higher than in uncomplicated RDS.

Treatment

The ventilator strategy must be individualized. In some cases, a baby with smaller lungs may have relatively normal compliance, high pulmonary vascular resistance, and sometimes cardiac dysfunction. In other cases, the pulmonary hypoplasia may be associated with markedly abnormal lung structure, poor compliance, and impaired gas exchange. In the initial hours after birth, the goal should be to avoid excessive intrathoracic pressure from lung overdistention which may lead to catastrophic pneumothorax and which may also further aggravate pulmonary hypertension and right-sided heart afterload. Permissive hypercapnia is appropriate, and high-frequency ventilation may be necessary for adequate ventilation and reduction of very high arterial P_{CO_2} levels.

For preterm infants with pulmonary hypoplasia, exogenous surfactant should be considered early because there may be concomitant surfactant deficiency. One of the main causes of pulmonary hypoplasia in preterm infants is PPROM. When PPROM occurs at 15 weeks' gestation, the incidence of pulmonary hypoplasia is 80%, and when it occurs at 19 weeks, the incidence is 50%, whereas after 26 weeks the incidence is near zero.[81] The survival rate of infants born at less than 32 weeks' gestation after rupture of membranes before 24 weeks' gestation has been reported as 75% to 90%.[82,83] The major predictors of neonatal survival are later gestational age at PPROM and adequate residual amniotic fluid levels.[84]

To avoid adding hypoxic pulmonary vasoconstriction to the already high pulmonary vascular resistance, the preductal arterial oxygen saturation should be kept at 92% or higher. Although acidosis may compound hypoxic pulmonary vasoconstriction, hypercapnia is usually well tolerated and will allow a less aggressive ventilator strategy. Routine paralysis is usually not needed and leads to third spacing of fluid. Spontaneous breathing may be beneficial, and some infants with pulmonary hypoplasia have been managed with nasal CPAP.[85]

Because increasing ventilator support for persistent hypoxemia may exacerbate lung injury, efforts should be directed toward treatment of pulmonary hypertension, even though the component that is reversible is unpredictable. There are several small studies demonstrating the beneficial effects of iNO in pulmonary hypoplasia (the majority in preterm infants born after PPROM),[86,87] suggesting a therapeutic trial of iNO therapy is warranted in this population. Although not statistically significant, there was a 33% reduction in mortality among extremely preterm neonates with pulmonary hypoplasia and pulmonary hypertension in a recent cohort study.[88] Other medications such as sildenafil and milrinone may also minimize pulmonary hypertension. Frequent echocardiography should be used to assess response to these medications. Because systemic hypotension causes worse right-to-left shunting,

cardiotonic agents may be helpful, acknowledging that these medications may increase both systemic and pulmonary vascular pressures. For more detail, see the review by de Waal[86] which includes a good description of cardiovascular support agents, mechanisms of action, and physiologic targets. Before administering agents such as inhaled nitric oxide that are intended to increase pulmonary blood flow, severe left ventricular dysfunction with increased left atrial pressure (often indicated by left-to-right atrial level shunting in the setting of pulmonary hypertension) should first be corrected to avoid pulmonary edema and minimize the risk of pulmonary hemorrhage.[89]

In cases of profound pulmonary failure, extracorporeal membrane oxygenation may be appropriate for late-gestation preterm and term infants if there is potential for clinical improvement over the course of several days.

Pneumonia

Incidence and Etiology

Given the lack of specific signs, the diagnosis of pneumonia is often made based on supportive evidence of infection (prolonged rupture of membranes, maternal fever, neonatal lethargy and temperature instability, abnormal laboratory studies, etc.). In the absence of a positive culture, the diagnosis is rarely unequivocal. Nonetheless, the incidence of pneumonia has been estimated at ~2% in extremely low birth weight (ELBW) preterm infants (see Fig. 42.1).[75] Across all gestational ages, the same study suggests that pneumonia is the third most common acute respiratory disorder in newborns, behind RDS and transient tachypnea of the newborn (TTNB) (see Table 42.1). When a bacterial pathogen is isolated, group B streptococcus was the most frequent pathogen (36%) identified from data collected between 2005 and 2014, followed by *Escherichia coli* (25%) and viridans streptococci and other streptococci (21%). Others included *Haemophilus influenzae* (5%), *Staphylococcus aureus* (4%), enterococcus (3%), *Listeria monocytogenes* (1%), and klebsiella (1%).[90] Newborns can also acquire postnatal pertussis and viral pneumonia, commonly due to respiratory syncytial virus, adenovirus, and rhinovirus. Pneumonia can also begin in utero with infectious agents such as cytomegalovirus and herpes simplex virus (see Chapter 34).

Clinical, Laboratory, and Radiographic Signs

Bacterial pneumonia is usually accompanied by bacteremia because newborns are frequently unable to confine bacteria to the lung, and therefore, some infants will exhibit clinical signs of sepsis or shock, including poor perfusion and hypotension, in addition to respiratory failure. Blood culture findings will be positive in some newborns with pneumonia, but the presence of maternal antibiotics in the blood of the newborn reduces confidence in a negative result.

Leukopenia, increased percentage of immature granulocytes, and elevated levels of inflammatory markers increase the likelihood of sepsis/pneumonia, but with poor positive predictive value. Tracheal aspirate culture (but not Gram stain) obtained immediately after placement of an endotracheal tube may help with diagnosis and guide therapy, especially when the blood culture result is negative.[91]

Newborns with pneumonia often have diffuse pulmonary infiltrates on chest radiograph, but the radiographic appearance may be difficult to distinguish from RDS and TTNB. Because newborns are unable to localize pulmonary infection, lobar infiltrates are rarely an indication of pneumonia in this age group—plugging of airways with secretions is more likely.

Treatment

Administration of antibiotics directed at the most common organisms (see earlier discussion) should be started immediately when pneumonia is suspected. Ampicillin and gentamicin provide appropriate initial coverage but should be tailored to sensitivities if bacteria are isolated. If the blood culture result is negative, and the mother has been pretreated with antibiotics, some practitioners advocate continuing antibiotics beyond 18 to 24 hours (e.g., 5 to 7 days) if the clinical course is strongly suggestive of pneumonia.

Air Leak Syndromes

Leakage of air from the bronchoalveolar airspace can lead to a variety of air leak syndromes. Additionally, an aberrant connection can form between the pleural space and areas of the airway more proximal to the alveolus (bronchopleural fistula). In this section, we will focus on the most common air leak syndrome in the neonatal period: pneumothorax.

Etiology and Incidence

A pneumothorax is a collection of air within the pleural space, between the parietal pleural of the chest wall and the visceral pleural of the lung. It occurs more frequently in the neonatal period than any other epoch of life. Pneumothorax is associated with increased morbidity and mortality, especially in the extremely preterm infant where it is associated with prolonged hospitalization and higher rates of mortality, BPD, and intraventricular hemorrhage.[92,93] The risk of BPD is further increased in the setting of persistent pneumothorax (>7 days).[94] Although antenatal steroids and surfactant have reduced the rate of pneumothorax, it remains an important cause and complication of respiratory distress in newborns.

A major risk factor for pneumothorax is prematurity. Spontaneous pneumothorax occurs in 1% of term births and increases to 7% to 12% at gestational ages below 29 weeks.[95,96] Other risk factors include male sex, low birth weight, mechanical ventilation, elective cesarean delivery prior to 29 weeks' gestation, and underlying lung disease (i.e., RDS, pneumonia, MAS, pulmonary hypoplasia, pulmonary interstitial emphysema).[97,98] Chorioamnionitis and prolonged rupture of membranes are also independent risk factors for pneumothorax in preterm and very low birth weight (VLBW) infants, respectively. Protective factors include antenatal steroid treatment, surfactant administration, and, when mechanical ventilation is utilized, the use of high-frequency positive pressure ventilation, synchronization, volume-targeted ventilation, and low tidal volumes with higher rates.[99,100]

The association between the use of CPAP and rates of pneumothorax is the subject of debate. Some studies, like the COIN trial,[23] suggest that the use of CPAP increases the risk of pneumothorax, especially in late preterm and term neonates who have increased lung elasticity, decreased chest wall elasticity, increased lung distensibility, and decreased surface tension.[23,101] In the COIN trial,[23] extremely preterm infants (25 to 28 weeks' gestation) were randomized to either CPAP of 8 or intubation after delivery. Infants randomized to CPAP had a higher incidence of

pneumothorax. However, other studies,[102] like the SUPPORT trial,[24] do not endorse this association.

Clinical, Laboratory, and Radiographic Signs

Small pneumothoraces may be asymptomatic. When symptomatic, most are diagnosed in the first 3 days of life due to increased work of breathing and/or increased respiratory support, particularly an increase in supplemental oxygen requirement.[92] In addition, clinical exam may reveal asymmetric aeration. In the setting of a pneumothorax under tension, increased intrathoracic pressure may displace heart tones and the point of maximal impulse of the heart and lead to diminished cardiac return, causing hypotension and bradycardia. Knowledge of the exact onset of a pneumothorax is difficult to ascertain; one study suggests that there can be a delay of 1 to 10 hours between onset and diagnosis.[103]

Although transilluminating the chest may help detect a pneumothorax, its accuracy and reliability are not known. On blood gas assessment, infants may present with hypercarbia and hypoxemia; this is more common in preterm infants.[93] Radiographically, there are asymmetric lung markings in the setting of pneumothorax with the affected side appearing more translucent (black) due to the accumulation of air outside the lung. A visceral pleural edge may be seen with a paucity of more peripheral lung markings. In the setting of a tension pneumothorax, the mediastinum will shift away from the affected side. Small pneumothoraces may be difficult to discern on an anteroposterior chest radiograph and their detection can be improved with the use of a lateral or lateral decubitus (suspected side up) radiograph. Although ultrasound is becoming increasingly popular to augment the diagnosis of pneumothoraces, its use remains institutionally dependent.[104]

Treatment

There are three mainstays of treatment for pneumothoraces: observation, thoracentesis, or thoracostomy and chest tube placement. Use of Fio_2 at 1.0 has not been shown to hasten the resorption of a pneumothorax and may result in unnecessary supplemental oxygen exposure.[105,106]

Asymptomatic pneumothoraces in newborns without lung disease do not require treatment and often go undiagnosed. Additionally, Litmanovitz and Carlo showed that a subset of symptomatic neonates requiring respiratory support could also be managed expectantly.[107] Determinants of successful expectant management included increased gestational age, lower respiratory support requirements, and normal blood gases.

When active treatment is deemed necessary, insufficient evidence exists to recommend thoracentesis over thoracostomy and chest tube placement, or vice versa. A review of two studies recently suggested that initial thoracentesis may avoid the eventual need for thoracostomy and chest tube placement, particularly if an angiocath is used and is left in-situ.[108] In support of these findings, a more recent randomized trial also suggested thoracostomy and chest tube placement can be avoided in some infants initially treated with thoracentesis, with a number needed to treat of only three.[109] Complications of thoracostomy and chest tube placement include lung injury, insertion site and pulmonary infection, phrenic nerve injury, chylothorax, hemorrhagic pericardial effusion, and breast deformities later in life.[110,111] For persistent or difficult-to-control pneumothoraces, additional treatment options include pleurectomy, chemical pleurodesis, and selective intubation of the bronchus to the unaffected lung.

Transient Tachypnea of the Newborn

Definition and Etiology

TTNB is among the most common causes of respiratory distress in the newborn period, affecting 0.5% to 4% of all late preterm and term neonates.[112] The symptoms of respiratory distress typically start within the first several hours after birth and result from failure of adequate absorption of fetal lung fluid. Studies have consistently shown that risk factors for TTNB include prematurity, birth by cesarean delivery, and male sex.[113–115] Among babies born by elective cesarean delivery, a recent study suggests that delivery before 39 weeks increases the risk of TTNB by more than twofold.[116]

Early theories of lung fluid clearance focused on the role of thoracic compression during vaginal delivery and were supported by the observation that TTNB is more common among babies born by cesarean delivery.[117] However, more recent studies have demonstrated that the complex process of lung liquid clearance begins well before term birth.[118] During fetal life, the lung epithelium is responsible for the production of a substantial volume of alveolar fluid, a process that is essential for normal fetal lung growth.[119] With parturition, increased levels of epinephrine, glucocorticoids, and other hormones cause the lung epithelia to transition from a secretory to a resorptive phenotype.[120,121] Activated endothelial sodium channels (ENaC) at the apical surface of lung type II epithelial cells transport sodium and water from the alveolar space into the type II cells.[122,123] Sodium is then actively moved from the type II cell into the interstitium by sodium-potassium pumps (Na/K-ATPase), causing passive movement of water, which is then resorbed into the pulmonary circulation and lymphatics. Supporting a possible role for abnormal activity of ENaC and Na/K-ATPase in TTNB, genetic polymorphisms in β-adrenergic receptor–encoding genes (which regulate expression of these channels) are more common in babies with TTNB.[124]

Diagnosis

The diagnosis of TTNB remains challenging for clinicians. The most typical presenting symptoms, tachypnea/respiratory distress and the need for supplemental oxygen, are common among most neonatal respiratory disorders, and, unfortunately, there exist no specific diagnostic tests for TTNB.[125] For those reasons, the diagnosis remains one of exclusion, and vigilance for other, more severe disorders is imperative. Typically, symptoms of TTNB develop within the first several hours after birth. The degree of respiratory impairment, including the respiratory rate, use of accessory respiratory muscles, and impairment in gas exchange, differs widely. CXRs should be considered in any baby presumed to have TTNB. Although radiographs commonly show prominent perihilar markings and fluid in the fissures, clinicians and radiologists often disagree in their interpretation of these findings in TTNB.[126] Recent studies suggest a potential role for ultrasound in differentiating TTNB from other causes of neonatal respiratory disorders.[127,128]

Treatment

Antenatal steroids for mothers who deliver at 34 to 36 weeks' gestation decrease the incidence of TTNB.[16] Once a presumptive diagnosis of TTNB has been made, treatment is largely supportive.

Oxygen should be provided to maintain normal arterial oxygen saturations. The degree of tachypnea and respiratory distress should determine the amount of respiratory support needed and whether a baby is allowed to feed by mouth. Some recent studies suggest that prophylactic CPAP in the delivery room for late-preterm and term newborns born via cesarean section decreases duration of symptoms and NICU admissions in suspected TTNB.[129,130] A recent Cochrane review evaluating inhaled epinephrine has not shown a decrease in the duration of symptoms or length of hospital stay.[131] Other Cochrane reviews assessing the utility of inhaled beta 2 agonists and inhaled steroids have shown more promising results but warrant further study.[132,133] If there is a suspicion of pneumonia or sepsis, empiric antibiotic therapy should be considered. Furosemide administration to accelerate clearance of lung fluid has not been shown to attenuate the course of TTNB.[134] Prospective studies suggest that moderate fluid restriction for the first 72 hours reduced the duration of respiratory support and cost of hospitalization.[135,136]

Prognosis

Alternative or additional diagnoses should be considered in any infant who is deteriorating or requires mechanical ventilation. With supportive care, full recovery is to be expected after TTNB. However, compared with well infants of a similar gestational age, newborns with TTNB have a significantly prolonged hospital course.[113] Moreover, recent epidemiologic studies have suggested that newborns with TTNB are at increased risk of hospitalization due to respiratory syncytial virus bronchiolitis in the first year of life[137] and increased risk of the later development of asthma.[138–140]

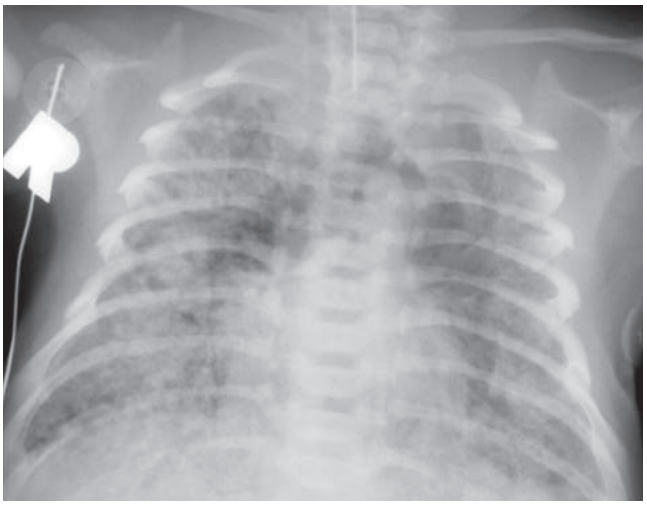
Aspiration Syndromes

Normally, there is a net egress of fluid from the fetal lung into the amniotic cavity, contributing significantly to the amniotic fluid volume. Hypoxemia or acidemia in utero can result in gasping, which may cause the fetus to aspirate amniotic fluid components. This may lead to a variety of aspiration syndromes depending upon the constituents of the amniotic fluid that is aspirated (i.e., sterile, infected, meconium-stained, and/or bloody fluid). This section will focus on the prototypical aspiration syndrome in the neonatal period: MAS.

Definition and Etiology

MAS is associated with inhalation of meconium and amniotic fluid during fetal life or at delivery and is often complicated by significant pulmonary hypertension. It is among the most common causes of hypoxemic respiratory failure in term newborns who require intensive care (Fig. 42.9). The incidence of MAS in babies born after 37 weeks' gestation ranges from 0.1% to 0.7%,[141–143] and one study suggests that the rate of MAS may have been declining in recent years.[144] Among babies born after 39 weeks' gestation with lung disease requiring mechanical ventilation, more than half have MAS.[145] Moreover, MAS is the primary diagnosis for a significant proportion of those newborns who require extracorporeal membrane oxygenation (ECMO) in the United States (26%) and the United Kingdom (51%).[146]

Although a significant percentage of term births are complicated by the passage of meconium before or at delivery, less than 10% of those exposed to meconium develop MAS. Among that 10%, fetal acidemia is believed to cause increased intestinal peristaltic activity that results in passage of meconium and fetal gasping, which draws meconium-contaminated amniotic fluid deep into the lungs. Supporting this theory, autopsy studies of babies who died of MAS demonstrate distal muscularization of small pulmonary arterioles, suggesting long-standing hypoxemia.[147] Recent work suggests that activation of inflammatory cascades may worsen the severity of MAS.[148,149] Particulate meconium in the distal airways causes check-valve obstruction of air passages and leads to regional hyperinflation and atelectasis. In addition, meconium inactivates surfactant, leading to secondary surfactant deficiency.[150] Moreover, babies with MAS are at high risk of persistent pulmonary hypertension, which significantly increases their morbidity and complicates their management.



• **Fig. 42.9** Chest Radiograph of Term Newborn With Severe Meconium Aspiration Syndrome. Note the anterior pneumomediastinum and small left anteromedial pneumothorax.

Prevention

Historically, prevention of MAS has focused on decreasing exposure of the fetal and newborn lung to the noxious effects of intrapulmonary meconium-contaminated amniotic fluid. Infusion of saline into the amniotic cavity (i.e., amnioinfusion) during labor has been studied as a means of both diluting meconium and relieving pressure on the umbilical cord, a potential cause of fetal acidemia. The largest trial investigating this practice[151] found no reduction in the risk of MAS. An alternative strategy for decreasing lung exposure to meconium is intrapartum oropharyngeal and nasopharyngeal suction of fetuses born through meconium-stained amniotic fluid. Although this practice was widely adopted in the 1970s, more recent studies have failed to demonstrate benefit,[152] and the practice is no longer endorsed by the American College of Obstetricians and Gynecologists.[153] Current American Academy of Pediatrics/American Heart Association guidelines for cardiopulmonary resuscitation and emergency cardiovascular care of the neonate no longer recommend intubation and tracheal suctioning for nonvigorous newborns born through meconium-stained amniotic fluid,[154–156] and there has been no increase in incidence or severity of MAS following this practice change.[142,143]

Clinical and Radiographic Signs

The clinical signs of MAS differ widely among babies and may relate to the degree of prenatal compromise, the timing, volume, and consistency of aspirated meconium, and the presence of associated problems. Clinical signs of MAS typically present immediately after birth with tachypnea, increased work of breathing, and cyanosis. Other common associated findings include metabolic acidosis, cardiac dysfunction and hypotension, and postductal desaturation indicative of right-to-left shunting of blood at the ductus arteriosus caused by pulmonary hypertension. Because of the potential for check-valve obstruction of small airways and failure to empty distal lung segments, pneumothorax can complicate the clinical picture. The risk of pneumothorax among ventilated babies with MAS ranged between 10% and 24%.[157,158] Like the degree of clinical signs, CXR findings differ widely. The classic CXR shows diffuse, fluffy infiltrates. However, some babies have milder initial radiographic findings, and there is often progression of visible parenchymal disease over time, likely related to secondary surfactant dysfunction.

Treatment

Approximately half of babies with MAS require mechanical ventilation. The ventilator strategy should be individualized to each baby and to the disease evident on the CXR. In general, because of the likelihood of increased airway resistance, a conventional strategy using slower rates with long inspiratory and expiratory times allows better gas dispersion and more adequate emptying during expiration. Gas trapping and regional or generalized hyperinflation can occur, particularly when rapid rates are used with a conventional mode of ventilation. Some babies respond better to ventilation with a high-frequency device, although there is also a significant risk of hyperinflation. When severe, hyperinflation impairs gas exchange, limits systemic venous return (adversely affecting cardiac performance), increases the risk of pneumothorax, and may exacerbate pulmonary hypertension.

The use of surfactant in MAS continues to be an area of active investigation. Surfactant lung lavage (SLL) demonstrated some promise in improving lung function in an animal model of MAS.[159] Although not definitive, clinical trials suggest that both bolus surfactant administration and SLL may favorably impact the duration of mechanical ventilation and hospital stay and the need for ECMO.[160,161] Furthermore, one recent multicenter retrospective study reported improved surfactant function during therapeutic hypothermia.[162]

In addition to management of parenchymal lung disease in MAS, special consideration must be given to other associated problems, particularly pulmonary hypertension. The risk of PPHN is quite high, exceeding 50% in some series. It has been demonstrated that iNO treatment improves oxygenation in MAS and is particularly efficacious when combined with a ventilator strategy that focuses on improving lung recruitment such as high-frequency oscillatory ventilation.[163]

Treatment with systemic antibiotics is often considered in babies with MAS given that intrauterine infection might be a precipitating factor in the initial passage of meconium, and in vitro studies suggest that the presence of meconium might facilitate the growth of bacteria in the lung. However, a recent Cochrane review and meta-analysis have not shown a benefit to antibiotic administration in neonates born through meconium-stained fluid or in neonates with MAS without evidence of sepsis.[161,164]

In spite of the availability of iNO treatment and high-frequency modes of ventilation, some babies do not respond to medical therapy and require treatment with ECMO. Babies with MAS treated with ECMO have a 94% survival rate, markedly higher than for newborns treated for other respiratory conditions.[165]

Congenital Diaphragmatic Hernia

CDH is a complex clinical syndrome caused by a developmental defect in the diaphragm, resulting in a spectrum of potentially severe cardiopulmonary abnormalities. The estimated incidence of CDH ranges from approximately 1 in 2500 to 1 in 7000 liveborn babies.[166] Approximately 80% to 85% of diaphragmatic hernias occur on the left side. In rare circumstances, they may be bilateral. As many as 30% to 40% of babies with CDH have additional congenital anomalies, most commonly of the heart, central nervous system, and genitourinary system.[166] The remaining 60% to 70% of babies have isolated CDH without other identifiable major anatomic malformations. CDH sometimes occurs in several well-recognized syndromes or in children with a chromosomal abnormality (particularly trisomy 18).

Medical and surgical management of the newborn with a CDH remains one of the most complex and challenging situations in the neonatal intensive care unit. A host of interrelated issues must be considered, including optimal mechanical ventilation strategies, the presence and treatment of pulmonary hypertension, evaluation of cardiac performance, and consideration of support with ECMO. (These management issues along with the clinical features and diagnosis of CDH are discussed in greater depth in Chapter 44.)

Surfactant Protein Deficiency

Overview

The details of surfactant biology and of RDS in premature newborns were presented earlier in this chapter. Term newborns may rarely present with a clinical syndrome dominated by respiratory failure that may be indistinguishable from surfactant deficiency in preterm infants. In the setting of unexplained and protracted respiratory failure in term infants, genetic alterations of surfactant-associated proteins, particularly surfactant protein B (SP-B), surfactant protein C (SP-C), and adenosine triphosphate (ATP)–binding cassette subfamily A member 3 (ABCA3), should be considered and ruled out. In addition, defective signaling of granulocyte–macrophage colony-stimulating factor, which regulates alveolar macrophages and surfactant catabolism, may cause progressive interstitial lung disease in infants.[167]

As described later, there is considerable overlap in the clinical presentation of babies with mutations of SP-B, SP-C, and ABCA3. Although onset, severity of clinical signs, and a family history of lung disease may offer clues to the underlying disorder, a full histologic and genetic evaluation should be considered. Genetic analysis of blood or buccal swabs for mutations of the genes encoding SP-B, SP-C, and ABCA3 are the definitive tests for each disease, but analysis is both costly and time consuming. Moreover, analysis is not routinely available, necessitating transport of specimens to a small number of laboratories specializing in these analyses. In addition, bronchoalveolar lavage (BAL) aspirate

should be analyzed by enzyme-linked immunosorbent assay for the presence of SP-B and pro-SP-C. Lung biopsy, performed by both standard microscopy and electron microscopy, can also provide important clues to the underlying diagnosis.

Surfactant-Associated Protein B Deficiency

Mature SP-B is a small hydrophobic protein (79 amino acids) that plays several key roles in the processing and function of normal pulmonary surfactant.[168] Transgenic mouse models suggest that SP-B is critical for phospholipid packaging into lamellar bodies, the formation of tubular myelin, and spreading/function of the surfactant monolayer. In addition, normal SP-B appears to be essential for normal processing of SP-C.[169] In all babies described to date with SP-B deficiency, the genetic mutation has been inherited from the parents (autosomal recessive) rather than occurring as a spontaneous mutation.[170] Although multiple genetic mutations have been described, approximately 70% of affected babies carry the 121ins2 mutation.[157] In a series of term babies referred for genetic evaluation of unexplained respiratory failure, 2 of 17 had detectable mutations of the SP-B sequence.[171]

Almost all babies with recognized deficiency of SP-B develop clinical signs of severe respiratory distress, including tachypnea, grunting, and retractions, within the first several hours of life. As for preterm newborns with RDS, CXRs classically reveal diffuse, hazy air space disease with visible air bronchograms. Severe PH may be a prominent feature of the disease. Treatment with surfactant is either ineffective or unsustained, and progressive respiratory failure is the rule with near 100% lethality in the first 6 months of life without lung transplant.[172] Lung transplant is currently the only effective long-term treatment, with a reported 5-year survival of 56%.[173] There is the potential for gene therapy in the future, but, to date, studies have been limited to animal models.[174–176]

Analysis of fluid obtained by BAL/tracheal aspirate from babies with SP-B deficiency should fail to detect any immunoreactive SP-B. The presence of pro-SP-C increases suspicion of SP-B deficiency because intact SP-B is necessary for normal posttranslational processing of the protein to mature SP-C. Histologic findings include alveolar cell hyperplasia, interstitial thickening, and variable degrees of fibrosis and alveolar proteinosis. Staining of lung tissue for pro-SP-B is variable but staining for mature SP-B should be minimal (because of cross-reactivity with epitopes on pro-SP-B) or absent. Initial DNA analysis focuses on the 121ins2 mutation. More exhaustive testing for other known mutations is warranted if initial testing is negative.

Surfactant-Associated Protein C Deficiency

Mature SP-C is a 35 amino acid hydrophobic protein. SP-C is believed to enhance spreading of surfactant and to participate in normal surfactant catabolism.[167] As with SP-B, multiple mutations of SP-C have been described; however, most of these mutations of the gene encoding SP-C arise spontaneously and result in sporadic disease. Whether the abnormal phenotype associated with SP-C deficiency arises from dysfunction of the alveolar surfactant or from accumulation of abnormal cellular SP-C and consequent type II cell injury is not known.

In contrast to babies with SP-B deficiency, babies with SP-C deficiency have a wide range of clinical presentations. Some may develop symptoms within the first several hours of life, similar to SP-B deficiency, whereas others present later in childhood or in adulthood with interstitial lung disease. In one series, half of children with SP-C deficiency presented in the neonatal period, and mortality was reported as 15%.[177] The reasons that underlie the variable onset, presentation, and severity of SP-C deficiency are not fully understood, but it is suggested that those individuals whose SP-C mutation lies within the specific BRICHOS chromosomal domain are at higher risk of neonatal presentation.[178] Corticosteroids are the mainstay of treatment of infants with SP-C deficiency, although many centers also administer azithromycin and/or hydroxychloroquine.[178]

In common with SP-B deficiency, the lung histopathologic features of patients with SP-C deficiency are nonspecific and widely variable. Common findings include accumulation of alveolar protein and macrophages and epithelial cell hyperplasia. Ultrastructural examination may reveal disorganized lamellar bodies with aggregates of small vesicles with electron-dense cores in the type II cells.[179] Allele-specific testing using the polymerase chain reaction for the most common 173T mutation provides an initial screen for SP-C deficiency. If the screen is negative, direct sequencing of the entire SP-C gene should be undertaken.

Adenosine Triphosphate–Binding Cassette Subfamily A Member 3 Deficiency

The ATP-binding cassette transporter proteins are essential for normal transport of compounds in numerous biologic systems.[180,181] Deficiencies of individual ATP-binding cassettes have been associated with clinical diseases in a number of different organ systems. ABCA3 is highly expressed in the lungs and is involved in the transport of lipids. Individuals lacking the gene for ABCA3 have abnormal accumulation of surfactant-rich lamellar bodies within their type II alveolar cells, with apparent inability to transport surfactant into the alveolar space. Shulenin et al.[182] detailed a variety of mutations within the ABCA3 gene in a substantial portion of term infants with unexplained respiratory failure and suspected surfactant protein deficiency.

The age at presentation for individuals with ABCA3 deficiency is highly variable, ranging from the immediate newborn period to later in childhood. There may be a family history of consanguinity. Clinical manifestations of disease in the neonate may be indistinguishable from those of neonates with SP-B deficiency, with onset of respiratory failure within hours of birth. The disease may be progressive and fatal. In addition, ABCA3 deficiency may also manifest as severe PPHN.[183] The treatment options for ABCA3 deficiency are limited. Some children undergo lung transplant, and recent case reports suggest that some patients may respond to corticosteroids, azithromycin, or hydroxychloroquine.[184–186] It has been reported that milder, transient neonatal symptoms may not prompt a diagnostic evaluation in the newborn period, although recurrent pulmonary symptoms may lead to later investigation.[187] These reports raise the possibility that deficiency of ABCA3 may be underrecognized in infants or children with a mild or normal phenotype.

The predominant pathologic findings of neonates with ABCA3 deficiency with neonatal respiratory failure include alveolar proteinosis,[188] type II cell hyperplasia with dense lamellar bodies, and accumulation of alveolar macrophages in the distal air space. Inheritance is believed to be autosomal recessive. Unlike SP-B deficiency, a single predominant mutation has not been described; rather, multiple distinct mutations affecting different protein domains have been identified.[189–191]

Thyroid Transcription Factor 1 Gene Mutation

Recent studies demonstrate that mutations of the gene (*NKX2-1*) encoding thyroid transcription factor-1 (TTF-1), which is expressed in the thyroid, lung, and brain, can result in neonatal respiratory failure. TTF-1 regulates structural lung development and plays a key role in the expression of SP-B, SP-C, and ABCA3. Histologic findings from these patients are heterogeneous and not diagnostic. Hamvas et al.[192] reported a series of 21 patients with *NKX2-1* mutations, 17 of whom had neonatal RDS (with or without PPHN). Some of these patients also had thyroid and/or brain abnormalities, while others had only respiratory disease evident at the time of diagnosis. Like with other interstitial lung disorders, treatment of symptomatic patients with steroids, azithromycin, and hydroxychloroquine may be beneficial.[193]

Surfactant-Associated Proteins A and D

Surfactant-associated proteins A (SP-A) and D (SP-D) are part of the innate immune system of the lung and maintain surfactant lipid homeostasis.[167] Although lung structural abnormalities have been described in mice lacking SP-A and SP-D, no phenotype attributable to abnormalities of either of these surfactant-associated proteins has been described in human infants.[194] Recent work suggests a role for mutations in the gene that encodes SP-A in adult-onset pulmonary fibrosis in rare circumstances.

Other Interstitial Lung Diseases

The interstitial lung diseases that occur in infancy are a heterogeneous group of disorders that overlap with the surfactant protein disorders and may involve one or more of the several components of the lung.[195] All produce considerable morbidity and mortality. Detailing each individually is beyond the scope of this chapter, but two entities, alveolar capillary dysplasia with misalignment of pulmonary veins (ACD/MPV) and pulmonary interstitial glycogenosis (PIG), have attracted considerable attention in recent years.[167,192,196]

Alveolar Capillary Dysplasia/Misalignment of Pulmonary Veins

ACD/MPV results from severe developmental abnormalities of the structure of the pulmonary circulation. In particular, alveolar capillary density is reduced, and capillaries typically reside in the center of abnormally widened alveolar walls rather than immediately proximate to the alveolar epithelium and air space. An additional histologic hallmark of the disease is abnormally positioned pulmonary veins, which are adjacent to the terminal bronchioles.[197] Most newborns with ACD/MPV experience respiratory failure and PPHN within the first several days of life. For reasons that are poorly understood, presentation may be delayed in some babies. The typical course of ACD/MPV is refractory respiratory failure and pulmonary hypertension, which ultimately leads to death. Some babies may respond to pulmonary vasodilators but only transiently.[198] Stankiewicz et al.[199] documented deletions of the Forkhead Box transcription factor (FOXTF) gene cluster on chromosome 16 in a series of ACD/MPV patients, and the FOXTF has recently been proposed to function through STAT3 signaling to promote lung angiogenesis.[200] Depending on the underlying chromosomal abnormality, 50% to 75% of patients with ACD/MPV have other malformations, most notably of the gastrointestinal, genitourinary, and cardiovascular systems.[195]

Pulmonary Interstitial Glycogenosis

PIG is an interstitial lung disease thought to be unique to infants that is characterized by a thickened lung interstitium and the accumulation of intracellular glycogen in immature interstitial cells.[201,202] It has been postulated that PIG represents a developmental lung disorder rather than one resulting from inflammation or other precipitants. Infants with PIG typically develop respiratory symptoms in the first few days of life, including tachypnea and hypoxemia; many require a period of assisted ventilation; and many have pulmonary hypertension.[203] In a series of 24 patients with PIG, Liptzin et al.[204] reports 63% had structural heart disease and 92% were treated with steroids. Many infants with biopsy-proven PIG treated with pulse steroid therapy have their respiratory symptoms abate or resolve altogether in the first year of life. Whether steroid treatment accelerates the abatement process has not been established and remains controversial.[205]

Summary

The initial evaluation of respiratory distress/hypoxemia in the newborn presents one of the most difficult challenges faced by pediatricians and neonatologists. An ordered approach using information derived from the history, physical examination, pulse oximetry measurements, radiographic and laboratory measurements, and echocardiography can help elucidate the cause of hypoxemia and respiratory failure and direct each step of clinical management. Recognizing the important contributions of parenchymal lung disease, pulmonary vasoconstriction, and cardiac performance is critical to successful clinical management of the newborn with respiratory failure.

Acknowledgment

The contributions of J. Craig Jackson to the previous edition of this chapter—particularly the sections on respiratory distress syndrome, pulmonary hypoplasia, pneumonia, and pulmonary hemorrhage—are gratefully acknowledged.

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Chronic Neonatal Respiratory Disorders

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KEY POINTS

- Bronchopulmonary dysplasia (BPD), as determined near term corrected age in former preterm newborns < 32 weeks' gestational age is a marker for chronic respiratory morbidity.
- Newborns that are most immature and those born from an adverse intrauterine environment, affected by maternal vascular disorders and decreased intrauterine growth, are most susceptible to early neonatal respiratory illness.
- Early neonatal respiratory illness, marked by increased exposure to supplemental oxygen and use of mechanical ventilation, is associated with BPD and later respiratory morbidity.
- Former preterm newborns have evidence of lung dysfunction near the time of initial hospital discharge.
- Lung dysfunction, immune deficits and environmental factors confer vulnerability to lower respiratory tract infection and wheezing illness in infancy and early childhood.
- Composite respiratory morbidity outcomes ascertained during infancy primarily reflect resource utilization due to respiratory disease, vary widely in prevalence based on population of interest, and reflect a broad spectrum of severity.
- Dysplastic development of both the lung and airway in former preterm newborns occurs. However, the most prominent long-term morbidity of former preterm newborns is obstructive lung disease and wheezing illness or asthma.

Introduction

Chronic lung disease of infancy following preterm birth was first described by Northway and colleagues in 1967.[1] These investigators identified clinical, radiographic and histopathological findings of lung fibrosis and airway disease in former preterm newborns with death or ongoing pulmonary dysfunction after exposure to prolonged mechanical ventilation and hyperoxia. They termed this disorder bronchopulmonary dysplasia (BPD), reflecting dysplastic extrauterine development of the lung parenchyma and airways following preterm birth prior to or early in the alveolar stage of lung development. Subsequent investigations (1) confirmed and expanded the description of post-mortem lung histopathology, noting pulmonary vascular changes consistent with observed right ventricular hypertrophy and cor pulmonale, and (2) demonstrated fixed and reactive airway obstruction, with symptomatic lung disease, susceptibility to infection and chest wall deformity in survivors with BPD at a mean age of 18 years.[2,3] Many of the newborns cared for in these reports were born at ≥30 weeks' gestational age (GA). However, as approaches to preterm infant care evolved, rates of survival and successful separation from respiratory support improved for infants at these gestational ages and lower, prompting investigations of a clinical marker for subsequent morbidity and mortality. In a retrospective analysis of former preterm infants born at 25 to 32 weeks' GA, Shennan et al. evaluated the ongoing use of supplemental oxygen up to 38 weeks' post-menstrual age (PMA) as a predictor for later respiratory morbidity or death up to 2 years of age.[4] They proposed 36 weeks' PMA as the most appropriate time point to discriminate between those infants with and without morbidity and mortality. The use of supplemental oxygen with or without positive pressure at 36 weeks' PMA evolved into the clinical definition of BPD, used as a benchmark and quality metric for neonatal care, and as an endpoint for clinical trials designed to improve respiratory outcomes in extremely preterm newborns.[5,6]

With further advances in perinatal and neonatal care, including the use of antenatal steroids, exogenous surfactant and lung protective strategies for early respiratory support, histopathological airway disease and lung fibrosis became less prominent post-mortem findings in former preterm newborns.[7] The changing landscape of respiratory illness in low and extremely low gestational age newborns (ELGAN) and interest in further discriminating respiratory outcomes in former preterm infants were addressed in a National Institutes of Health (NIH) sponsored expert workshop in June 2000. Attendees developed a tiered, severity-based approach to the definition of BPD, proposing categories of mild, moderate, and severe BPD based on level of respiratory support at 36 weeks' PMA (degree of supplemental oxygen and use of positive pressure) for infants born at < 32 weeks' GA.[8] Investigators from the National Institute of Child Health and Human Development (NICHD) Neonatal Research Network (NRN) subsequently validated this NIH consensus definition in follow-up of survivors of preterm birth at 18 to 22 months corrected age.[9] They demonstrated increasing proportions of infants with use of respiratory medications, rehospitalization for respiratory cause, growth failure and neurodevelopmental impairment with increasing severity of BPD. Notably, the addition of an abnormal chest radiograph demonstrating parenchymal lung disease did not improve discrimination for these later morbidities.

The NIH consensus definition has raised concerns as to its usefulness at both lower and higher levels of severity. Specifically, the discrimination of infants classified as no versus mild BPD may not be clinically important. Infants classified as severe BPD may represent too broad a category to be clinically meaningful, as it includes a spectrum spanning infants receiving support by nasal cannula and those remaining on mechanical ventilation. In October 2016, an NICHD expert panel proposed a modification of the prior consensus definition of BPD, still defined at

36 weeks' PMA, using various cut-offs for inspired oxygen concentration (FiO_2) and nasal cannula flow at different levels of BPD. Notable features of this modification were a change in terminology to Grades I-III, with the highest grade, Grade III, including only infants on mechanical ventilation with $FiO_2 > 21\%$ or infants requiring noninvasive pressure support with $FiO_2 \geq 30\%$ (e.g., continuous positive airway pressure [CPAP], high flow nasal cannula (HFNC) > 3 L/min); a special category IIIA would be assigned to infants that died prior to 36 weeks' PMA due to lung disease.[10] Alternatively, Abman and colleagues from the US-based Bronchopulmonary Dysplasia Collaborative proposed retaining the original NIH consensus categories while subdividing severe BPD into two subtypes: severe type 1 BPD would encompass infants supported by low flow nasal cannula with an $FiO_2 \geq 30\%$ or any administration of noninvasive positive pressure support or HFNC, and severe type 2 BPD for all infants supported with mechanical ventilation.[11] Neither group evaluated the ability of these particular cut-offs to predict later respiratory morbidity, but this task was taken on by Jensen and colleagues from the NICHD NRN, using the outcome of death or severe respiratory morbidity ascertained at 18 to 26 months of age.[12] Their exploration of discriminatory cut-offs for various levels of respiratory support at 36 weeks' PMA yielded three levels of BPD, termed Grades 1 to 3, defined solely by mode of support without regard for FiO_2. Grade 3 includes all infants on mechanical ventilation, Grade 2 all infants on noninvasive positive pressure or nasal cannula flow > 2 LPM and Grade 1 all infants on nasal cannula flow ≤2 LPM. Similarly, Isayama and colleagues found that the dichotomous classification of use of supplemental oxygen or any mode of positive pressure respiratory support (including nasal cannula flow >1.5 LPM) was the strongest predictor of respiratory outcome at 18 to 21 months corrected age, in data from the Canadian Neonatal Network.[13]

These proposed definitions of BPD as either a predictor or surrogate outcome for later respiratory morbidity do not address the underlying pathophysiology of chronic respiratory illness in former extremely preterm newborns. The evolution of the histopathology related to early respiratory insufficiency, with alveolar simplification accompanying less prominent fibrosis, generated the concept of a "new BPD," that develops in vulnerable preterm newborns born during the late canalicular or early saccular stage of lung development and characterized by extrauterine arrested alveolar and microvascular development.[7,14,15] Yet symptomatic and functional airway obstruction remains the most prominent manifestation of persistent pulmonary disease in former preterm newborns and is not explained by arrested lung growth nor the recovery from that arrest.[16] Additional contributions to chronic respiratory illness may be pulmonary vascular disease, susceptibility to infection, environmental exposures and the influence of other socioeconomic circumstances, each of which are variably related to lung parenchyma versus airway development and dysplasia.[17–19] Thus, early dysplastic, arrested lung growth and ongoing clinical airway disease suggest that these conditions may themselves be related but separate co-travelers that lay the path to chronic respiratory illness in former preterm infants.

Epidemiology of Bronchopulmonary Dysplasia and the Vulnerable Preterm Lung

The primary risk factors for BPD and its severity are related to the degree of immaturity at birth, reflected by gestational age or birth weight.[20–22] Various US-based publications including contemporary data from the population-based California Perinatal Quality Care Collaborative (CPQCC), the multicenter NICHD NRN and the nationwide Vermont Oxford Network (VON) collaborative, demonstrate these important relationships, and provide estimates of the incidence and severity of BPD in extremely preterm newborns born at 22 to 29 weeks' GA (Table 43.1). Overall incidence of (1) BPD or death at 36 weeks' PMA was 47% in CPQCC, (2) BPD among survivors at 36 weeks' PMA was 45% in the NICHD NRN, and (3) Grade 1/2 BPD (any use of nasal cannula or noninvasive positive pressure) was 41% and Grade 3 BPD (mechanical ventilation) was 4% in survivors at 36 weeks' PMA in VON. Given the variability in both the patient populations and the diagnostic criteria for BPD, these estimates are remarkably consistent, with outcomes reported in over 44,000 infants spanning 12 birth years. These estimates were determined by clinical prescription of respiratory support and could be affected by provider variability. However, the use of a physiologic challenge consisting of a monitored decrease in nasal cannula flow and FiO_2 at 36 weeks' PMA does not substantively affect the proportion of infants classified with BPD.[23–25]

In addition to immaturity, elements of the fetal environment influence early respiratory morbidity of BPD. Investigators in the multicenter ELGAN Study classified over 1000 singleton pregnancies into one of six presenting conditions leading to delivery at < 28 weeks' gestation. Their analyses produced two primary phenotypes for preterm delivery, an inflammatory phenotype, and a vascular (placental) phenotype, associated with maternal preeclampsia or fetal indications for delivery.[26] These delivery indications were used to evaluate risk of BPD, along with birth weight z-scores, reflecting fetal growth. Low birth weight z-scores (<−2 and between −2 and −1) conferred increased odds of BPD, with the greatest effect seen in newborns with the most severe intrauterine growth restriction, even when adjusting for delivery indication.[27] Other single center studies similarly reported that pre-eclampsia greatly increased the odds of BPD while adjusting for birth weight z-score and severe intrauterine growth restriction in combination with maternal vascular disease greatly increased the odds for BPD and BPD or death.[28,29] In the EPIPAGE-2 cohort, a French prospective population-based study of outcomes of prematurity, birth weight z-score was an independent predictor of BPD (decreasing z-score increased odds of BPD) while adjusting for GA and vascular/placenta-based complications of pregnancy.[30] Consistent with these findings, maternal vascular underperfusion identified in the placentas of extremely preterm newborns was also associated with increased odds of BPD.[31] These findings support the concept that the adverse fetal environment affects intrauterine lung growth and development, increasing the vulnerability of the immature lung to further insults.

When considering chronic neonatal respiratory disorders, it is important to recognize the interface of early respiratory support and supplemental oxygen due to respiratory insufficiency, with the markers and effectors of lung dysfunction and dysplasia a precursor to chronic lung disease. Laughon et al. demonstrated that the pattern of supplemental FiO_2 over the first 14 days of life is highly associated with later risk of BPD in newborns 23 to 27 weeks' GA enrolled in the ELGAN Study.[32] Regardless of the mode of respiratory support, newborns with consistently low FiO_2 (≤25%) in the first 14 days had an incidence of BPD of only 17%, whereas those with higher early and escalating FiO_2 had a BPD incidence of 67%, and newborns with an initial decrease and then escalation of FiO_2 had an intermediate BPD incidence of 51%. In refinement of this risk and vulnerability, these investigators also showed that

TABLE 43.1 Epidemiology of Bronchopulmonary Dysplasia (BPD)

		CPQCC[a]			NICHD NRN[b]		VON[c]		
		2007–2011 22–29 wk 400–1500 g			2008–2012 22–28 wk 401–1500 g		2018 22–29 wk		
		n	BPD or Death (%)		Survivors (*n*)	BPD (%)	Survivors (*n*)	Grade 1/2 BPD (%)	Grade 3 BPD (%)
Birth weight category (g)	<750	4531	80.7						
	750–999	4391	49.3						
	1000–1249	3828	25.1						
	1250–1500	2302	13.1						
Gestational age (wk)	22				24	87.5	148	69.6	20.9
	23				266	78.9	931	76.6	12.7
	24				801	68.9	1833	69.4	10.6
	25				1101	57.3	2550	60.3	8.2
	26				1310	50.2	3006	53.0	4.5
	27				1581	36.4	3740	40.2	2.9
	28				1770	23.5	4732	29.3	1.9
	29						5382	20.0	0.8

[a]California Perinatal Quality Care Collaborative; Receiving supplemental oxygen at 36 weeks' post-menstrual age (PMA) or earlier discharge or mortality prior to 36 weeks' PMA.[20]
[b]National Institute of Child Health and Human Development Neonatal Research Network; Survivors receiving supplemental oxygen at 36 weeks' PMA or earlier discharge.[21]
[c]Vermont Oxford Network; Survivors receiving specified mode of support at 36 weeks' PMA or earlier discharge. Grade 1/2 nasal cannula or noninvasive positive pressure, Grade 3 mechanical ventilation.[12]

within each of these patterns, those newborns with birth weight z-score < −1 had an elevated risk of BPD.[33] In a secondary analysis of data from the Trial of Late Surfactant (TOLSURF) that enrolled preterm infants who remained mechanically ventilated at 7 to 14 days of life, cumulative supplemental oxygen exposure over the first 14 days of life accurately predicted later outcomes of BPD or death and BPD in survivors.[34] In multivariate modeling, cumulative mean airway pressure over the same 14-day time period was also an independent predictor of these adverse respiratory outcomes. Using data from the NICHD NRN for newborns 23 to 30 weeks' gestation, Laughon et al. found that gestational age, birth weight, mode of respiratory support and FiO_2 were all selected as important predictors of BPD or death over the first 28 days of life, but the importance of these variables shifted over time.[35] At days 1 and 3, gestational age and birth weight were the most influential factors, but at days 7 to 28, mode of respiratory support was the most important factor, and FiO_2 was the second most important factor at days 21 and 28. Taken together, these studies highlight the risks associated with the need for early or prolonged respiratory support, and the chronic consequences of that support.

Randomized trials designed to evaluate limitation of exposure to invasive mechanical ventilation and oxygen supplementation in extremely preterm infants have shown modest effects on the occurrence of BPD (primary noninvasive respiratory support vs. mechanical ventilation and surfactant). Moreover, these approaches may have unintended consequences (lower vs. higher oxygen saturation targets) and may not decrease later respiratory morbidity in at-risk populations. Two meta-analyses published in 2016 describe the effects of the primary strategy of providing noninvasive positive pressure (CPAP) versus intubation and mechanical ventilation.[36,37] These analyses had different approaches and their results differed somewhat. The meta-analysis from the Cochrane Library, using data from three trials, demonstrated decreased risk ratios for BPD (0.89; 95% CI 0.79, 0.99) and BPD or death (0.89; 95% CI 0.81, 0.97) with the strategy of applying and continuing CPAP soon after birth.[37] This strategy was accompanied by significant reductions in the use of mechanical ventilation and surfactant administration. The other study applied network meta-analyses to 2 of the 3 studies included in the Cochrane analysis, producing network odds ratios that had similar effect sizes as the reported risk ratios, but confidence intervals for BPD and BPD or death that crossed the null boundary. The implementation of noninvasive strategies has been widespread and short-term benefits may increase with use of noninvasive surfactant administration, for newborns meeting specific criteria[36]. However, broad clinical implementation of a strategy to limit invasive mechanical ventilation has been also been associated with an increase in overall duration of respiratory support and worse lung function parameters at school age.[38]

The international Neonatal Oxygenation Prospective Meta-analysis (NeOProM) collaborative aligned study protocols and shared data to allow for an individual patient data meta-analysis

of 5 double-blind randomized, controlled clinical trials.[39] These studies compared oxygen saturation targets of 85% to 89% versus 91% to 95% for extremely preterm newborns receiving supplemental oxygen. Although the primary outcome of the collaborative was the effect of oxygen saturation targets on neurodevelopmental disability, the group analyzed secondary outcomes and subgroup effects.[40] Importantly, mortality was increased in the lower oxygen saturation group up to 18 to 24 months corrected age, including at 36 weeks' PMA (risk difference 2.5%; 95% CI 0.5, 4.5%). Severe necrotizing enterocolitis (NEC) was also increased with lower oxygen saturation targets (risk difference 2.3%; 95% CI 0.8, 3.8). Among survivors, BPD, defined as oxygen supplementation at 36 weeks' PMA, was decreased by 5.6% (95% CI −8.5, −2.7%), relative risk (RR) 0.81 (95% CI 0.74, 0.90), an effect that was even greater in SGA infants (adjusted RR 0.54 (CI 0.37, 0.77). These results provide more direct insight into the effects of oxygen supplementation on the development of chronic respiratory disease in these vulnerable populations. However, the increase in mortality and NEC when targeting oxygen saturations < 90% suggests that more targeted strategies will be needed to improve respiratory outcomes.

Additional Risks to the Developing Lung

Pulmonary vascular disease (PVD) with or without pulmonary hypertension (PH) is a co-morbidity that impacts respiratory status and potential for recovery in former extremely preterm newborns, particularly in those with BPD.[41,42] Based on experimental models and human histopathology, aberrant vascular growth with a restricted vascular bed would be expected to accompany the dysplastic parenchymal lung development following preterm birth.[7,14,15,43,44] Published studies in this area pose a challenge to describing risks and outcomes for PVD/PH due to (1) heterogeneous study population inclusion criteria, (2) timing of evaluation, and (3) variable diagnostic criteria for PVD/PH, which lack standardization by echocardiography in this population (the diagnostic mode most commonly employed).[42,45–50] As a result, there is a broad range of estimated prevalence of PVD/PH in former preterm newborns at or around term corrected age, with higher prevalence among infants with a concurrent diagnosis of BPD, and increasing prevalence with increasing severity of BPD. In a meta-analysis of 25 published studies, Arjaans et al. provide prevalence estimates for the following populations: extremely preterm newborns with or without BPD (12%; 95% CI 9, 15%); extremely preterm infants with BPD (20%; 95% CI 14, 25); extremely preterm infants by NIH consensus BPD classification [none 2% (95% CI 0, 8%); mild 6% (95% CI 1, 13); moderate 12% (95% CI 4, 24%); severe 39% (95% CI 29, 49%)].[41]

The underpinnings of the development of PVD/PH in former preterm infants are of great interest as PVD/PH is strongly associated with the presence and severity of BPD, yet it does not affect all infants within any BPD classification equally. Arjaans et al. analyzed risk factors for the diagnosis of PVD/PH in their meta-analysis and found that newborns that were SGA had an increased risk of PVD/PH regardless of the diagnosis of BPD.[41] This is consistent with increased prevalence of PVD/PH in infants born from pregnancies with placentas affected by maternal vascular underperfusion, further implicating the adverse fetal environment as a critical area of vulnerability for extremely preterm newborns with respect to the lung.[31,51] Two additional prospective investigations further support the concept that the early clinical condition of extremely preterm newborns predisposes them to adverse respiratory outcomes. In survivors to 36 weeks' PMA, Mourani et al. performed echocardiograms at 7 days of age and demonstrated that interventricular septal wall flattening (a sign of elevated right heart pressure) at 7 days of age was associated with the later classification of BPD and with echocardiographic diagnosis of PVD/PH at 36 weeks' PMA.[52] Mirza and colleagues performed serial echocardiograms approximately every 48 hours until 14 days of age.[53] They found that 55% (32/58) of newborns had a delayed perinatal transition at 72 to 96 hours of age, defined as estimated right-sided pressure ≥50% of systemic blood pressure. This factor was the primary influence on the outcome of BPD or death in multivariate modeling, although delayed transition was also inversely related to gestational age. Further, of the 10 infants that had persistent PVD/PH by echocardiogram at 14 days, all ultimately died or were classified with BPD, consistent with the findings from Mourani et al. Newborns with concerning echocardiographic findings at the assessment time points of 72 to 96 hours and 7 days of age also had increased levels of respiratory support compared to those with more reassuring findings.[52,53] Overall, it is clear that the vulnerability conferred by immaturity and an adverse intrauterine environment creates susceptibility to PVD/PH, and that PVD/PH confers further risk of adverse outcomes for extremely preterm newborns with chronic respiratory disease.

The diagnosis of PVD/PH is associated with increased mortality. The meta-analysis of Arjaans et al. identified a risk ratio for mortality of 4.7 (95% CI 2.7, 8.3) in infants with and without a diagnosis of BPD.[41] Individual investigations have further delineated this relationship. In a single center study, Arjaans and colleagues demonstrated that among 28 infants with BPD complicated by PVD/PH, those with suprasystemic right-sided pressure estimates by echocardiogram at ≥36 weeks' PMA had worse survival curves than those with subsystemic pressure estimates (1-, 3-, and 7-month survival of 80%, 50%, and 30% vs. 94%, 83%, and 76%; log-rank $P = .03$).[54] Overall mortality was 39%. Similarly, mortality was 28% in 61 infants with BPD and PVD/PH in another single center cohort.[55] Finally, in a multicenter cohort of infants with severe BPD, Vyas-Read and colleagues identified that infants with interventricular septal wall flattening on echocardiogram had elevated mortality compared with infants with normal septal position (19% vs. 8%).[42] Septal flattening was a specific (82%) but not sensitive (37%) predictor of mortality relative to normal or missing echo findings. Beyond the echocardiographic assessment of PVD/PH and its relationship to BPD, data regarding vascular dysfunction lends further insight into the interface of structural and functional lung disease. Functional pulmonary vascular differences as assessed by reactivity at cardiac catheterization are present in former preterm newborns with BPD. Vasoreactivity in response to acute vasodilator testing was present in 35% to 77% of those infants selected for cardiac catheterization.[56–58] Frank and colleagues reported only one death among nine infants with a vasodilatory response, compared to five deaths among 17 nonreactive infants, a difference that was statistically significant when combined with the outcome of tracheostomy (11% vs. 53%, $P = .04$).[57] For abnormal vasoconstriction, increased pulmonary artery pressure has been described with withdrawal of supplemental oxygen in children with BPD during cardiac catheterization, similar to what is seen with respect to worsening PH by echocardiogram during respiratory infection (consistent with vasoconstriction triggered by the inflammatory stimulus).[59,60] Thus, PVD/PH in former preterm infants with BPD is both a structural and a

functional phenomenon, and the ongoing risks of PVD/PH may further influence the developing lung.

Individual infants may have multiple major comorbidities of prematurity following extremely preterm birth, with the most immature newborns at greatest risk. In a California population-based cohort of extremely preterm newborns (22 to 28 weeks' GA), the proportion of infants with more than one major neonatal morbidity increased with decreasing gestational age, while rates of survival free of major morbidity increased with increasing gestational age.[61] These results mirror data from the NICHD NRN and the French population-based EPIPAGE-2 cohort study.[21,62] BPD also is a strong predictor of impaired neurodevelopmental outcome, with early respiratory support metrics influencing later neurological status.[9,13,63] Consistent with these patterns, inflammatory conditions, such as sepsis and NEC, are overrepresented in preterm newborns that are later classified with BPD, and NEC is also a risk factor for PVD/PH.[22,41,64] These inflammatory conditions may impede extrauterine lung development and contribute to early and later respiratory insufficiency. Early inflammation is also an effector of airway disease; in data from the Tucson Children's Respiratory Study (TCRS), both bronchiolitis and pneumonia occurring at < 3 years of age are implicated in persistent clinical and functional obstructive airway disease in children born at term without neonatal respiratory illness.[65,66] Thus, inflammatory insults that occur before and after initial discharge may contribute to the later respiratory manifestations of extremely preterm birth, represented by airway obstruction and wheeze.

Pharmacological Prevention of Bronchopulmonary Dysplasia

Many controlled trials have investigated pharmacologic interventions to prevent BPD in high-risk preterm newborns.[6] However, relatively few interventions have consistently shown short-term benefit (i.e., decrease in incidence of BPD or BPD or death, or increase in survival without BPD), despite utilizing meta-analyses to increase power. Even fewer interventions have demonstrated longer-term respiratory benefit (reduction in incidence of respiratory morbidity after hospital discharge). Proposed pharmacological interventions have generally targeted decreasing inflammation and/or direct promotion of lung growth and repair; several drug classes have demonstrated short-term benefit with or without consideration of longer-term effects.

With respect to strategies to limit inflammation, systemic, inhaled, and instilled glucocorticoids have been studied to prevent BPD. There is well-known controversy regarding the use of systemic dexamethasone, as its administration has been associated with increased rates of neurodevelopmental impairment (NDI), including cerebral palsy, compared to unexposed newborn controls. This risk may be modified by (1) the timing of exposure, (2) the total dose of dexamethasone, and (3) the underlying risk of BPD, with dexamethasone conferring lower risk or no increased risk of NDI in newborns and infants at higher risk of BPD.[67,68] Early dosing (in the first week of life) of systemic hydrocortisone has also been studied. A recent meta-analysis demonstrated that early hydrocortisone modestly increased survival without BPD (risk ratio 1.19; 95% CI 1.04, 1.35; number needed to treat [NNT] 13) and decreased BPD (risk ratio 0.84; 95% CI 0.72, 0.98; NNT 14), but substantially increased rates of intestinal perforation (risk ratio 1.76; 95% CI 1.09, 2.84; number needed to harm [NNH] 28).[69] This increased risk of intestinal perforation was attributed to proximate or concurrent medical treatment of patent ductus arteriosus (PDA), although the meta-analysis also demonstrated a substantial decrease overall in treatment for PDA in newborns exposed to early hydrocortisone (risk ratio 0.66; 95% CI 0.49, 0.88; NNT 11).[69,70] Further, survival without moderate-to-severe NDI was higher in infants previously treated with hydrocortisone (risk ratio 1.14; 95% CI 1.03, 1.27; NNT 13). Early hydrocortisone was also evaluated in an individual patient data meta-analysis including four of the five studies analyzed in the conventional meta-analysis.[71] These analyses identified that the relationship of hydrocortisone and intestinal perforation only held in newborns that were also treated with indomethacin for PDA, and it demonstrated a modest increased in odds of late onset sepsis with hydrocortisone (odds ratio 1.34; 95% CI 1.02, 1.75; NNH 15).

The effects of late hydrocortisone (after the first week of life) were evaluated in the previously cited conventional meta-analysis, with data from only two studies. No significant effects were seen on either BPD-related or later follow-up outcomes.[69] A third trial of late hydrocortisone conducted by the NICHD NRN investigators has completed enrollment, but results are not yet available (NCT01353313, ClinicalTrials.gov, accessed November 17, 2021).

Inhaled budesonide, initiated in the first day of life and continued until 32 weeks' PMA, decreased the combined outcome of BPD or death with marginal statistical significance (40.0% vs. 46.3%; relative risk 0.86; 95% CI 0.75, 1.00).[72] Death was higher and survival with BPD was lower following inhaled budesonide. In follow-up at 18 to 22 months corrected age, mortality remained significantly increased in the inhaled budesonide group (relative risk 1.37; 95% CI 1.01, 1.86).[73] This effect on mortality has not been explained, although local or systemic effects of this potent glucocorticoid on susceptibility to infection have been considered.[74] There was no difference in rates of NDI nor several respiratory morbidities at follow-up, including use of selected respiratory medication and home respiratory support. In other work, early administration of up to 6 doses of instilled budesonide suspended in surfactant (beractant, AbbVie Inc., North Chicago, IL) decreased BPD or death compared to surfactant alone in very low birth weight (VLBW) newborns intubated with respiratory distress syndrome (risk ratio 0.58; 95% CI 0.44, 0.77).[75] Newborns treated with budesonide plus surfactant also transiently had lower levels of inflammatory markers in tracheal aspirate fluid and lower rates of treatment of PDA, an effect also demonstrated with inhaled budesonide.[72,75] There were no differences in neurodevelopmental or medical outcomes assessed at 2 to 3 years corrected age. Pilot studies of this formulation and a dose-response study of repeated doses of budesonide in calfactant (ONY Biotech, Amherst, NY) showed limited absorption of budesonide.[76,77]

Other trials have focused on pharmacologic interventions that directly enhance normal lung growth and development after preterm birth. Intramuscular Vitamin A, administered over the first 4 weeks of life, showed benefit for prevention of BPD or death in a trial conducted by the NICHD NRN in 1996–1997 (relative risk 0.89; 95% CI 0.80, 0.99; NNT 14).[78] A recent re-analysis of these trial data accounting for risk of BPD or death utilizing a contemporary risk calculator demonstrated that newborns in the lowest risk quartile were more likely to benefit from Vitamin A therapy.[79] Later outcomes have not been published.

Inhaled nitric oxide (iNO) has also been studied extensively, with broad variation in dose and timing of iNO exposure across trials. Due to this variability, no single point estimate was provided for iNO effect on BPD or death in the most recent meta-analysis

reported from the Cochrane Collaboration.[80] With early rescue therapy, early preventative therapy or later preventative therapy in higher risk newborns, risk ratios ranged from 0.92 to 0.94 and all 95% confidence intervals just crossed the null. In a subsequent individual participant data meta-analysis from three of the trials that included diverse populations, the analyses focused on evaluating differential benefits of iNO by maternal race. With these composite data, infants of non-Hispanic white mothers had no benefit of iNO on BPD or death (relative risk 0.99; 95% CI 0.87, 1.12) and infants of Black mothers had a significant benefit (relative risk 0.77; 95% CI 0.65, 0.91; NNT 7). The overall effect in these three trials was similar to that identified using the conventional meta-analysis approach (relative risk 0.89; 95% CI 0.81, 0.97), and a significant interaction p value indicated differential benefit of iNO across the four racial/ethnic groups evaluated: non-Hispanic white, Black, Hispanic, and other.[81]

Caffeine was found to be effective in the prevention of BPD in the Caffeine for Apnea of Prematurity (CAP) Trial (odds ratio 0.63; 95% CI 0.52, 0.76; NNT 10), with early initiation at <3 days more effective than later initiation.[82,83] In the CAP Trial, caffeine lowered the postmenstrual age at discontinuation of invasive ventilation, positive pressure ventilation and supplemental oxygen use, and decreased rates of treatment for PDA.[82,83] Caffeine stimulates diaphragmatic activity and increases sensitivity to carbon dioxide, together resulting in more effective respiratory effort. Thus decreased exposure to the effects of respiratory support is the most likely mechanism for caffeine's efficacy in prevention of BPD, although caffeine has shown variable direct effects on lung development and inflammation in experimental models of BPD. There are not published data on the effects of caffeine on later respiratory morbidity.

Overall, currently available pharmacological therapies leave at least 40% of at-risk preterm infants with the composite outcome of BPD or death. The effects on later respiratory morbidity are even less well-described, with a lack of broadly accepted definitions of outcomes following the well accepted classification of BPD at 36 weeks' PMA. Of interest, effective therapies for BPD also decrease treatment rates for PDA. It seems likely that safe and effective therapies for BPD, along with implementation of nonpharmacological practices that promote extrauterine lung and vascular growth and development, will best mitigate the short- and long-term effects of prematurity and adverse intrauterine and extrauterine environments.

Persistent Respiratory Morbidity in Former Preterm Newborns

The characterization of persistent respiratory morbidity in infancy and early childhood among former preterm newborns has predominantly been done through collection of resource utilization attributed to respiratory disease and presence of respiratory symptomatology, using medical record review, administrative databases, surveys of medical providers or standardized parent/caregiver-completed questionnaires. Domains of interest include:

- Home respiratory support
- Hospitalization for respiratory illness
- Respiratory medication use
- Respiratory symptoms

In the symptom domain, the occurrence of wheeze (and less frequently cough) is assessed, with specification for symptoms that occur with and without other signs of illness (e.g., cough or wheeze "apart from colds") and with minimum frequency (e.g., "more than twice per week").[84,85] Outcomes after reaching term equivalent age inform and validate the classification of chronic respiratory illness (BPD) as former preterm newborns approach term corrected age, at 36 and 40 weeks' PMA. Beyond this goal, however, there is interest in capturing this information to aid in counseling and support for children and families after hospital discharge and for consideration of standardized metrics to evaluate neonatal clinical practices and outcomes for clinical trials.[86] This is particularly the case as former extremely preterm newborns may have considerable morbidity even if they do not meet criteria for BPD.[18] However, drawbacks to this approach include a similar critique to those levied at the BPD classification: chronic respiratory morbidity is defined by the prescription of its treatment, which varies broadly across clinical settings and does not define the physiological respiratory dysfunction.[87] Even when confining this approach to infants with severe BPD followed in US-based tertiary subspecialty clinics, there is considerable variation seen in all domains of respiratory resource utilization by site.[88] Thus translating morbidity outcomes into specific pathophysiology is a challenge. Conversely, these metrics assess the burden of the chronic respiratory disorder and are likely to be meaningful to families of these children, lending support to their broad application.

Specific medication use may lend insight into pathophysiology. For instance, the report of bronchodilator and controller medications for asthma or wheezing, in combination with recent symptoms or reported physician-diagnosis of asthma, has been used as a component of asthma or recurrent wheezing definitions in epidemiological studies.[89–92] Medication use over the first 1 to 2 years following initial hospital discharge has been collected from various cohorts of extremely preterm newborns. Table 43.2 shows data from various studies for proportions of high-risk former extremely preterm infants reporting morbidity in each domain in the first two years. Although variably defined in these studies, wheeze and cough were reported in 29% to 58% of the infants.[84,93–96] In a similar cohort, the EPICure Study in the United Kingdom and Ireland reported respiratory symptoms "nearly all the time" in 32% and 14% of infants surveyed at 1 and 2 years of age, respectively.[97] These rates are likely elevated, based on data from large populations (over 30,000 infants) studied in the International Study of Wheezing in Infants, and the Vitamin D Antenatal Asthma Reduction Trial (VDAART).[91,98] Reported medication use for obstructive lung disease is also prevalent in high-risk former preterm infant cohorts; 40% to 50% of infants received bronchodilators in the first 2 years after discharge, 22% to 56% reported inhaled corticosteroid exposure and systemic steroids were reported in up to 16% of infants.[84,88,93–96] In data from linked national registries in the Netherlands, medications for obstructive airway disease were prescribed to 30% of infants born at < 32 weeks at 6 to 12 months of age, and only 16% of former full term newborns.[99] Similar rates were identified in a population-based study from Israel, using data extracted from the electronic medical record; rates of prescribed respiratory medications were 51% and 62% following initial hospital discharge in the first and second year of life among former VLBW newborns, compared to 41% and 44% among former term control infants.[100] In data from the Prematurity and Respiratory Outcomes Program (PROP), Ryan and colleagues further describe that the proportion of infants with medication exposure in the first year of life decreases with increasing gestational age. Seventy-two percent of

TABLE 43.2 Post-Discharge Respiratory Resource Utilization and Symptoms in Former Extremely Low Gestational Age Newborns

Publication, Cohort	Marlow 2006[93] UKOS	Hibbs 2008[94] NO CLD	Stevens 2014[84] SUPPORT	Keller 2017[95] TOLSURF	Ryan 2019[96] PROP	Collaco 2021[88] BPD Collaborative
Characteristics	<29 wk MV <1 hr	500–1250 g MV or CPAP 7–21 d	24–27 wk <1 hr	≤28 wk MV 7–14 d	<29 wk ≤7 d	≤28 wk severe BPD[a]
Follow-up age[b]	22–28 mo *n* = 373	12 mo *n* = 455	18–22 mo *n* = 916	12 mo *n* = 450	12 mo *n* = 641	≤2 yr *n* = 119
Respiratory Medication						
Any				63%	55%	
Diuretic		24%	6%	21%	12%	44%
Bronchodilator	40%	47%		51%	46%	
Inhaled corticosteroid	24%	26%	26%	31%	22%	56%
Systemic steroid		14%	9%	16%	15%	
Pulmonary vasodilator					0.8%	15%
Home respiratory support		44%	22%	46%		59%
Respiratory hospitalization		22%	29%	28%		19%
Respiratory Symptoms						
Wheeze	37%	53%	58%	52%		
Cough	50%		29%	44%		

[a]Severe BPD by NIH consensus criteria.[8]
[b]Age corrected for prematurity except for Collaco et al.[88]
BPD, Bronchopulmonary dysplasia; *CPAP*, continuous positive airway pressure; *MV*, mechanical ventilation (invasive); *PROP*, prematurity and respiratory outcomes program; *SUPPORT*, surfactant positive pressure and pulse oximetry trial; *TOLSURF*, trial of late surfactant; *UKOS*, United Kingdom oscillation study.

infants born at 23 to 24 weeks' GA reported exposure to at least one medication in the first year of life, declining to 40% of infants born at 28 weeks' GA.[96] This pattern was consistent for exposure to diuretics, inhaled bronchodilators and inhaled corticosteroids, and to a lesser extent, systemic steroids.

Respiratory hospitalization rates are also high in these high-risk cohorts, ranging from 19% to 29% in the first 2 years, by parent report (Table 43.2). Re-hospitalization for any indication in former preterm newborns is a substantial burden. Two European retrospective cohort studies reported hospitalization rates of 40% to 46% in year 1 and 25% in year 2 for infants born < 32 weeks' GA, while only 14% of infants born at term were hospitalized in the first year of life.[99,101] Raiser et al. reported that respiratory infection accounted for 42% and 47% of these hospitalizations among former preterm newborns in years 1 and 2.[101] Similarly, Houweling et al. reported that the most frequent discharge diagnoses for re-hospitalizations in former preterm infants in the first year of life were respiratory illness (acute bronchitis and bronchiolitis, acute upper respiratory infection).[99] The proportion of infants born < 32 weeks' GA with these hospital discharge diagnoses was 7%, compared to 3% in infants born at term.

The spectrum of morbidity changes over the first year after discharge. In the PROP cohort, the proportion of infants with exposure to at least one respiratory medication after discharge increased modestly over that year (25%, 32%, 32%, and 34% at 3, 6, 9, and 12 months corrected age).[96] Rates of exposure to inhaled bronchodilators increased from 13% to 31% and inhaled corticosteroids from 9% to 14%, while exposure to diuretics decreased over this time frame, from 11% to 2%. There was a similar pattern in data from the TOLSURF cohort, with a fall in use of home respiratory support over the same time. Respiratory hospitalizations fell from 3 to 9 months corrected age, and then increased slightly at 12 months.[95]

Composite Respiratory Morbidity Outcomes

Composite respiratory morbidity outcomes have been developed to include different combinations of measures from various respiratory morbidity domains, with incorporation of repeated events or exposures when measured at multiple time points. Table 43.3 shows the distribution of these composite outcomes and their description in both high-risk and broader populations of low gestational age newborns. These represent a broad spectrum of composite morbidity, ranging from outcomes requiring a single event, medication or diagnosis to others requiring persistent need for respiratory support or monitoring at or beyond the second year of life.[12,13,18,95,100,102] For those outcomes that might be considered less serious, two-thirds to three quarters are affected, even in

TABLE 43.3 Composite Respiratory Outcomes at 1–2 Years' Corrected Age in Infants Born Preterm

Publication, Cohort and Characteristics	Follow-Up Age[a]	Adverse Outcome	Outcome Description
Isayama 2017[13] Canadian Neonatal Network <29 wk Structured interview	18–21 mo *n* = 1503	Serious respiratory morbidity or death 7% (serious respiratory morbidity 6%)	Tracheostomy or ongoing home respiratory support or monitoring, ≥3 respiratory hospitalizations, death after initial hospital discharge
Keller 2017[95] TOLSURF ≤28 0/7 wk MV 7–14 d Questionnaire at 3, 6, 9, and 12 mo	12 mo *n* = 439 *n* = 426	Any pulmonary morbidity 75% Persistent pulmonary morbidity 36%	Respiratory medication, home respiratory support, or respiratory hospitalization at any time point Respiratory medication, home respiratory support, or respiratory hospitalization at ≥3 time points
Keller 2017[18] PROP <29 wk, ≤7 d Questionnaire at 3, 6, 9, and 12 mo	12 mo *n* = 724	Postprematurity respiratory disease 69%	Respiratory medication, home respiratory support, respiratory hospitalization, or symptoms (wheeze, cough) at least once per week at ≥2 time points or death due to cardiopulmonary cause ≥36 weeks' PMA
Morrow 2017[102] Tertiary US centers (Colorado and Indiana) ≤34 wk, 500–1250 g, ≤7 d Questionnaire at 6, 12, 18 and 24 mo	2 yr *n* = 524	Late respiratory disease 71%	Respiratory medication, respiratory hospitalization or physician-diagnosis of asthma, reactive airway disease or BPD exacerbation
Jensen 2019[12] NICHD Neonatal Research Network <32 wk Structured data collection	18–26 mo *n* = 2677	Serious respiratory morbidity or death 26% (serious respiratory morbidity 21%)	Ongoing home respiratory support or monitoring, ≥2 respiratory hospitalizations, tracheostomy at any time, continued hospitalization for respiratory indication ≥50 weeks' PMA, death ≥36 weeks' PMA
Littner 2021[100] Israel (health maintenance organization) <1500 g Electronic medical record	1 yr *n* = 5146 2 yr *n* = 5056	Composite respiratory morbidity 59% Composite respiratory morbidity 68%	Respiratory medication, subspecialty (pulmonology, cardiology) clinic visit, respiratory diagnosis (asthma or reactive airway disease, recurrent wheezing >3 times in 12 mo, bronchiolitis, bronchitis, pneumonia, stridor)

[a]Age corrected for prematurity except for Littner et al.[100]

BPD, Bronchopulmonary dysplasia; *PMA*, postmenstrual age; *PROP*, prematurity and respiratory outcomes program; *TOLSURF*, trial of late surfactant.

patient populations that are not selected for neonatal illness severity.[18,95,100,102] Conversely, in the high-risk TOLSURF cohort, 25% of infants have no respiratory morbidity reported by 12 months corrected age while ~50% of term-born children are free of respiratory morbidity in an Israeli population-based cohort at 1 and 2 years.[95,100] The relative risk of this composite respiratory morbidity outcome was 1.22 (95% CI 1.19, 1.26) and 1.30 (95% CI 1.27, 1.34) at these time points for former VLBW newborns. In contrast, composite outcomes representing more serious or pervasive morbidity with or without mortality occurred in 7% to 36% of infants; mortality ranged from 0.8% to 4.1% in these cohorts.[12,13,18,95] Generally, the more serious composite outcomes could be more useful in discriminating those infants with greater repercussions of chronic neonatal respiratory disease.

There has been interest in defining the severity of respiratory morbidity in former preterm newborns by their use of post-discharge therapies. Gage and colleagues surveyed pediatric pulmonologists from academic medical centers in North America, asking them to rank eight components of post-discharge respiratory morbidity.[103] From the cumulative ranks of these eight components (home supplemental oxygen > respiratory hospitalization > diuretic use > daily bronchodilator use > daily inhaled corticosteroid use > intermittent bronchodilator use > intermittent corticosteroid use), they developed a 8.5 point severity score which they applied to 940 former preterm infants born at < 30 weeks' GA or with a diagnosis of BPD at 4 to 9 months corrected age. Using their proposed cut-offs, only 7% of infants met the threshold for severe chronic lung disease and 58% were classified with no morbidity. The PROP investigators created a consensus Respiratory Morbidity Severity (RMS) scale, for which infants were assigned to one of 3 mutually exclusive categories based on their most severe morbidity component (Table 43.4).[18] In cross-tabulation with the postprematurity respiratory disease (PRD) composite outcome, 81% of infants (184/227) infants with no PRD were classified minimal/none on the RMS and 10/227 were classified as severe. Infants with PRD were almost evenly divided between moderate/mild RMS (237/497) and severe (233/497) RMS. The overall distribution of RMS was 28% minimal/none, 38% moderate/mild and 35% severe. Thus, the RMS does have greater discrimination than PRD. More recently, O'Brodovich et al. utilized the Delphi methodology to develop a Chronic Lung Disease of Prematurity Severity Scale (CLDPSS), employing the expertise of 91 pediatric pulmonologists, neonatologists, and pediatricians that provide outpatient care for former preterm newborns with chronic lung disease.[104] After proposing many of the same components as Gage et al, they iteratively developed a 100-point scale with individual items weighted by the relative importance the participants placed on that item. The CLDPSS is meant to be scored at 12 months corrected age but it has not yet been applied to any cohort and it is not yet clear how it will account for the trajectory of illness over

TABLE 43.4 Published Severity Scales for Chronic Respiratory Morbidity in Former Premature Newborns

Respiratory Condition		PROP RMS[a] (Expert Consensus)			CLDPSS[b] (Delphi Method)	
		Severe	Moderate/Mild	Minimal/None	Relative Importance	Score
Death from cardiopulmonary cause		Yes			NA	
Home respiratory support		Tracheostomy with MV		None	MV/ BiPAP/ NIPPV	23
			Tracheostomy without MV		Nasal cannula oxygen ≥2 LPM or CPAP	21
		Nasal cannula oxygen >3 mo CA	Nasal cannula oxygen ≤3 mo CA		Nasal cannula oxygen <2 LPM	16
Hospitalization		≥2 any time	1 any time	None	≥1 within 3 mo	15
ED visit without hospitalization		NA	NA	NA	≥1 within 3 mo	12
Medication	Pulmonary vasodilator	Any	None	None	Daily	14
	Systemic steroids	Any	None	None	Any	9
	Diuretic	NA	NA	NA	≥3 d/wk	9
					PRN	6
	ICS	≥2 time points *with* symptoms ≥1 d/wk	Any	None	≥3 d/wk	9
					PRN	5
	BD		≥2 time points	<2 time points	≥3 d/wk	8
					PRN	5
Symptoms			≥2 time points, symptoms ≥1d/wk	<2 time points or symptoms <1 d/wk	na	

[a]PROP RMS, Prematurity and Respiratory Outcomes Program Respiratory Morbidity Severity.[18]
[b]CLDPSS, Chronic Lung Disease of Prematurity Severity Scale.[104]
BD, Bronchodilator; *BiPAP*, bilevel positive airway pressure; *CA*, corrected age; *CPAP*, continuous positive airway pressure; *ED*, emergency department; *ICS*, inhaled corticosteroid; *MV*, mechanical ventilation; *NIPPV*, noninvasive positive pressure ventilation.

the first year. Table 43.4 aligns the RMS criteria with the CLDPSS components, to allow for comparisons. Score cut-offs for different levels of severity for the CLDPSS have not been proposed.

Antecedents and Covariates for Persistent Respiratory Morbidity

Multiple studies have demonstrated associations between BPD and increased rates of various markers of respiratory morbidity in the first several years after birth.[84,96,101] Infants with BPD enrolled in the PROP study had greater use of inhaled respiratory medication in the second half of the first year after initial discharge compared to infants without BPD; similar differences persisted into the second year of life in the UK-based EPICure Study.[96,97] These data are consistent with increased wheezing illness throughout childhood in infants with BPD.[92,97,105] Other relationships further shed light on early influences of later respiratory morbidity. In follow-up from UKOS at 24 months corrected age, lower birth weight z-score conferred increased odds for respiratory hospitalization, cough and inhaled medication use after adjustment for sex, BPD, and parental smoking.[106] Littner and colleagues showed a strong unadjusted relationship to GA for their composite respiratory morbidity outcome in over 5000 children born very low birth weight up to 2 years of age; this was also shown in univariate analysis of both PRD and RMS at 1 year in the PROP cohort.[18,100] In exploration of early neonatal illness severity, Stevens et al. identified that the highest quartile of supplemental oxygen exposure at days 1, 3, 7, 14, and 28 in former VLBW infants without a diagnosis of BPD was associated with increased rates of respiratory symptoms, medication use, and resource utilization at 12 months corrected age.[107] Further, in data from the PROP cohort, high supplemental oxygen exposure in the first 14 days of life independently predicted the composite outcome of PRD, and high and intermediate supplemental oxygen predicted severe or moderate/mild RMS; intrauterine growth restriction fell out of these multivariate models.[108] A subset of these infants underwent pulmonary function testing (PFT) at 1 year corrected age. High and intermediate oxygen exposure was associated with evidence of airway obstruction (lower $FEV_{0.5}$/FVC z-score). Consistent with these

findings, Mourani and colleagues investigated early illness markers for their 2-year composite morbidity outcome of late respiratory disease (LRD) from the Colorado/Indiana cohort.[19] They found that combined evidence of PVD/PH on echocardiogram and use of invasive mechanical ventilation were the most important predictor for adverse outcome. Together, these studies support that that early illness in the susceptible newborn contributes to chronic neonatal respiratory disease.

Two studies have undertaken extensive modeling of predictors of their composite respiratory morbidity outcomes at 1 and 2 years corrected age (the PROP and Colorado/Indiana cohorts, respectively).[18,102] Considering only characteristics that could be defined by 24 hours of age in the PROP cohort, perinatal models for PRD and RMS selected overlapping factors that increased the odds of adverse outcome, while adjusting for gestational age.[18] These included intrauterine growth restriction, male sex, parent history of asthma, intubation at birth, and public insurance anticipated for the infant's medical care. Maternal smoking during pregnancy was only selected as a predictor for PRD and when post-discharge environmental tobacco smoke exposure was substituted (determined by number of smokers in the home and smoking rules for home and vehicles) there was no substantial change in the effect of other characteristics on PRD. These modeling exercises support the concept that the vulnerable lung in extreme prematurity is a strong predictor of later respiratory morbidity, as intrauterine growth restriction reflects an adverse fetal environment, and intubation at birth occurs in those newborns that are more compromised (78% of this cohort). Cardiopulmonary resuscitation at birth (CPR) was not selected as an important factor in these analyses. Independently, 36-week models were developed incorporating additional infant characteristics available at 36 weeks' PMA. Almost identical perinatal characteristics were selected as PRD predictors. Additional variables selected for both 36-week models, which increased the odds of adverse outcome, included BPD (modified from the NIH consensus definition to no/mild, moderate, and severe), growth failure at 36 weeks' PMA and low breast milk exposure during hospitalization. Of interest, when comparing changes in odds ratios (at least 20%) from perinatal and 36-week models for PRD, decreased effects were found for gestational age, intrauterine growth restriction and male sex, and parent history of asthma dropped out. These changes suggest that these perinatal influences impact the intermediate outcome of BPD, which had similar accuracy for predicting PRD as models at both time points.

Morrow and colleagues presented a multivariate model for LRD at 2 years in the Colorado/Indiana cohort.[102] The models for PRD and LRD share some variables in common, and the direction of effect for each of these is consistent between the two cohorts, although co-variates differ between models. The Colorado/Indiana investigators also compared multivariate models for BPD and LRD. In this comparison, several factors had a substantial change in effect (change in odds ratio ≥20%) between these two outcome time points. Gestational age, birth weight z-score, cesarean section delivery and maternal preexisting hypertension had lesser effect in the LRD model, whereas maternal diabetes and chorioamnionitis had increased effect. LRD and the other composite long-term outcomes are comprised of events that occur after initial hospital discharge, so additional influences on their occurrence are likely to be important. For example, sociodemographic characteristics are associated with at least some of the components of these outcomes, including hospitalization and wheezing illnesses, and some of these characteristics may be markers for environmental influences on respiratory disease that were otherwise not measured.[92,109–111] Further delineation of different aspects of sociodemographic factors may help elucidate the underpinnings of the racial disparities found in these later outcomes in both studies.

Lower respiratory tract infection (LRTI), and hospitalization due to infection is common among former preterm newborns. Eighteen percent of infants born < 33 weeks' gestation were hospitalized for upper or lower respiratory tract conditions in the first year after discharge in a French-based registry.[111] These data are consistent with the proportion of children with respiratory hospitalization up to 1 to 2 years in the high-risk extremely low gestational age cohorts described in Table 43.2 (19% to 29%). Of children < 2 years admitted to a children's hospital pediatric intensive care unit with a respiratory illness, 17% were born at < 32 weeks' gestation, and 53% of these former preterm newborns had an LRTI as the cause for admission.[112] The susceptibility of former preterm newborns to LRTI may be due to various causes; T-cell dysmaturation due to extremely preterm birth was associated with later respiratory morbidity in a subset of former extremely low gestational age newborns enrolled in the PROP cohort.[113]

In another subset of infants from PROP, respiratory system function was assessed by respiratory inductance plethysmography just prior to initial hospital discharge.[114] Although infants classified with BPD had lower time to peak expiratory flow over total expiratory time (Tpef/Te) and other subtle findings indicating lower lung compliance or upper airway obstruction, these findings did not predict PRD. Other studies have shown obstructive lung disease prior to discharge in former preterm newborns across a range of illness severity and gestational ages.[87,115]

The short- and long-term follow-up of children enrolled in TCRS provide important context for considering the relationship of lung function in infancy to later symptomatic respiratory disease. The cohort is largely term born and children had no significant respiratory illness at birth. In the initial TCRS publication, the investigators demonstrated that infants who later went on to develop symptomatic LRTI with wheezing in the first year of life had diminished lung function at mean ~8 weeks of life as measured by Tpef/Te and respiratory conductance, when compared to infants without symptomatic LRTI.[116] At 6 years of age, children with persistent wheeze had a more atopic profile and greater exposure to maternal smoking than children with no history of wheezing illness.[117] In later follow-up, the TCRS investigators showed that airway obstruction in infancy as assessed by $Vmax_{FRC}$ continued to correlate with a pattern of obstructive airway disease up to 22 years of age.[118] Finally, those infants with an episode of pneumonia at < 3 years of age had worse lung function at 11 to 26 years of age than children without LRTI, with a more pronounced decrement in lung function than that found in children with LRTI but no pneumonia.[66] The children with pneumonia also had significant increased odds of current asthma and active wheeze up to age 29, compared with children without LRTI, whereas those children with LRTI without pneumonia had only increased odds for active wheeze. The known susceptibility of former preterm newborns to LRTI and wheeze early in life and their decreased lung function lay the foundation for chronic, prolonged, and recurrent wheezing illness and obstructive lung disease in these children.

Wheeze, Asthma, and Lung Function in Former Preterm Newborns

Meta-analyses solidify the data regarding increased rates of recurrent wheezing illness or asthma and airway obstruction in former preterm newborns. Been and colleagues identified an adjusted OR 1.46 (95% CI 1.2, 1.65) for wheezing illness/asthma throughout childhood; an estimate for children born < 32 weeks' GA was almost twice the overall odds for all former preterm children (OR 2.81; 95% CI 2.52, 3.12).[90] A meta-analysis demonstrated that percent predicted forced expiratory volume in 1 second ($\%FEV_1$) for children born preterm was consistently decreased throughout childhood, with this decrement more pronounced in children with a diagnosis of BPD at 36 weeks' PMA.[119] In addition to biological vulnerabilities, environmental exposures are likely an important modifier of chronic respiratory disorders after preterm birth. In the high-risk TOLSURF cohort, Wai and colleagues demonstrated that a diagnosis of BPD, public insurance status, and maternal breast milk exposure were mediators of persistent wheeze.[92] Well-established risk factors for increased risk of wheezing illness, such as atopic predisposition and environmental tobacco smoke, were not identified as mediators in this secondary analysis.

With respect to environmental tobacco smoke, Collaco et al. found that hair nicotine levels in former preterm newborns with BPD at < 3 years of age were more strongly associated with adverse respiratory outcomes than parental report of household smoking.[17] As expected, children with smokers in the home had higher hair nicotine levels than children without smokers in the home. However, 22% of the children without reported smokers in the home had hair cotinine levels above the proposed cut-off for smokers in the home, suggesting there may be additional sources of environmental tobacco exposure in these at-risk children, possibly related to socioeconomic factors such as neighborhood or housing conditions. Thus better objective measures of environmental risks may further elucidate which former preterm newborns are at highest risk for chronic respiratory illness after initial hospital discharge.[111]

Several studies have continued to follow former extremely preterm infants into later childhood and adolescence. At 6 years of age, the EPICure cohort described current wheeze in 36% of children with a diagnosis of BPD, 20% of children without BPD diagnosis and 13% of classmate controls.[97] Peak expiratory flows were decreased in former extremely preterm newborns with BPD compared to those without BPD, and those without BPD compared to controls. At 11 years of age, former preterm newborns had increased rates of current asthma compared to controls (25% vs. 13%), but differences in wheezing outcomes between children with and without a diagnosis of BPD were no longer statistically significant.[89] All measures of airway obstruction were impaired in former preterm newborns compared to controls, and for those with BPD compared to those without BPD. Further, metrics of obstructive airway disease were correlated with an asthma diagnosis. In follow-up data from UKOS, investigators describe overall rates of wheezing of 15% at 11 to 14 years and an asthma diagnosis of 9% at 16 to 19 years in these children born at < 29 weeks' gestation.[120,121] Pulmonary function studies at these ages also demonstrated considerable evidence of obstructive airway disease. Of interest, the primary research question in UKOS was focused on differences in lung function by initial mechanical ventilation mode, high frequency versus conventional. At 11 to 14 years, lung function was better in the high-frequency group, although rates of clinical illness did not differ. By 16 to 19 years, there was no difference in lung function between the groups and clinical illness favored the conventional ventilation group. In Australia, Doyle and colleagues evaluated three eras of population-based cohorts of extremely low birth weight/low gestational age newborns from 1991, 1997, and 2005.[38] They describe a transition in clinical respiratory support strategies to increased use of noninvasive support (CPAP), accompanied by increased total duration of assisted ventilation and supplemental oxygen use and increased diagnosis of BPD. At 8 years of age, rates of current wheeze range from 23% to 26% and were not different by cohort era. There was also worse pulmonary function in the most recent eras, as evidenced by more severe airway obstruction. A single center US-based study assessed 47 children born < 33 weeks' gestation (~70% moderate-severe BPD) from 4 to 20 years of age.[122] Both $\%FEV_1$ and percent predicted functional vital capacity (%FVC) increased over time, indicating ongoing lung growth. However, %FVC improved more rapidly than $\%FEV_1$, resulting in decreased FEV_1/FVC; this decrease was correlated with the duration of invasive mechanical ventilation. Taken together, these studies show increased prevalence of symptomatic lung disease (wheeze and asthma) at later follow-up (6 to 20 years) in former preterm newborns, and to a greater extent, functional evidence of obstructive lung disease. These long-term consequences may not be modifiable by changing neonatal respiratory strategies.

Conclusions

The precursors of symptomatic lung disease and diminished lung function at initial hospital discharge in former preterm newborns are due to immaturity and an adverse fetal environment related to placental vascular derangements, maternal smoking, and possibly other environmental exposures. The intensity of early respiratory support influences further dysplastic lung development, in part due to the degree of impaired lung and vascular development and function at birth. A diagnosis of BPD, regardless of the specific criteria used for this diagnosis, increases the risk for later morbidity complications, which are likely related to the degree of lung dysfunction (particularly expiratory airflow obstruction), immune deficits, and environmental factors. Risks of chronic neonatal respiratory disease can be identified at birth. Renewed focus on later interventions throughout infancy to improve long-term outcomes is indicated, given the recognition of obstructive lung disease while lung growth is ongoing.[16]

Suggested Readings

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Anatomic Disorders of the Chest and Airways

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KEY POINTS

- Prompt diagnosis and early intervention are key in salvaging infants with life-threatening surgical disorders of the airway and chest.
- Improved prenatal ultrasound has shifted the diagnosis and management of disorders of the chest and airways into the fetal period.
- Increased utilization of video-assisted thoracoscopic surgery for congenital thoracic lesions has made surgical intervention on the infant chest less invasive and more tolerable.

Anomalies of the Airways

Nasopharyngeal Obstructive Disorders

Respiratory distress caused by nasal obstruction may manifest as a serious, life-threatening event shortly after birth. Because newborns are preferential nasal breathers for the first 2 to 3 weeks after birth, nasal obstruction may cause severe cyanosis, particularly during oral feedings, with airway obstruction relieved only when the mouth is open to cry.[1] There are several causes of neonatal nasal obstruction, including congenital choanal atresia, nasal pyriform aperture stenosis, nasolacrimal duct cyst, and nasal hypoplasia. Buckling or, less commonly, dislocation of the nasal septum due to birth trauma can also cause breathing problems; most cases respond to decongestant and steroid nasal drops, but dislocations require surgical manipulation.[2]

Congenital Choanal Atresia

Caused by persistence of the buccopharyngeal membrane, congenital choanal atresia occurs in between 1 in 5000 and 1 in 9000 births and has a significant female preponderance. In the majority of choanal atresia cases, the obstructing membrane is of mixed bony and membranous composition.[3] Choanal atresia is most commonly unilateral, right-sided, and with female predominance (in ~2/3 of cases); bilateral malformations are more serious and constitute an emergency at birth.[1,4] Over half of all cases are associated with other congenital anomalies, bilateral cases more so than unilateral.[5] Choanal atresia can be associated with the *CHARGE association*, a constellation of the following anomalies:

- Colobomas of the eyes
- Heart defects
- Atresia of the choanae
- Retardation of growth or development
- Genitourinary defects
- Ear anomalies associated with deafness

The association was officially named *CHARGE syndrome* in 2004, when a common mutation in the *CHD7* gene on chromosome 8 was identified in 60% of cases.[6]

Because the newborn is a preferential nasal breather, there may be serious difficulties soon after birth, especially in cases of bilateral atresia. Unilateral atresia may present simply with unilateral discharge and feeding difficulties but may not present until later in childhood. While insertion of a 5- or 6-French catheter through the nose or visualizing airflow using a mirror under the nostril remains a common test, nasofiberscopy or nasal rigid endoscopy is the diagnostic tool of choice for confirmation of atretic choana by directly visualizing point of obstruction.[7] Computed tomography (CT) of the nasopharynx with intranasal contrast can also confirm diagnosis, evaluate the nature and severity of nasal obstruction, and help in surgical planning.[7]

Emergent management of choanal atresia is focused on ensuring that the oropharyngeal airway is patent, which may necessitate endotracheal intubation. A McGovern nipple, an orogastric tube, or a modified endotracheal tube can be used to overcome the seal between the palate and the tongue.[4,8] Tracheostomy is rarely necessary and typically only required when associated with other anomalies.[9]

Surgical repair is the mainstay of treatment and can be performed within a few days of birth. Patency can be established by various methods according to surgeon preference. With the improvement in transnasal technique with modern endoscopic biting and drilling instruments, it is currently the favored approach for both initial and revision surgeries.[7,10] Correction is accomplished by relieving the obstruction using dilators and removing hypertrophied posterior septal bone under endoscopic visualization.[10,11] The transnasal endoscopic approach works best with thin buccopharyngeal membranes and has low restenosis rate.[12] The transpalatal approach entails surgical correction of the

offending defect and is typically performed for thick bony membranes in older patients. However, despite the minimal rate of reoperation, this approach is associated with a higher rate of palate growth deformities.[13] While temporary stenting has been used to decrease restenosis rates, the use of stents remains controversial. It may not prevent restenosis and can be associated with complications, including alar injury, columellar tear, and vestibular stenosis.[14] Mitomycin C has also been used as an adjunct to surgical repair to improve patency, but its use is controversial and so it is not routinely used.[15]

Congenital Nasal Pyriform Aperture Stenosis

Nasal pyriform aperture stenosis is a rare cause of nasal obstruction and should be suspected when encountering difficulty in passing a nasal catheter. Pyriform aperture stenosis is characterized by excessive bone formation in the medial nasal processes of the maxillary bone. The condition may be isolated or associated with other anomalies, such as a solitary median maxillary central incisor tooth or, more seriously, midline defects such as pituitary hypoplasia with endocrine insufficiency, diabetes insipidus, or other manifestations of holoprosencephaly and craniosynostosis.[16] Similar to choanal atresia, an oral airway may be necessary to relieve the breathing difficulty. Although the obstruction can be suitably demonstrated by CT of the nasopharynx, because of its high association with holoprosencephaly, a karyotype analysis and brain CT and/or magnetic resonance imaging (MRI) may be required if brain abnormalities are suspected.[17] In most cases, nasal obstruction is mild and may respond to nasal decongestants. In refractory cases of obstruction, usually when the pyriform aperture is less than 5.7 mm, sublabial surgery is necessary to remove excessive bone, and nasal stenting is typically placed.[18] Novel methods of rapid maxillary expansion to enlarge the pyriform aperture are being investigated including the use of custom palate expansion devices or immediate surgical transverse enlargement of maxilla.[19,20]

Pierre Robin Syndrome (Robin Sequence)

Although this pattern of upper airway problems was first described by Pierre Robin in 1923, characterization of Pierre Robin syndrome remains difficult and controversial. A constellation of anomalies within the spectrum of cleft palate, micrognathia, and glossoptosis that results in airway obstruction, the Pierre Robin syndrome been linked to mutations in the *SOX9* and *KCNJ2* genes.[21,22] Given the varying definitions published, the incidence of Pierre Robin syndrome is difficult to pinpoint; it has been reported to occur in anywhere from 1 in 8500 to 1 in 30,000 births.[23] The hypoplastic development of the mandible is of clinical significance as the micrognathia can lead to airway obstruction and cyanosis.[24] Obstruction is common when the infant is in the supine position, during feeding, and in active sleep, when pharyngeal muscle tone is absent. Excessive air swallowing, followed by gastric distention, vomiting, and tracheal aspiration, is a frequent problem. The pharyngeal obstruction is maintained by the generation of large negative pressures in the lower pharynx during inspiration and swallowing.[25] Chronic obstruction leads to carbon dioxide retention, failure to thrive, and development of pulmonary hypertension with right ventricular failure.[26,27]

As with the imprecise diagnosis of Pierre Robin syndrome, the severity of respiratory obstruction and the management indicated are varied. Mild cases may present with only mild glossoptosis, and because oral feeds are tolerated without respiratory obstruction, these cases can be managed by side-to-side nursing and prone positioning.[28,29] In the event of respiratory symptoms with feedings or failure to thrive, a nasogastric tube for feedings may be required.[28] In severe cases of respiratory distress, nasopharyngeal intubation should be performed, typically by passing a 3.5-mm tube through the nose and into the hypopharynx.[30] This prevents the generation of negative pressure and greatly relieves the respiratory difficulty. The nasopharyngeal tube may be left in place for weeks or even months with proper management. Other treatments include tongue–lip adhesion surgery (glossopexy) to hold the tongue forward and tracheostomy if a nasopharyngeal tube does not adequately relieve the obstruction.[31,32] Mandibular distraction and velar extension appliances can be performed in attempts to avoid tracheostomy.[32,33] Nutrition can be maintained with a hypercaloric formula fed by nasogastric or gastrostomy tube. With adequate airway management and the passage of time, the problem becomes less threatening, especially after a few months, when the infant gains better control of the tongue.[34,35] Oral feedings can then be introduced, usually with a long lamb's nipple to help hold the tongue forward. With adequate nutrition and growth of the mandible, the problem usually resolves by 3 to 12 months of age, when cleft palate repair can safely take place.

Glossoptosis–Apnea Syndrome

Pierre Robin syndrome is not the only condition characterized by mechanical obstruction by the tongue. Infants with Beckwith–Wiedemann syndrome may have considerable breathing difficulties and apnea due to the associated macroglossia.[36] Infants with a normal-sized tongue who also have conditions such as unilateral choanal atresia, choanal stenosis, or swelling of the nasal mucosa may generate considerable negative pressure in the pharynx; this, combined with inadequate muscular control over the tongue, may lead to pharyngeal obstruction with respiratory distress, cyanosis, and severe episodes of apnea.[37]

Pharyngeal Incoordination

While the tongue provides the majority of force to move a food bolus into the esophagus, weakness and incoordination of the pharyngeal musculature can lead to disordered swallowing.[38] This incoordination causes choking and cyanosis with feedings and may be complicated by aspiration pneumonia.[39] Affected infants have difficulties in swallowing their own secretions. The condition may be seen in infants with severe hypoxic–ischemic encephalopathy and pseudobulbar palsy, Arnold–Chiari malformation, Möbius syndrome, and other facial malformations. Electromyography of the facial and pharyngeal muscles during rest, crying, and eating can aid in diagnosis.[40] Specifically, it can be used to assess the sucking and swallowing coordination in infants during bottle feeding. Drugs with antimuscarinic effects, such as atropine, can decrease secretions and may produce some relief. Although some infants may gradually improve, long-term management may require initiation of tube feedings or even gastrostomy in children who fail to improve.

Laryngeal Deformities

Laryngomalacia (Congenital Laryngeal Stridor)

A relatively common condition, congenital laryngeal stridor or laryngomalacia is the most frequent cause of stridor in infants.[41] Laryngomalacia is characterized by the prolapse of poorly supported supraglottic structures—the arytenoids, the aryepiglottic folds, and the epiglottis—into the airway during inspiration,

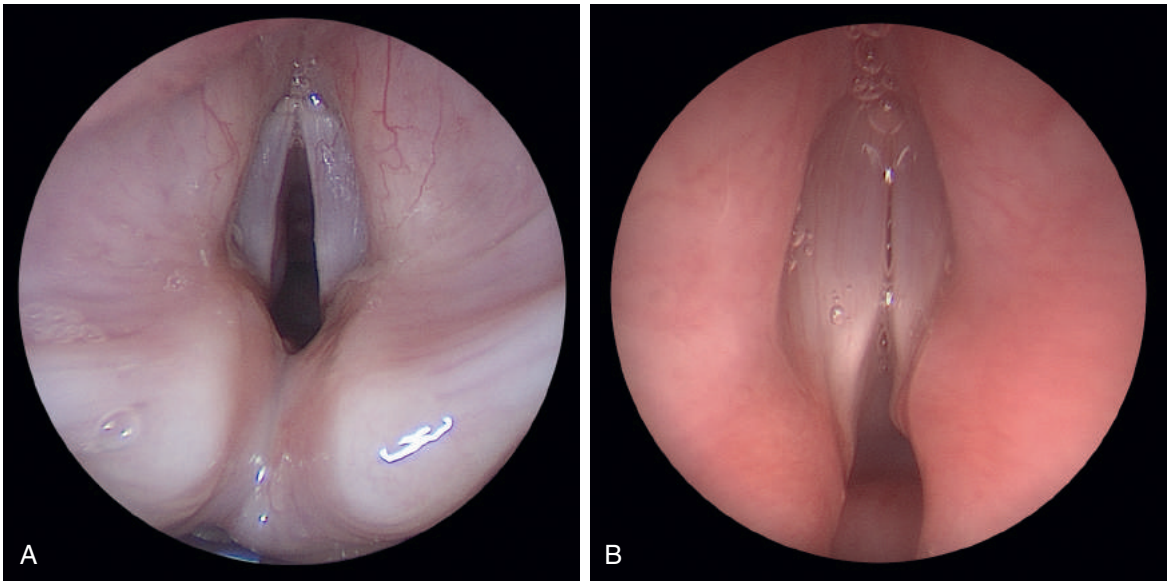
causing respiratory obstruction and difficulty with feeding.[42] Despite loud, high-pitched inspiratory stridor and significant chest retractions that typically present during the first month of life, the infant seldom has cyanosis, hypercarbia, notable feeding difficulty, failure to thrive, or an abnormal cry.[43] Laryngomalacia is worse in the supine position, with the neck flexed, and subsides in the prone position, with the neck extended.[43] Obstruction is worse during episodes of agitation and lessens when the infant is calmed. Severe forms of laryngomalacia may cause apneic events, pulmonary hypertension, or difficulties with feeding and/or weight gain.

Although a CT scan is effective at demonstrating the abnormal prolapse of the aryepiglottic folds supporting the diagnosis, confirmation should be obtained with flexible fiberoptic laryngoscopy.[44,45] Laryngeal ultrasound is an adjunctive tool for diagnosis that is non-invasive, and dynamic.[46] In some cases, gastroesophageal reflux or episodes of obstructive apnea may be associated with this condition.[43] Synchronous airway abnormalities are found in up to 30% of infants, including vocal cord paralysis, tracheomalacia, and subglottic stenosis.[47] Thus, the evaluation of stridor must include the examination of the entire upper airway and upper digestive tract. Stridor associated with laryngomalacia is usually loudest at 4 to 8 months, and most cases resolve with conservative management around 12 to 24 months.[48] Conservative therapy entails prone positioning, feeding and weight monitoring, and acid suppression therapy and most demonstrate improvement over roughly 18 months.[49] Approximately 20%, however, will have severe obstructive apnea, cor pulmonale, and/or failure to thrive and require a surgical intervention. In these cases, supraglottoplasty may be indicated, with 71% having complete resolution of symptoms and 94% having symptom improvement postoperatively.[50] Tracheostomy is reserved for supraglottoplasty failures.[51]

Vocal Cord Paralysis

Unilateral cord paralysis is usually left sided and typically presents without marked stridor or retractions manifesting as aspiration.[52] The infant may cough and choke during feedings, as laryngeal closure with swallowing is impaired. The condition is most frequently secondary to iatrogenic injury, usually caused by excessive stretching of the neck during delivery or injury during surgery; however, it can also be due to neurologic disorders.[53] Right-sided vocal cord paralysis has been reported as a complication of cervical extracorporeal membrane oxygenation (ECMO) cannulation.[54] Ligation of a patent ductus arteriosus (PDA) has also been associated with a high rate of left-sided vocal cord paralysis, probably secondary to recurrent laryngeal nerve injury during dissection.[55] A recent systematic review reported that 32% of low birth weight infants who undergo PDA ligation have left vocal cord paralysis demonstrated on laryngoscopy.[55] This increases their risk of developing bronchopulmonary dysplasia (BPD), reactive airway disease, and feeding problems requiring a gastrostomy tube. Laying the infant on the paralyzed side may decrease the amount of stridor, as the affected cord falls away from the midline. Diagnosis can be confirmed with flexible nasolaryngoscopy but may require direct laryngoscopy in the operating room (Fig. 44.1). The condition tends to improve over a period of several weeks or months, and speech and swallow therapy can be used in cases where improvement is not seen. Generally, medialization of the vocal cord (including injection laryngoplasty and thyroplasty) is not recommended in neonates less than 6 months of age; however, in children with gross aspiration and severe phonotory difficulty, it may be required.[56] Tracheostomy is preferred in severe cases of respiratory obstruction.[52]

Bilateral cord paralysis is a much more serious condition, accompanied by high-pitched inspiratory stridor; frequently, however, the cry is normal. Usually, severe central nervous system problems are to blame, such as hypoxic–ischemic encephalopathy, cerebral hemorrhage, Arnold–Chiari malformation, hydrocephalus, or brainstem dysgenesis. Associated problems may include pharyngeal incoordination with swallowing difficulty and esophageal dysfunction, recurrent apnea episodes, and tracheal aspiration of mucous secretions and formula. The stridor may resolve slowly if brain swelling subsides after birth, as is the case with ventriculoperitoneal shunt placement. The diagnosis may be suspected at laryngoscopy but should be confirmed by flexible fiberoptic bronchoscopy, rigid bronchoscopy, or ultrafast cine CT



• **Fig. 44.1** Vocal cord paralysis. (A) Bronchoscopy of a neonate demonstrating a normal glottis. (B) Bronchoscopy of an infant with bilateral true vocal cord paralysis causing obstruction. (Courtesy Dr. Jamie Funamura, UC Davis Health System, Sacramento, CA.)

scan. Tracheostomy is frequently required in about 50% to 60% of patients, and the prognosis usually is poor secondary to the underlying problems.[57,58]

Laryngeal Atresia

Laryngeal atresia is the result of failed recanalization of the larynx during embryologic development, resulting in a newborn with complete laryngeal obstruction presenting with severe respiratory distress. In some cases, the larynx may be completely obstructed by a laryngeal web seen in the delivery room during attempts to intubate the cyanotic infant. An endotracheal tube sometimes can be forced beyond the obstruction into the trachea. Otherwise, a large-bore needle should be inserted percutaneously into the trachea to maintain marginal gas exchange while preparations for emergency tracheostomy are made. Most infants with laryngeal atresia have other lethal malformations.[59,60] Most cases are now diagnosed prenatally from ultrasound findings consistent with congenital high airway obstruction syndrome (CHAOS), such as polyhydramnios and enlarged hyperechoic lungs with an associated flattened or inverted diaphragm.[61,62] The mother may be evaluated for ascites or hydrops fetalis due to impaired venous return to the heart, and the amniotic fluid lecithin level may be very low in such cases. In the absence of other lethal malformations, the characteristic ultrasound diagnosis may permit preparations for emergency tracheostomy after delivery of the infant or an ex utero intrapartum treatment (EXIT)-to-airway procedure, discussed later. Intrapartum fetoscopic tracheal decompression is being investigated.[63,64]

Congenital Subglottic Stenosis

Congenital subglottic stenosis, manifesting as inspiratory stridor from birth, is caused by partial obstruction of the cricoid probably due to incomplete canalization of the cricoid ring. In a full-term infant the normal subglottic lumen is 4.5 to 5.5 mm in diameter, whereas that of a preterm neonate is 3.5 mm in diameter. A subglottic diameter of 4 mm or less in a full-term infant or 3 mm or less in a premature infant is consistent with a diagnosis of subglottic stenosis. Subglottic stenosis is diagnosed by direct laryngoscopy supplemented with rigid bronchoscopy and chest radiography to evaluate other airway lesions and/or concomitant lung disease, as the latter may be common in the premature infant. Treatment consists of balloon dilation or endoscopic lysis with a carbon dioxide laser in cases of membranous stenosis. However, most cases severe enough to require intervention are cartilaginous and require an anterior cricoid split, obviating the need for and complications of tracheostomy in most cases.[65,66]

Congenital Subglottic Hemangioma

Subglottic hemangiomas are rare, accounting for 1.5% of all congenital abnormalities of the larynx.[67] They often occur in association with a cutaneous hemangioma and may cause inspiratory stridor and expiratory wheezing that progress with enlargement of the tumor.[68] This diagnosis is suspected when asymmetric subglottic narrowing is seen on plain radiographs and is confirmed by flexible and rigid endoscopy demonstrating a sessile vascular lesion, most commonly in the posterolateral subglottis.[69,70] Although intralesional injections of steroids is relatively successful over systemic administration of steroids, in many cases intubation or tracheostomy is eventually required.[71] Results of removal by carbon dioxide or potassium titanyl phosphate (KTP) laser have been encouraging, enabling treatment without tracheostomy; however, associated complications such as subglottic stenosis have been reported.[70,72] Finally, case reports using beta blockers (propranolol and acebutolol) have been successful in causing regression of subglottic hemangiomas, preventing the need for tracheostomy altogether.[73]

Laryngotracheoesophageal Cleft (Congenital Laryngeal Cleft)

In laryngotracheoesophageal cleft, a longitudinal communication is present between the airway and the esophagus, stretching from the larynx into the upper trachea or sometimes as far as the carina. This rare condition is reported in 1 in 10,000 to 1 in 20,000 births and is caused by failed fusion of the two lateral growth centers of the posterior cricoid cartilage, preventing proper fusion of the posterior cricoid lamina.[74] Affected infants have respiratory distress with inspiratory stridor and cyanosis, associated with tracheal aspiration of saliva and feedings. Chest radiographs may show evidence of aspiration pneumonia, and the cine esophagogram shows contrast material spilling into the trachea. The diagnosis can be established with direct laryngoscopy and bronchoscopy. Given the high association with other congenital anomalies and syndromes, such as tracheal atresia, tracheoesophageal fistula, and Opitz–Frias syndrome, a thorough evaluation of all organ systems and genetic karyotype are recommended.

Laryngotracheoesophageal clefts are classified, by severity of symptoms, into four groups (types I to IV), which are used to determine the management strategy and the need for surgical intervention.[75] For all types, initial management involves adequately securing the airway, including with an endotracheal tube or tracheostomy if necessary. Mild cases can sometimes be managed by conservative therapy, including swallow rehabilitation and antireflux medication.[76] In those who fail conservative management, endoscopic therapy including injection augmentation of the cleft may be attempted.[77] More severe cases require extensive reconstruction employing an anterior translaryngotracheal approach or even a partial upper sternotomy. Despite these reconstruction attempts, mortality remains high, up to 25% among all types of clefts and higher in cases with associated congenital anomalies and type IV clefts.[78,79]

Tracheal Deformities and Other Tracheal Disorders

Tracheal Agenesis

Tracheal agenesis is a rare condition that occurs in less than 1 in 50,000 births.[80] The trachea can be atretic just below the vocal cords but is most often absent all the way down to the carina.[81] Clinical manifestations include severe distress, absence of vocal sound, and severe cyanosis. Affected infants usually have additional congenital anomalies within the VACTERL association, including tracheoesophageal fistulas, severe cardiac malformations, and sometimes renal and anal anomalies.[82] Prenatal diagnosis is difficult, but prenatal presentation may manifest as CHAOS. If diagnosed prenatally, delivery via EXIT should be arranged to maximize the chances for survival.[82,83] Postnatal diagnosis should be suspected in cases of respiratory distress with immediate hypoxia, no audible cry, and a mechanical inability to intubate.[84] Despite the presence of a larynx, intubation cannot be accomplished at delivery; however, if the tracheal tube is positioned in the esophagus and connected to a mechanical ventilator,

reasonable gas exchange can be obtained via the tracheoesophageal fistula.[85] When the tracheal atresia is high, a tracheostomy can be performed in the remnant tracheal tissue. If survival seems possible, gastric division and a gastrostomy for feeding should be performed. Reconstructive surgery is not likely to be successful, however, and the prognosis is extremely poor, if not because of poor ventilation, then because of the underlying malformations.

Congenital Tracheal Stenosis

In congenital tracheal stenosis, a segment of the trachea is narrowed, usually starting in the subglottic region. The affected segment may be short or long; occasionally, the entire trachea is hypoplastic, and the bronchi may be involved. This disorder affects 1 in 64,500 live births and is usually caused by complete or nearly complete cartilage rings that narrow the trachea.[86] Affected patients may have inspiratory stridor, expiratory wheezing, feeding difficulties, and experience cyanotic episodes. Mild inflammation and small mucous plugs may cause life-threatening deterioration. In up to 60% of cases, other congenital malformations are also present, such as vascular ring anomalies, congenital heart defects, tracheoesophageal fistula (especially the H type), and hemivertebrae.[87] There also is an association with pulmonary agenesis.[87]

Patients with this deformity usually can be intubated, but the endotracheal tube cannot be advanced and should not be forced. Mechanical ventilation with generous levels of positive end-expiratory pressure (PEEP) may help stabilize the infant. Tracheostomy is not indicated and interferes with making the diagnosis.[88] Sometimes the diagnosis can be made by inspiration and expiration chest radiographs, using air as the contrast medium. However flexible fiberoptic bronchoscopy in the neonatal intensive care unit (NICU) or rigid bronchoscopy in the operating room is usually required for diagnosis.[87] Because it is important to examine the lower limits of the stenosis, it may be necessary to proceed with tracheobronchography, but this may sometimes cause acute decompensation and is controversial.[89] Ultrafast cine CT scan and MRI have become useful diagnostic techniques to define the lower limits of the stenosis.[90] In addition, three-dimensional (3D) CT reformats provide valuable information on the trachea–cardiovascular relationship and can allow for 3D printing of models to rehearse complex surgical interventions.[91]

In most cases, the stenosis requires treatment of some kind in the operating room. Balloon dilation alone is not likely to be successful in the case of a complete tracheal cartilage ring, because cartilage cannot be stretched. For short-segment stenosis, balloon tracheoplasty may be sufficient, where serial dilations during rigid bronchoscopy split the complete tracheal rings.[92,93] Some groups have used metallic stents to prevent restenosis with long-term success; however, granulation tissue over the stent can make removal difficult.[94] For longer-segment stenosis, slide tracheoplasty has become standard of care.[95] Backer et al. described the successful use of free autografts of resected trachea.[96] Additionally, rib cartilage and pericardial patch have been used for tracheoplasty.[97,98] Tissue bioengineering as future treatment with decellularization of trachea and recellularization with stem cells is currently being studied.[99]

The use of cardiopulmonary bypass has improved treatment and is advocated by some as averting the need for complex anesthesiology techniques.[100,101] Premature infants with congenital tracheal stenosis cannot undergo tracheal resection and tracheoplasty with cardiopulmonary bypass procedures. For these patients, aggressive balloon dilations are recommended, with splitting of the weaker posterior aspect of the tracheal rings.[102] With improvements in surgical techniques and the addition of ECMO to complex repairs, survival rates have improved to greater than 80%.[101] Those patients undergoing slide tracheoplasty who have concurrent distal bronchomalacia is predicted to have more adverse outcomes.[101]

Tracheobronchomalacia (Tracheomalacia)

Tracheobronchomalacia is characterized by dynamic collapse of the trachea during breathing secondary to delayed development of tracheal cartilage. This condition may be primary or associated with tracheoesophageal fistula, BPD, extrinsic tracheal compression, or prolonged intubation.[103] Tracheobronchomalacia should be suspected in infants presenting with respiratory distress, cyanotic spells, or persistent respiratory symptoms including expiratory stridor, recurrent respiratory infections, or persistent or recurrent wet cough. Chest radiographs are usually normal, with only a 62% sensitivity in diagnosing trachebronchomalacia.[104] With improvements in multidetector CT scanners and ultrafast cine CT scanners, diagnosis can be made with improved accuracy.[105,106] Bronchoscopy remains the gold standard for diagnosis, which shows approximation of the anterior and posterior walls of the trachea during expiration.[107] The bronchoscope may support the walls of the trachea, alleviating the respiratory distress by passage of the bronchoscope to the carina, while potentially disguising the extent of the abnormalities.

Factors associated with the development of tracheomalacia include immaturity, higher mean airway pressure, and prolonged mechanical ventilation.[108] Affected infants may have significant dynamic compression of the trachea. Because the trachea of premature infants is very compliant and may be excessively stretched and injured during mechanical ventilation, very immature infants are particularly prone to tracheomalacia. Some premature infants have greatly enlarged tracheas or tracheomegaly after prolonged mechanical ventilation.[109]

Many infants with tracheomalacia spontaneously improve by 1 to 2 years of age, when the cartilage has become strong enough to support tracheal patency and airway caliber increases. Severe cases with life-threatening episodes of airway obstruction necessitate tracheostomy with an elongated tracheostomy tube. However, current treatments for milder cases include tracheal intubation with continuous positive airway pressure (CPAP) or PEEP, which prevents tracheal collapse, and anterior aortopexy (fixation of the aorta to the sternum), which has the effect of supporting the attached trachea.[110,111] When aortopexy is performed, the majority occur via a left anterior thoracotomy, however there are reports of right anterior and muscle sparing approaches as well as thoracoscopic aortopexies.[112] Many of the most severely affected patients respond well to aortopexy. Posterior tracheopexy has also been performed for severe tracheomalacia with posterior membranous tracheal intrusions, and can be performed in conjunction with aortopexy.[113] Both thoracoscopic and muscle sparing thoracotomy approaches for posterior tracheopexy have been employed.[114,115] Tracheal stents have been limited due to higher failure rate than aortopexies and serious long-term complications including migration and erosion of stents.[116] 3D printed bioresorbable external splinting for airway support is currently being investigated.[117]

Tracheal Compression by Vascular Rings

Tracheal compression can be caused by several factors: (1) a double aortic arch, (2) a right aortic arch, (3) a left-sided origin of the (right) innominate artery, (4) a right-sided origin of the left common carotid artery, or (5) an anomalous origin of the left

pulmonary artery from the right pulmonary artery.[118] With a right aortic arch, the trachea is compressed by the main pulmonary trunk, aortic arch, and ligamentum arteriosus. The anomalous innominate or common carotid arteries form a tight crotch, which impinges on the anterior trachea. The anomalous left pulmonary artery returns to the left by passing between the esophagus and the trachea, compressing the trachea between the right and the left pulmonary arteries. Infants with tracheal compression have inspiratory stridor and expiratory wheezing, with symptoms usually appearing later in the neonatal period. Affected infants often lie with the head and neck hyperextended to stretch the trachea and make it less compressible. If the esophagus is compressed, feeding is associated with regurgitation.

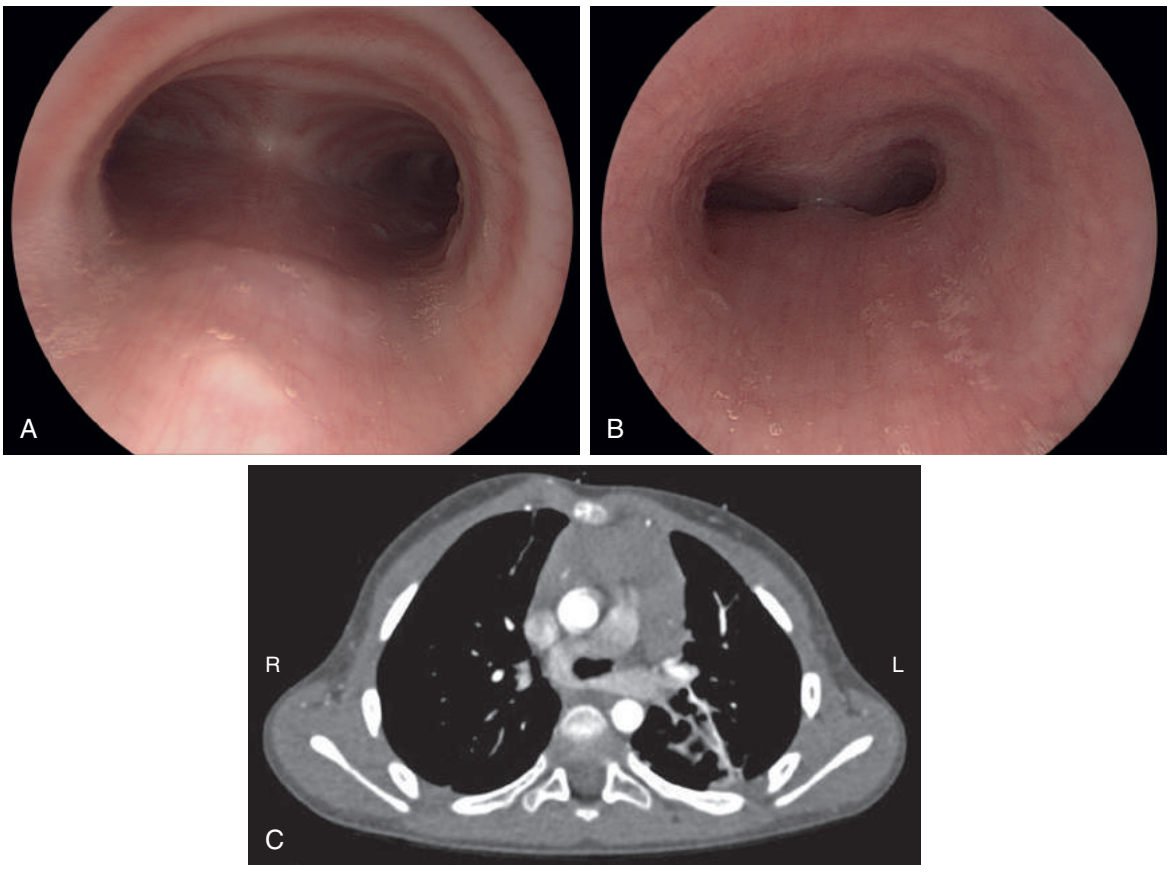
There are several methods of diagnosis. The chest radiograph may show mild overinflation, a right-sided aorta, and, with appropriate technique, evidence of tracheal narrowing. A barium swallow examination may show indentation of the esophagus. Bronchoscopy should reveal a pulsatile mass with narrowing near the carina (Fig. 44.2A,B). Cross-sectional imaging with CT and MRI has proven to be accurate in defining most vascular malformations and can give detailed imaging of the surrounding anatomy (Fig. 44.2C).[118] While echocardiography is less reliable in making the diagnosis of a vascular ring, it has been demonstrated that prenatal diagnosis by ultrasound avoided unnecessary delays in the repair of symptomatic vascular rings and that repair on identification of symptoms prevented the development of secondary tracheobronchomalacia.[119,120] Treatment involves surgical division of the vascular ring. Recently, minimally invasive techniques including a video-assisted thoracoscopic approach and endoscopic robotic-assisted techniques have been employed.[121,122] After surgical division of the vascular ring, the respiratory distress may persist for weeks or longer because of localized tracheal deformity (either stenosis or tracheomalacia), emphasizing the need for immediate repair on diagnosis. In cases of isolated vascular rings, repair provides treatment with minimal mortality and limited postoperative complications.[123]

Tracheal Compression by Extrinsic Masses

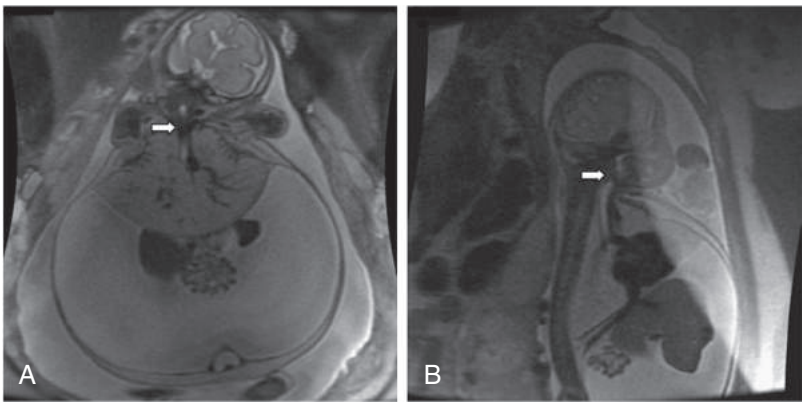
The trachea may also be compressed by a bronchogenic cyst, an enteric duplication cyst, a thoracic neurogenic tumor, or a mediastinal teratoma. These may be demonstrated by anteroposterior and lateral chest films and are especially apparent on CT scan.[124] Such masses may also compress the esophagus and can be demonstrated with a barium swallow.

Congenital High Airway Obstruction Syndrome (CHAOS) and the Ex-Utero (3HD) Intrapartum Treatment (EXIT) Procedure

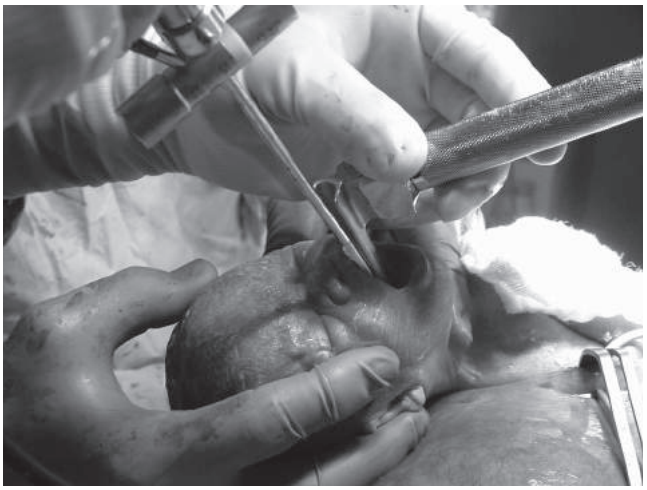
CHAOS describes a spectrum of rare anomalies, including laryngeal web, laryngeal atresia, laryngeal cyst, and tracheal atresia



• **Fig. 44.2** Pulmonary artery sling. (A) Bronchoscopy of a normal distal trachea just above the level of the carina. (B) Bronchoscopy of a trachea with external compression by a vascular ring; in this case a pulmonary artery sling. (C) Contrast-enhanced computed tomography demonstrating the anomalous left pulmonary artery passing behind the trachea and in front of the esophagus. Compression of the trachea can be seen. (Courtesy Dr. Jamie Funamura, UC Davis Health System, Sacramento, CA.)



• **Fig. 44.3** Fetal MRI in the coronal (A) and sagittal (B) planes of a patient with fetal CHAOS. Note the atretic laryngeal segment (arrows). This lesion measured 7 mm and was deemed a poor candidate for fetal tracheoplasty. (From Saadai P, Jelin EB, Nijagal A, et al. Long-term outcomes after fetal therapy for congenital high airway obstructive syndrome. *J Pediatr Surg*. 2012;47(6):1095–100.)



• **Fig. 44.4** Direct laryngoscopy, bronchoscopy, and esophagoscopy are performed during an ex utero intrapartum treatment procedure. Using these methods, airway obstruction can be overcome and endotracheal intubation can be performed. (From Hirose S, Farmer DL, Lee H, et al. The ex utero intrapartum treatment procedure: looking back at the EXIT. *J Pediatr Surg*. 2004;39:375–380.)

or stenosis. Most cases are sporadic and the true incidence is unknown; thus the natural history of this disease is not well known. CHAOS is characterized by enlarged lungs, dilated distal airway, everted diaphragm, ascites, and ultimately nonimmune hydrops fetalis. Prenatal diagnosis is becoming more common with the progress of ultrasound and MRI techniques (Fig. 44.3).[125] The exact nature of the airway obstruction may not be entirely clear, and the time required to establish a safe airway soon enough after delivery carries various risks, including anoxic brain injury.

As with any anomaly that causes either direct respiratory airway obstruction or airway compression by means of mass effect, CHAOS poses a difficult problem for the clinical team during delivery. The EXIT procedure was developed as a solution to this problem: by preserving fetoplacental circulation throughout a scheduled cesarean section delivery, a safe fetal airway can be established before umbilical cord ligation (Fig. 44.4).[126] Infants with CHAOS still require postnatal airway reconstruction after delivery, but once a tracheostomy is in place, laryngeal or tracheal reconstruction is essentially an elective procedure and can be performed once the patient's overall status is optimized.[127] With EXIT, infants with CHAOS have a high chance at survival with low rates of hypoxemia-related complications in some small case series which show promising results.[128]

Disorders of the Mediastinum

Table 44.1 outlines the most common etiologies of mediastinal masses in children by location.[129]

Thymus

The thymus occupies the upper anterior mediastinum, and it is more prominent in the newborn period than at any other time of life. It may be so large as to reach the diaphragm or obscure both cardiac borders on radiographs. The normal thymus can be distinguished from an abnormal mass by the absence of tracheal deviation or compression. The thymus changes in position with respiration and is less prominent with deep inspiration. It also involutes with stress as well as with corticosteroid therapy. Absence of the thymic shadow in an infant should alert the clinician to the possibility of severe combined immune deficiency syndrome or DiGeorge syndrome with hypocalcemia and cardiac anomalies.[130]

The cardiothymic-to-thoracic ratio provides an index of thymic size. The shadow of the enlarged thymus is the most common radiopaque mass visualized in the anterior mediastinum of the newborn. The enlarged thymus causes little if any trouble in the neonatal period. Fletcher et al. and Gewolb et al. noted that a large thymus is present on the first day of life in infants at risk for hyaline membrane disease, presumably because of less-than-normal levels of glucocorticoids before birth.[131,132] The effect that glucocorticoids have on the thymus is also supported by a study that found that prenatal steroid administration causes thymic atrophy.[133] This steroid-induced atrophy, however, does not appear to have any clinical significance.

Thymomas and thymic carcinomas are extremely rare in the pediatric population, with the median age at diagnosis of 11.5 years.[134] The presence of myasthenia gravis occurs only 7% of the time, compared to roughly 50% of the time in adults.[135] There are not existing consensus guidelines for the management

TABLE 44.1 Mediastinal Mass Etiology by Location

Mediastinal Location	Type of Lesion	Most Common
Anterior	Solid	1. Thymic hyperplasia 2. Thymoma 3. Thymic carcinoma 4. Lymphoma 5. Teratoma
	Cystic	1. Thymic cyst 2. Lymphatic malformation
	Fatty	1. Lipoma 2. Thymolipoma
Middle	Cystic	1. Congenital foregut duplication cyst 2. Bronchogenic cyst 3. Esophageal duplication cyst 4. Neurenteric cyst
	Lymphadenopathy	1. Lymphoma 2. Metastatic disease 3. Infectious
Posterior	Solid	*Sympathetic ganglion tumors* 1. Neuroblastoma 2. Ganglioneuroblastoma 3. Ganglioneuroma *Nerve sheath tumors* 1. Schwannoma 2. Neurofibroma 3. Malignant peripheral nerve sheath tumor

of these rare tumors, however complete primary resection is preferred which may be accomplished thoracoscopically for thymomas. For thymic carcinoma, a malignant tumor of the thymus, the addition of multimodal therapy consisting of chemotherapy and radiotherapy is used if needed.[135,136] Unfortunately, despite this the prognosis remains grim with 21% 5-year overall survival with thymic carcinoma.

Congenital Mediastinal Teratoma

Mediastinal teratomas arise from primordial germ cells and are classified as being mature or immature. Mature teratomas are more common and contain all three embryonic cell layers (ectoderm, mesoderm, and endoderm). Immature teratomas are characterized by mature elements of all three germ layers but also have immature embryonic tissue interspersed.[137] Mediastinal teratomas rarely cause symptoms in the newborn infant. However, when an anterior mediastinal mass is associated with respiratory distress in the newborn, the strong likelihood is that the lesion is a mediastinal teratoma.[138] Teratomas can also cause chest pain, cough, and recurrent post-obstructive pneumonia secondary to bronchial obstruction. Chest radiographs show an anterior mediastinal mass with calcification; however, CT and MRI are the primary modes of diagnosis. Mature teratomas are invariably not malignant, and surgical resection via median sternotomy, posterolateral thoracotomy, or thoracoscopy is sufficient treatment.[139] Immature teratomas in children fortunately behave similar to mature teratomas and should be completely excised; however, there may be a role for chemotherapy before resection.

Prenatal diagnosis of a mediastinal teratoma is becoming more common with use of fetal ultrasound and fetal MRI. Large masses can cause compression of the mediastinum, leading to nonimmune hydrops and fetal demise.[140] If hydrops does not occur and the pregnancy is carried to term, delivery can be complicated by airway compression and cardiopulmonary failure. These cases can be managed by in utero resection in the case of hydrops or via an EXIT procedure near term followed by resection after stabilization.[141,142]

Congenital Bronchogenic Cysts

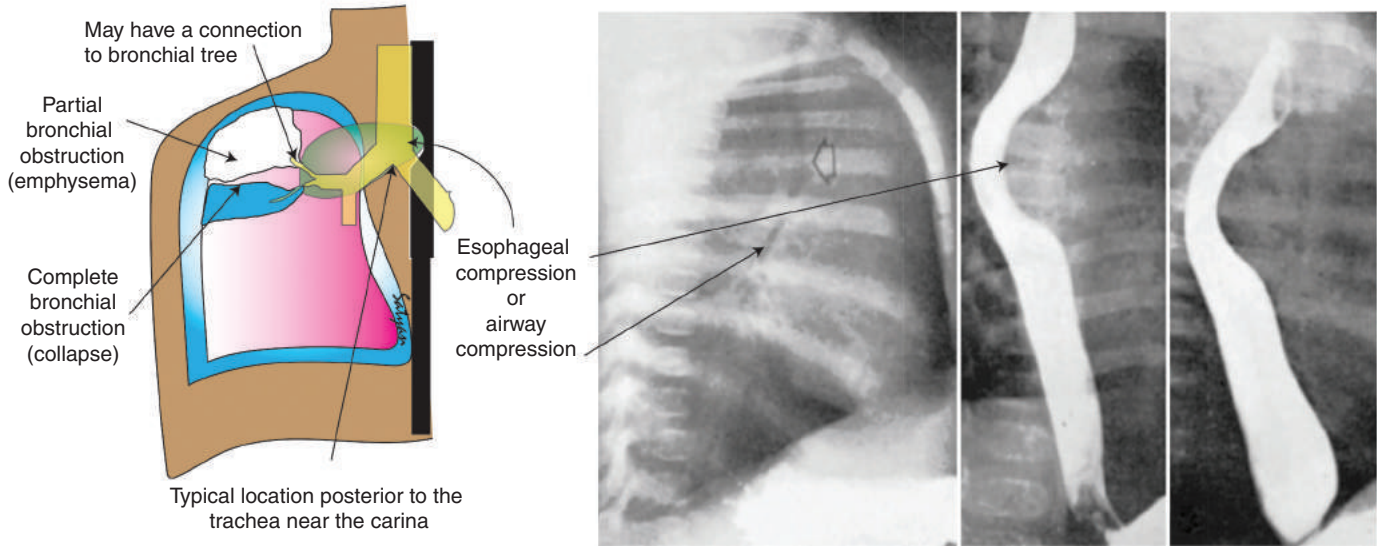
Bronchogenic cysts arise from the foregut during embryologic development and can be located in the mediastinum (just above the tracheal bifurcation), pericardium, abdomen, neck, and within the diaphragm.[143] While the majority are extrapulmonary, a minority of bronchogenic cysts are found within the lung, and some pathologists believe that these may not be distinct from intralobar sequestrations or type 1 congenital pulmonary airway malformations (CPAMs).[144]

Bronchogenic cysts can be seen on prenatal ultrasound, although they are more likely to present later as they increase in size over time, with airway compression, recurrent infection, hemoptysis, or pneumothorax.[145] Intrauterine airway compression can result in congenital lobar emphysema (CLE; see later discussion). In the newborn, bronchogenic cysts are encountered infrequently, and most do not come to the attention of the practitioner until later in infancy or during the second decade of life. Bronchogenic cysts seldom attain a large size and often contain clear fluid and debris. They are lined with columnar or cuboidal epithelium, and their walls generally contain smooth muscle, glands, and cartilage, the latter indicating their bronchial origin.[144] These cysts lie near the carina in the middle mediastinal space. They produce lung overdistension or atelectasis, depending on whether airway obstruction is complete or partial, and this is accompanied by respiratory distress in the newborn infant.

Imaging techniques and findings include:

- Radiographic examination: show a mass lesion at or just above the carina and displacing the lower trachea forward (Fig. 44.5).
- CT images: show sharply marginated cystic masses of soft tissue or water attenuation.
  - Specific criteria exist to distinguish these cysts from other mediastinal masses on CT.[146]
- MR images: hyper- or isointense to cerebrospinal fluid on both T1 and T2 images, with cyst wall enhancement with gadolinium.[146]
- Fetal ultrasound: Anechoic unilocular intrathoracic cyst, can be accompanied by polyhydramnios or hydrops.[147]
- Barium swallow examination: shows indentation of the esophagus, from external compression of the cyst pushing it backward at the level of the carina (see Fig. 44.5).
- Bronchoscopy: shows compression of the trachea and sometimes of one major bronchus, usually from the posterior aspect.

Sometimes a bronchogenic cyst may communicate with the airway and contain air. In the immediate newborn period, there may be retention of fetal lung fluid in the lung compromised by the bronchogenic cyst; the fluid may take days to clear. Treatment for bronchogenic cyst consists of surgical excision by partial or total lobectomy, either open or thoracoscopically, with uniformly good results. Early excision prevents eventual



• **Fig. 44.5** Bronchogenic cyst. Bronchogenic cyst is commonly located posterior to the carina and may be connected to the bronchial tree. Partial obstruction to the airway can result in overinflation (emphysema) of a lobe of the lung or retention of fetal lung liquid. Complete obstruction of the bronchus can lead to lobar collapse. These cysts can cause compression on the airway and be visible on a contrast upper gastrointestinal study. (Copyright Dr. Satyan Lakshminrusimha, UC Davis Health System, Sacramento, CA.)

development of symptoms in adulthood and malignant degeneration later in life.[148]

Esophageal duplication cysts are foregut cysts that are similar in development to a bronchogenic cyst, as both the foregut and the lungs arise from the same primordial tissue. Differentiation between the two occurs on histopathologic evaluation by determining the type of epithelium present, therefore an esophageal duplication cyst should remain on the differential when working up a patient with a mediastinal cyst concerning for a bronchogenic cyst.[149] Treatment consists of surgical excision, either open or thoracoscopically; esophagogastroduodenoscopy (EGD) may be a useful adjunct intra-operatively.

Neurenteric Cysts

Neurenteric cysts are rare lesions that result from the inappropriate separation of the embryonic notochordal plate and presumptive endoderm during development, including the persistence of the primitive foregut adhering to the notochord.[150] When the foregut descends from its early position in the neck, this adhesion to the notochord causes anomalies of the vertebral bodies. As the adhesion breaks off the duplicated portion of the foregut, it prevents complete descent into the thorax and abdomen along with the mature foregut. They often lie in the posterior mediastinum but with increasing size may project far into one or the other hemithorax. Their walls are composed of a mucosal layer, characteristic of their site of origin, and one or more muscular layers. They contain secreted fluid that is the same as that of their parent viscus.

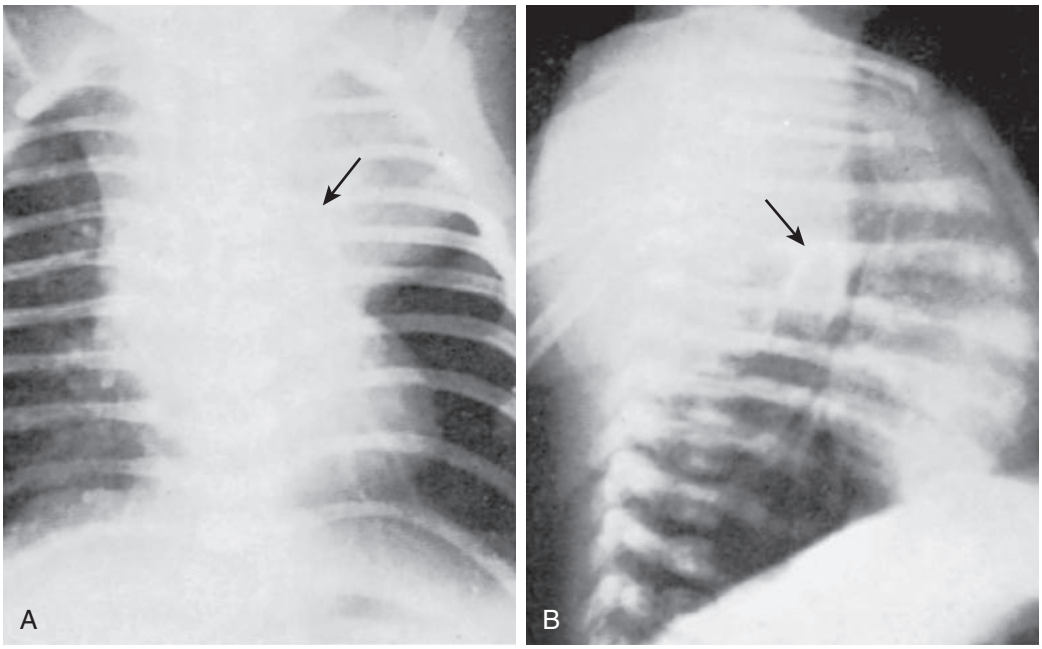
Clinical signs depend on the size and location of the cyst. Because most are posterior and lie close to the trachea, esophagus, and great vessels, they are seldom present without signs of abnormality. With their involvement with the spine, most have concurrent neurologic symptoms from spinal cord compression.[151] Cyanosis, tachypnea, and dyspnea often are present from birth.[152] Swallowing difficulties and vomiting are less frequent. Recurrent lower respiratory tract infections are findings in older infants with such cysts. Frank hemorrhage from the lungs or stomach is not uncommon. In most instances, hemorrhage indicates that the cyst is of gastrogenic origin, with peptic acid erosion into the trachea or esophagus.

It is recommended to perform careful radiographic evaluation of the spinal canal with CT scan and/or MRI, with MRI having proven superior in the delineation of the cyst and its relationship to the neural structures nearby.[153] Technetium scans may also be useful for delineating cysts lined with gastric mucosa. Radiographs of the chest show abnormal densities that are often difficult to distinguish from unusual cardiac contours. The barium swallow examination commonly shows displacement of the esophagus, usually forward because the mass is in the posterior mediastinum. The cyst may partially or totally compress the bronchus, with consequent lung overdistension or atelectasis. Sometimes the symptoms are intermittent as the cyst enlarges or empties. Bronchoscopy may show signs of external compression of the trachea or bronchus, usually from the posterior aspect.

Operative resection is indicated as soon as the diagnosis of a neurenteric cyst is made. It is neither necessary nor wise to delay exploration because all intrathoracic masses will eventually become symptomatic and have been known to cause paralysis and fatal meningitis. The goal of management is total surgical resection, most commonly via a posterior approach. Reports of recurrence range from 0% to 37%, with a link between partial resection and recurrence.[150]

Congenital Thoracic Neuroblastoma

Neuroblastoma, the most common solid tumor in the mediastinum of infants, arises from sympathetic neural tissue along the vertebral column and is therefore located in the posterior mediastinum. While neuroblastomas can arise anywhere in the sympathetic nervous system, thoracic neuroblastomas account



• **Fig. 44.6** Congenital thoracic neuroblastoma. The mass *(arrow)* is in the left upper hemithorax (A) and in the posterior mediastinum (B) on chest x-ray. (From Hope JW, Koop CE. Differential diagnosis of mediastinal masses. *Pediatr Clin North Am*. 1959;6:379.)

for approximately 20% of cases.[154] They may extend into the lungs, causing respiratory distress, or the spinal canal, causing neurologic signs such as Horner syndrome and paralysis. The chest mass may be identified incidentally on chest radiograph or during evaluation of respiratory distress (Fig. 44.6A,B). In older infants the diagnosis may follow chest radiography for lower respiratory tract infection, or radiographs may be taken to investigate dyspnea with physical exertion. Approximately 80% of neuroblastomas will demonstrate tumor calcification on CT scan. While CT scan can aid in diagnosis, MRI is essential to assess the degree of intervertebral tumor extension and can also evaluate for lymphatic spread, chest wall involvement, and liver metastases.[155] Distant boney metastasis is assessed by metaiodobenzylguanidine (MIBG) scintigraphy.[156] Diagnosis of thoracic neuroblastomas is also possible in the prenatal period using ultrasound.[157]

Despite some characteristic imaging findings specific to neuroblastomas, differentiation from other posterior mediastinal masses may be impossible before exploration. Encasement of the aorta or major branches, compression of the trachea or bronchus, and intraspinal tumor extension supports a diagnosis of neuroblastoma. Neuroblastoma clinical markers include:

- Elevation of urinary vanillylmandelic acid and homovanillic acid: may be present, but this finding is less common in the newborn, and its absence does not rule out neuroblastoma.
- Elevation of epinephrine, norepinephrine, and dopamine: may result in systemic hypertension, but this has not been reported in a thoracic neuroblastoma.
- Assays for various clinical biologic markers may be elevated, but not typically in the newborn:
  - Elevation of serum ferritin
  - Elevation of serum lactate dehydrogenase
- Bone marrow aspirate: obtained for cytologic evaluation of bone marrow involvement.
- Nuclear bone scan: performed to evaluate for bony metastases.
- Cytogenetic biologic markers: may be detected in the excised tumor tissue (e.g., cellular DNA ditetraploidy and increased N-*myc* oncogene copy number); these are markers of more aggressive disease, but are commonly negative in the newborn.[158]

Staging of neuroblastomas is typically done using the International Neuroblastoma Risk Group (INRG) staging based on clinical criteria and image-defined risk factors. Table 44.2 outlines the image-defined risk factors for neuroblastoma.[159]

The traditional International Neuroblastoma Staging System (INSS) categorizes the tumors into four stages, based on the extent of surgical resection. Neuroblastoma stages are as follows:

- L1: localized tumor not involving vital structures as defined by list of image-defined risk factors and confined to one body compartment
- L2: locoregional tumor with presence of one or more image-defined risk factors
- M: distant metastatic disease (except stage MS)
- MS: metastatic disease in children younger than 18 months with metastases confined to skin, liver, and/or bone

The associated risk stratification schema classifies tumors into low, intermediate, and high risk, depending on the histologic category of the tumor, as well as whether the tumor exhibits amplification of the *MYCN* gene and has favorable differentiation features. Based on these factors, tumors are then classified on a range from very low to high risk. Table 44.3 breaks down the INRG stages above with details including age, histologic category, grade of tumor, biologic markers, and pretreatment risk groups.

Regarding management of these tumors, treatment is guided by the Children's Oncology Group (COG) risk stratification which includes the above-mentioned low- to high-risk groups. Options for management include surgery, chemotherapy, radiotherapy, differentiation therapy, and immunotherapy.[160] Treatment for

TABLE 44.2 Pertinent Image-Defined Risk Factors for Neuroblastic Tumors

Location	Risk Factor
Ipsilateral tumor extension within two body compartments	Neck-chest, chest-abdomen, abdomen-pelvis
Neck	Tumor encasing carotid and/or vertebral artery and/or internal jugular vein Tumor extending to base of skull Tumor compressing the trachea
Cervico-thoracic junction	Tumor encasing brachial plexus roots Tumor encasing subclavian vessels and/or vertebral and/or carotid artery Tumor compressing the trachea
Thorax	Tumor encasing the aorta and/or major branches Tumor compressing the trachea and/or principal bronchi Lower mediastinal tumor, infiltrating the costovertebral junction between T9 and T12
Thoraco-abdominal	Tumor encasing the aorta and/or vena cava
Infiltration of adjacent organs/structures	Pericardium, diaphragm, kidney, liver, duodeno-pancreatic block, and mesentery
Conditions to be recorded, but *not* considered IDRFs	Multifocal primary tumors Pleural effusion, with or without malignant cells Ascites, with or without malignant cells

From Monclair T, Brodeur GM, Ambros PF, et al. The International Neuroblastoma Risk Group (INRG) staging system: an INRG Task Force report. *J Clin Oncol*. 2009;27:298-303.

low-risk is often surgical resection alone, which has proven curative for most patients with a 97% 5-year overall survival rate.[161] Traditionally, these excisions have been done via open thoracotomy; however, with improvements in thoracoscopic technology, several groups advocate the use of video-assisted thoracoscopic surgery (VATS) for resection of thoracic neuroblastomas and report good results with no documented recurrences and with minimal complications.[162,163] Intermediate-risk tumors which are not amenable for upfront resection will receive neoadjuvant chemotherapy primarily in order to improve resectability. There is no reported difference in overall survival among patients with complete resection, minimal residual disease, or biopsy only in this intermediate-risk group, thus suggesting aggressive attempts at complete resection are unnecessary.[164] COG protocols for high-risk neuroblastoma include induction chemotherapy followed by surgical resection with a goal of complete resection; however, studies have not shown substantial survival benefit in patients with high-risk neuroblastoma undergoing gross total tumor resection.[165,166] For the induction chemotherapy, it has been shown that high-risk patients who receive four cycles of chemotherapy before surgical resection have a superior overall survival than patients who receive two.[167] Following operative intervention, consolidation therapy consists of chemotherapy and stem cell transplant. Additional therapies, including radiotherapy and immunotherapy, are also options for consolidation or maintenance therapy. The 5-year overall survival rate for high-risk patients is 40% to 50%.[167]

The outlook for neuroblastomas in extra-adrenal locations is better than that for their adrenal counterparts.[164] The outlook for neuroblastomas manifesting in the first 18 months of life also is good, especially in the distinct subgroup of neuroblastoma known as MS disease in which the tumor has only spread to the skin,

TABLE 44.3 International Neuroblastoma Risk Group Staging

INRG Stage	Age (mo)	Histological Category	*MYCN*	11q Aberration	Ploidy	Pretreatment Risk Group
L1/L2		Ganglioneuroma maturing; ganglioneuroblastoma intermixed				A Very low
L1		Any, except ganglioneuroma maturing or ganglioneuroblastoma intermixed	NA Amp			B Very low K High
L2	<18	Any, except ganglioneuroma maturing or ganglioneuroblastoma intermixed	NA	No Yes		D Low G Intermediate
	≥18	Ganglioneuroblastoma nodular; neuroblastoma	NA NA Amp	No Yes		E Low H Intermediate N High
M	<18		NA		Hyperdiploid	F Low
	<12		NA		Diploid	I Intermediate
	12 to <18		NA		Diploid	J Intermediate
	<18		Amp			O High
	≥18					P High
MS	<18		NA NA Amp	No Yes		C Very High Q High R High

NA, Non-amplified; *Amp*, amplified; blank field = "any"; diploid (DNA index ≤1.0); hyperdiploid (DNA index >1.0 and includes near-triploid and near-tetraploid tumors); very low risk (5-year EFS > 85%); low risk (5-year EFS > 75% to ≤ 85%); intermediate risk (5-year EFS ≥ 50% to ≤ 75%); high risk (5-year EFS < 50%).
From Sokol E, Desai A. The evolution of risk classification for neuroblastoma. *Children*. 2019;6:27. doi:10.3390/children6020027, and Cohn SL, Pearson AD, London WB, et al. The International Neuroblastoma Risk Group (INRG) classification system: an INRG Task Force report. *J Clin Oncol*. 2009;27:289–297.

liver, and less than 10% of bone marrow involvement. Many of these tumors are cystic in nature, and the histologic examination suggests that the neuroblasts are arranged in clumps rather than in sheets; this "neuroblastoma in situ" carries a high likelihood of spontaneous regression and therefore a good prognosis. In general, prognosis for children presenting less than 18 months of age is improved compared to older children.

Disorders of the Chest Wall

Abnormalities of the bone and muscle of the chest wall may be a mechanical hindrance to ventilation.

Skeletal Disorders

Although abnormalities involving bone are rare, they may be recognized immediately and are sometimes amenable to operative correction.

Defects of Sternal Fusion

Defects in fusion of the sternum are uncommon, occurring in less than 1 in 100,000 live births.[168] Complete separation of the two halves of the sternum allows protrusion of cardiovascular structures, a condition known as *ectopia cordis*.[169] Lethal malformations of the heart are commonly associated with this condition. Upper sternal clefts are the most common type of sternal fusion defect.[168] Early operation is advised to shield the underlying structures from injury, improve respiratory mechanics secondary to paradoxical chest motion, and because of the greater ease of approximating the separated parts in the first days of life compared with later.[170] A lower sternal cleft and ectopia cordis with a congenital heart defect may be associated with congenital apertures in the upper abdominal wall, in the pericardium, and in the anterior diaphragm, with a Morgagni-type diaphragmatic hernia, the so-called pentalogy of Cantrell.[168]

Pectus Excavatum

The most common sternal defect is pectus excavatum, occurring 1 in 400 to 1000 live births.[171] It is characterized by a posterior curvature of the sternum and lower costal cartilages, resulting in a "funnel chest" appearance. Pectus is three to five times more common in males and is sometimes associated with syndromes such as Marfan syndrome, Noonan syndrome, and Turner syndrome.[171] A family history of some type of anterior thoracic deformity was found in up to 42% of patients.[171] Only approximately one-third of cases of pectus excavatum are present in infancy.[172] Even in those cases that are present at infancy, it is rarely a severe deformity, and symptoms are minimal. As the deformity worsens, the heart may be compressed between the sternum and the vertebral column and displaced to the left, impinging on the space of the left lung. This compression may result in exercise intolerance, chest pain, and shortness of breath.[173] In addition to the physical symptoms, the chest deformity often leads to cosmetic concerns and psychological distress sufficient to warrant intervention.

Although routine chest radiographs may suffice in evaluating the severity of chest wall deformity, chest CT is typically preferred because this modality provides the bony and cartilaginous anatomic details as well as any information regarding cardiac compression necessary when considering surgical intervention. Using chest CT allows one to calculate the Haller index (HI), or the ratio of the transverse distance to the anteroposterior distance. A normal HI is about 2, and most cases of pectus excavatum that qualify for operative correction are greater than 3.25.[174] However, the indications for operative correction are debatable. Those patients with severe cardiac or pulmonary compression, abnormal cardiac or pulmonary function studies, or failed previous repairs are candidates for repair.[173] Periodic evaluation of cardiovascular status with echocardiogram and electrocardiography in addition to assessment of pulmonary function is appropriate in the presence of progressive deformity and during periods of rapid growth such as adolescence. In our opinion, correction should not be undertaken until the child is several years of age and then only in those few children in whom the deformity appears to be progressing. Serial photographs are the best way to document changes in pectus excavatum. Surgical correction involves insertion of a metal bar underneath the sternum. This can be done most commonly via a minimally invasive approach with the Nuss procedure or by an open approach with the Ravitch procedure when not a candidate for the Nuss procedure; results are excellent in the majority of patients.[175] Surgery is almost always associated with improved self-image and perceived functional activity.[176,177] Recurrences are possible during later active growth and may require repeat intervention, often with excellent outcomes.[178]

Poland Syndrome

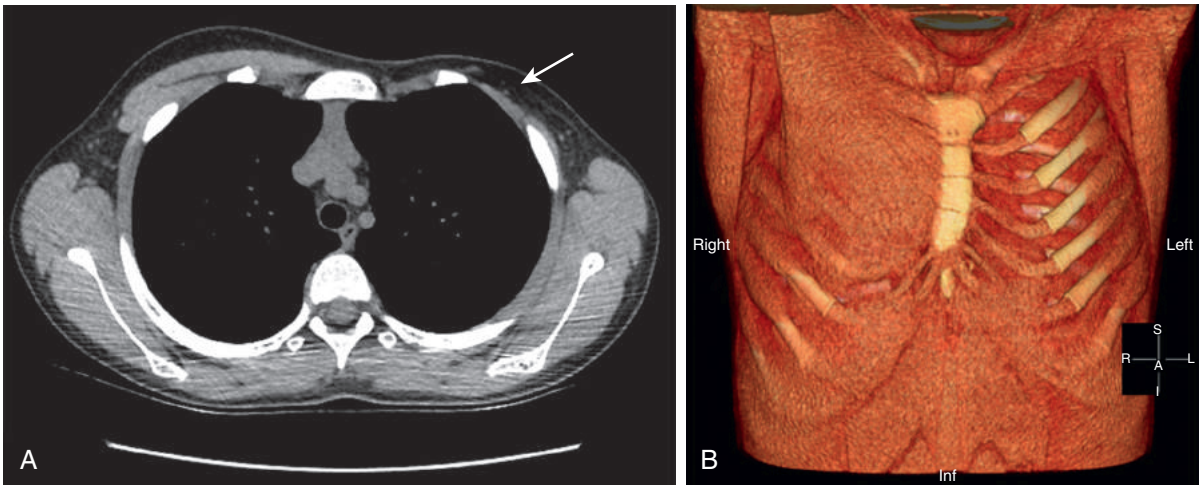
There is a great amount of diversity in the clinical manifestations of Poland syndrome; however, all children must have hypoplasia or absence of the pectoral muscles, typically only on one side. Additional manifestations include cartilage agenesis of the second through fifth ribs on the ipsilateral side, athelia, and amastia. There may be associated syndactyly, hemivertebrae, and scoliosis.[179] Breathing may be paradoxical and the cardiac impulse easily observed through the soft tissues, but there is rarely any severe respiratory distress that would necessitate emergent intervention. Later in childhood, although uncommon, there may be increasing respiratory symptoms with scoliosis-related lung disease and/or heart failure. CT scans are useful in assessing the configuration of the chest wall and its need for reconstruction when symptoms develop (Fig. 44.7A,B).[180] No operative intervention is required in infancy, although complex reconstruction with autologous rib grafts, chest wall implants, and latissimus dorsi flaps may be desirable in later childhood as well as mammoplasty in affected girls after puberty.[180,181]

Thoracic Dystrophies

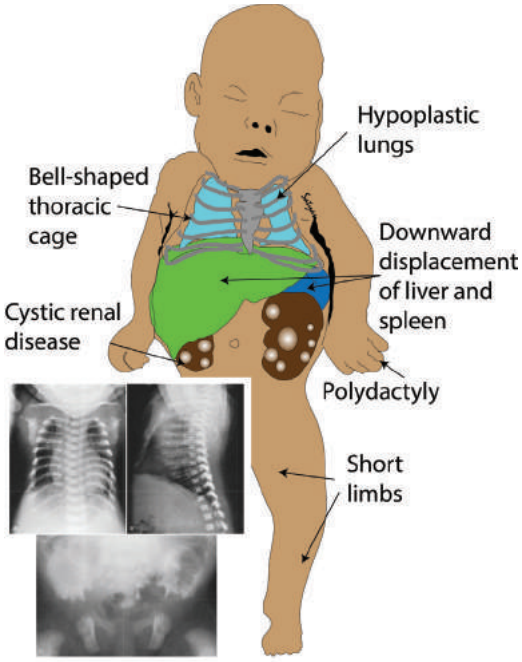
Asphyxiating Thoracic Dystrophy (Jeune Syndrome)

First described by Jeune et al., asphyxiating thoracic dystrophy is an autosomal recessive chondrodystrophy, associated with short-limbed dwarfism and often polydactyly.[182] It is a rare deformity, occurring in approximately 1 in 100,000 to 130,000 live births. The ribs are horizontal, hypoplastic, and short, with flared costochondral junctions (Fig. 44.8). The thorax is small, bell-shaped, and rigid; this results in displacement of the liver and spleen well into the abdominal cavity. Some degree of lung hypoplasia may be present and, if present, is often severe and lethal.[183] The pelvis shows flaring of the iliac wings and acetabular abnormalities. Renal cystic dysplasia may be present, resulting in hypertension and renal failure. Patients can also have variable pancreatic, hepatic, and retinal abnormalities. Prenatal diagnosis with ultrasonography is possible.[184]

In the past, most patients with this condition did not survive the first month. Keppler-Noreuil et al. reviewed 118 cases published in literature and noted almost equivalent number of living to deceased patients at the time of report.[185] In those less than



• **Fig. 44.7** Poland syndrome. (A) Axial CT scan and (B) three-dimensional CT reconstruction demonstrating a congenital absence of the pectoralis major and pectoralis minor muscle *(arrow)*. There is no underlying rib deformity. (Courtesy Dr. Chirag V. Patel, UC Davis Health System, Sacramento, CA.)



• **Fig. 44.8** Asphyxiating thoracic dystrophy. Infants born with this condition have a small, bell-shaped thoracic cage often associated with hypoplastic lungs and a displaced liver and spleen. Many infants have cystic renal disease. Other malformations such as short limbs and polydactyly are common in this condition. (Copyright Dr. Satyan Lakshminrusimha, UC Davis Health System, Sacramento, CA.)

2 years of age, respiratory etiology was most common cause of death. In those between 3 and 10 years of age, renal etiologies were primary cause of death. There is paucity in data in those greater than 20 years of age, but with one patient reported at age 32 years. Davis et al. reported an operative technique for lateral thoracic expansion in 10 patients with chest wall deformities limiting thoracic capacity—including 8 patients with classic Jeune syndrome.[186] Three were younger than 1 year of age at the time of surgery, and six were ventilator dependent. All of the infants older than 1 year of age at the time of surgery improved, with measured lung volumes increasing in 2 of 3 studied and thoracic volumes by CT increasing in 4 of 5 studied. The only deaths were in 2 infants younger than 1 year of age at the time of surgery, suggesting that lateral thoracic expansion is a safe and effective procedure for patients beyond the first year of age. However, given that the Davis technique was two-staged and sequential, there have been efforts to investigate one-stage bilateral thoracic expansion.[187] A vertical expandable prosthetic titanium rib procedure has also been introduced for the purpose of progressive expansion of the chest cavity in patients with thoracic insufficiency.[188] There also has been a case report describing use of the Nuss procedure, as used in pectus excavatum repairs, in two patients with Jeune syndrome.[189] However, long-term improvements in lung function, specifically in patients with Jeune syndrome, have yet to be reported.

Other Thoracic Dystrophies

Severe underdevelopment of the thoracic rib cage, accompanied usually by lethal pulmonary hypoplasia, may be seen in other conditions, such as thanatophoric dwarfism syndrome, short rib–polydactyly syndrome, and campomelic dwarfism syndrome. Affected infants do not usually survive for long after birth.

Neuromuscular Disorders

Other causes of thoracic dysfunction are diseases of the nerves and muscles, including congenital myasthenia gravis, congenital spinal muscular atrophy (Werdnig–Hoffmann disease), congenital myotonic dystrophy, glycogen storage diseases, and congenital spinal injury. Such conditions are usually recognized in the context of the associated systemic muscular weakness. Newborns with myasthenia gravis have episodes of muscle weakness, poor feeding, weak cry, hypoventilation, and apnea with a positive response to an anticholinesterase medication. In congenital spinal muscular atrophy, there is lung hypoplasia associated with absent fetal breathing, and as a result the thorax is small. Other features include severe hypotonia, muscle fasciculation, respiratory failure, and early death; the inheritance is autosomal recessive. In congenital myotonic dystrophy, there is lung hypoplasia from absent fetal breathing; affected infants have respiratory distress at birth, rapidly need mechanical ventilation, and are soon ventilator dependent. Mothers of these

infants have myotonia, difficulty in relaxing muscle contractions; the inheritance is autosomal dominant.

Disorders of the Pleural Cavity

The pleural cavity lies between the parietal pleura, lining the chest wall, and the visceral pleura, lining the lung and other structures. The blood supply to both pleural surfaces is systemic. Venous drainage of the parietal pleura is to the systemic system and the visceral pleura to the pulmonary system. The pleural surfaces filter fluid into the pleural space and the pleural lymphatics then resorb fluid from the pleural cavity.[190] This process is hindered in the setting of abnormal lymphatic development or abnormal systemic venous pressures (because the thoracic lymphatic system drains directly to the systemic veins), resulting in chylothorax. Chylothorax can also occur because of surgical disruption of the thoracic duct or in the setting of lymphatic malformations. Other causes of hydrothorax in the newborn include hydrops fetalis, transudative pleural effusions associated with congenital lung lesions or group B streptococcal pneumonia, empyema (usually associated with nosocomial pneumonia), or fluid extravasation from a displaced central venous catheter.

Congenital Chylothorax

Congenital chylothorax is the most common prenatal diagnosis of pleural effusion. Without oral fat intake chylomicrons, most common indicator for chylothorax, it is difficult to distinguish between chylous and non-chylous effusion. It is rare, occurring in 1 in 10,000 births, with mortality ranging between 20% and 60%; mortality can be as high as 98% if associated with hydrops fetalis.[191] A large fetal chylothorax may evert the diaphragm, and can be the cause of nonimmune hydrops fetalis secondary to hypoproteinemia, and impaired venous return or cardiac function in the setting of increased intrathoracic pressure. Additionally, large fetal chylothorax can result in pulmonary hypoplasia and significantly contribute to mortality. Fetal chylothorax portends a worse prognosis for survival if it is bilateral or associated with hydrops, and prognosis is better if the effusion resolves without reaccumulation.[192]

In utero intervention may be undertaken in cases of large or bilateral effusions, or hydrops, and has been shown to improve survival.[192] Classically, these interventions include transabdominal thoracentesis (with ongoing ultrasound monitoring for fluid reaccumulation) and placement of an indwelling thoracoamniotic shunt (if the effusion reaccumulates), to allow continued drainage of fluid. More recently, in utero pleurodesis has been successfully performed using Picibanil (OK-432), with recent long-term follow up study suggesting comparable survival and neurodevelopmental outcomes to thoracoamniotic shunts.[193] Maternal dietary modification with low-fat, high-medium-chain triglyceride may delay reaccumulation of chylothorax.[194] Fetuses diagnosed with chylothorax should be evaluated for associated conditions that may affect their prognosis, including Down syndrome, Noonan syndrome, and Turner syndrome.

Newborns with congenital chylothorax often present with severe respiratory distress, requiring immediate respiratory support and urgent drainage of pleural fluid. Chest radiographs will show either unilateral or bilateral fluid accumulation, often with shift of the mediastinum. Aspiration of the fluid can confirm the diagnosis. Before feedings have been started, the fluid will be straw colored but will change to the classic milky-white appearance after feedings have been initiated. Characteristics of a chylous aspirate include a high cell count with a lymphocytic predominance, a high triglyceride level (usually above serum levels, but not present unless enteral feeds initiated), and high protein content (Table 44.4). The introduction of small-volume fat-containing feeds with resultant elevated fluid triglyceride levels can confirm the diagnosis if biochemical indices are otherwise not confirmatory. A review of 39 cases of pediatric chylothorax revealed that the composition was consistent with classic descriptions as above.[195]

TABLE 44.4 Characteristics of Chylous Effusions

Characteristic	Description
Appearance	Clear yellow (milky with fat-containing feeds)
Cell count	>1000 cells/mm^3
Lymphocyte proportion	>80%
pH	7.4–7.8
Triglycerides	>110 mg/dL
Cholesterol	65–220 mg/dL
Albumin	1.2–4.1 g/dL
Total protein	2.2–5.9 g/dL

Adapted from Straaten HLM, Gerards LJ, Krediet TG. Chylothorax in the neonatal period. *Eur J Pediatr* 1993;152:2–5; Costa F. Quilotórax. In: Maksoud JG, editor. Cirurgia pediátrica. 2nd ed. Rio de Janeiro: Revinter; 2003. pp. 624–630; Brodman RF. Congenital chylothorax: recommendations for treatment. *N Y State J Med* 1975;75:553–557.

Management of Chylothorax

Neonatal management of a congenital chylothorax includes replacement of protein, clotting factors, and immunoglobulins as needed. Ongoing respiratory support may be needed, and often a chronic chest drain is required to decrease respiratory compromise. Feeds are usually restricted, either providing medium-chain triglycerides (MCTs) only (because MCTs are generally absorbed directly from the intestine without processing to chylomicrons) or a nonfat diet. If these measures fail, a period of total enteric rest is undertaken, with parenteral nutrition administered, until resolution of the chylous effusion. Some practitioners believe that a period of enteric rest is necessary to decrease strain on the lymphatic system, because lymphatic efflux is still increased even with nonfat-containing diets.[196] Once chest drainage has resolved, feeds are reintroduced with a MCT-only formula for 3 to 6 weeks before transitioning to a normal diet.

While there are no controlled studies demonstrating that one strategy is superior to others to hasten resolution of chylothorax, one retrospective study comparing total parenteral nutrition (TPN) to MCT suggested that TPN is more effective than oral MCT in the treatment of congenital chylothorax.[196] A conservative approach was successful in 80% to 85% of patients.[195,197] The primary risk of this non-surgical approach is infection, because these children have prolonged hospitalizations with central venous access, chest drains, no enteral feeds, and protein and immunoglobulin losses. Children who were less likely to have spontaneous resolution of chest drainage included those children with elevated

central venous pressure, children who had more prolonged effusions, and children with higher output than those with surgical injury.

An adjunctive medical approach is the administration of somatostatin or its analogue, octreotide, to decrease pleural drainage. Somatostatin is delivered only via continuous infusion, whereas octreotide can also be given via intermittent subcutaneous injection, because of its longer half-life. The mechanism of effect is thought to be via decreased intestinal secretions and absorption of triglyceride, and therefore intestinal efflux and lymphatic return.[198] Some practitioners proceed quickly to the use of these medications, believing they decrease the volume and duration of chest drainage compared with conservative management; however, this has not been studied in a controlled setting, so the efficacy and risks of this approach are unknown.[199] Others use these medications once conservative measures have failed. Recent metanalysis has found that octreotide was effective in 47% of cases.[199] Potential risks of therapy include hormonal effects of glucose instability and hypothyroidism, and gastrointestinal effects such as cholelithiasis, hepatocellular injury, nausea, diarrhea, abdominal distention, and decreased intestinal perfusion. In this regard, monitoring of serum glucose, thyroid function, liver enzymes, and indicators of cholestasis during therapy is recommended. These serious side effects raise concerns, particularly regarding concomitant feeding during administration of the medications. Octreotide can be administered either subcutaneously or as continuous intravenous infusion. In most cases, dosing is titrated up until chest drainage is minimal. The infusion is usually continued for several days after chest drainage is controlled and then weaned off over several days.

Sildenafil and sirolimus are being investigated as potential adjuncts to conservative treatment. Sildenafil is thought to facilitate resolution of chylothorax by promoting new lymphatic vessel formation and remodeling. There is a case report demonstrating success with sildenafil in late preterm infants with congenital chylothorax secondary to pulmonary lymphangiectasis that was refractory to octreotide.[200] Sirolimus targets mammalian target of rapamycin (mTOR), which is upregulated in number of atypical lymphatic pathology. It has shown some promise in adults and older children with chylothorax secondary to lymphatic malformation, but needs further study including age-appropriate dosing regimens and efficacy in neonates.[201] Additionally, propranolol has been used as off-label treatment of chylothorax and chyloperitoneum in multiple case studies, but no definitive dosing, safety, or efficacy has been established.[202] Propranolol is thought to decrease abnormal lymphatic proliferation by downregulating vascular endothelial growth factor (VEGF).

Most practitioners recommend about 3 to 4 weeks of maximal medical therapy before proceeding with an operative intervention, while others recommend intervention in patients who drain more than 100 mL/year of age per day without slowing down after 10 to 20 days.[195] Effusions are unlikely to resolve after 6 weeks, and waiting too long for operative intervention may result in significant compromise to the child. Operative interventions include thoracic duct ligation via thoracotomy or thoracoscopy, pleurodesis, and/or placement of a pleuroperitoneal shunt.[203] For chylothorax after iatrogenic injury to the thoracic duct, lymphovenous anastomosis is being investigated.[204] Percutaneous thoracic duct embolization is another approach that was developed as a less invasive alternative to surgical thoracic duct ligation, which involves diagnostic lymphangiography followed by transabdominal catheterization and embolization to occlude the thoracic duct proximal to the leak.[205]

Congenital Thoracic Masses and Cysts

Fetal lung masses vary in the degree of abnormality of parenchyma and vasculature and probably represent a spectrum of developmental abnormalities that may be difficult to distinguish prenatally.[144,206] In fact, some investigators have suggested classifying these lesions solely on the basis of the vascular supply (systemic vs. pulmonary) and whether or not lung structure is normal, because the histology and probably the developmental pathways leading to the individual lesions are overlapping.[207] Some level and degree of fetal airway obstruction have been implicated as the cause of many of these lesions.[144] In prenatal series, CPAMs of the lung are frequently the most common lesion reported.[207] However, in postnatal series, CPAMs are often less common (with various imaging modalities and pathology available to clarify the diagnosis after birth), particularly when only cystic lesions are considered.[143,144,208] There are multiple reports of "hybrid" lesions, with features of both CPAM and bronchopulmonary sequestration (BPS), emphasizing that these lesions constitute a spectrum of anomalies.[143,144]

Congenital Pulmonary Airway Malformation of the Lung

CPAMs are the most common congenital lung lesions and develop from an overgrowth of abnormal lung tissue, extending from different levels of the airway. In general, they are unilateral. The lesions may communicate with the airways, allowing them to transition from being fluid filled in utero to air filled postnatally, but they do not contain normal alveoli. CPAMs were previously known as congenital cystic adenomatoid malformations (CCAMs), which were originally classified into three types based upon the size of the cysts and their cellular characteristics.[208] Subsequent revisions of this classification system have included less common proximal and distal lesions and have suggested new nomenclature based on the fact that not all lesions are either adenomatoid or cystic.[144] The term "congenital pulmonary airway malformation," initially proposed by Stocker, has now become the accepted terminology for these lesions. Table 44.5 shows the stages of lung development with the corresponding airway malformation and CPAM type. Consistent with this classification, persistent epithelial expression of the nuclear regulatory protein thyroid transcription factor 1 has been found in type 1 and 3 CPAMs (see later discussion), indicating developmental arrest in the pseudoglandular stage, and targeted fetal airway overexpression of the growth factor fibroblast growth factor 10 in rats produces CPAM-like lesions.[208,209]

Congenital Pulmonary Airway Malformation Types

There are 5 types of congenital pulmonary airway malformations. Table 44.6 outlines the characteristics for each of the 5 types of congenital pulmonary airway malformations (Fig. 44.9). Clinically, Type 0 CPAM lungs may be normal weight or small, but, regardless, this lesion is rapidly lethal because of severe, intractable respiratory failure. It is diffuse and bilateral, recurs in families, and can be associated with other anomalies, suggesting a genetic cause.[210]

For type 1 CPAMs respiratory distress depends on the size of the lesion, with some lesions detected only by incidental imaging or following infection or malignant transformation (see Fig. 44.9). There does appear to be a risk of malignant transformation in unresected or residual CPAM tissue. The specific link to neoplasm has not been elucidated, though the diagnosis of bronchoalveolar

TABLE 44.5 Stages of Airway Branching and Lung Development With Corresponding Congenital Airway Malformation

		CPAM TYPE	
Developmental Stage	**Developmental Events**	**Stocker**	**Adzick**
Embryonic 0–7 wk	Formation of tracheal bud and growth and branching to segmental bronchi	Type 0 Tracheobronchial Type 1 Bronchial/bronchiolar	Macrocystic
Pseudoglandular 7–17 wk	Completion of airway branching to terminal bronchioles (preacinar); gland formation	Type 2 Bronchiolar	
Canalicular 17–27 wk	Formation of respiratory bronchioles to prealveolar structures	Type 3 Bronchiolar/alveolar duct	Microcystic
Saccular 28–36 wk	Formation of secondary septae	Type 4 Distal acinar	
Alveolar 36 wk–2 yr	Formation of alveoli		

CPAM, Congenital pulmonary airway malformation.
From Cha I, Adzick NS, Harrison MR, et al. Fetal congenital cystic adenomatoid malformations of the lung: a clinicopathologic study of eleven cases. *Am J Surg Pathol*. 1997;21:537–544; Hislop AA. Airway and blood vessel interaction during lung development. *J Anat*. 2002;201:325–334.

TABLE 44.6 Types of Congenital Pulmonary Airway Malformations

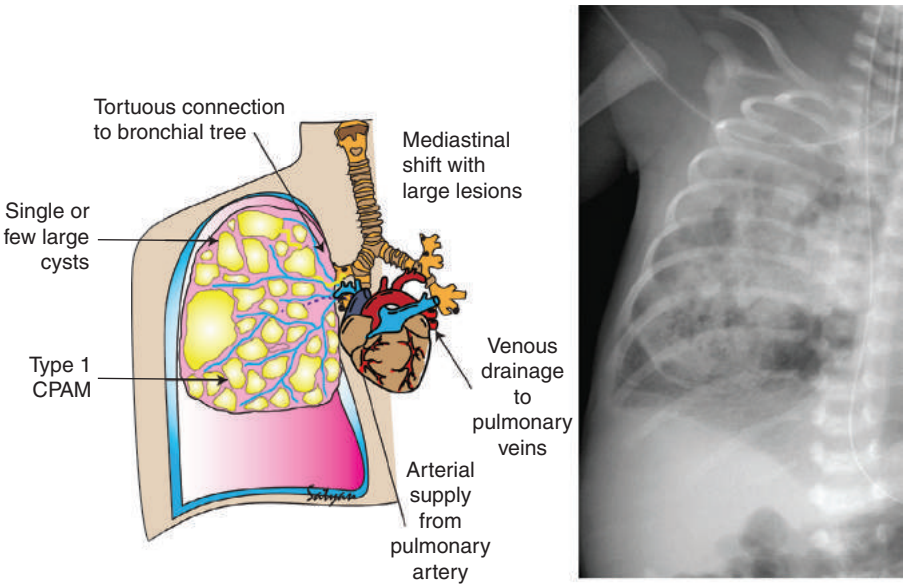
Type	Cases (%)	Characteristics	Cyst Size (cm)	Cellular Characteristics	Other
0	<2	Originate from tracheobronchial tree Normal lung lobulation No alveoli	<0.5	Ciliated pseudostratified epithelium	Affected usually die at birth
1	60–65	Originate from distal bronchi or proximal bronchioles Restricted to a single lobe Can be communicating	>2	Cuboidal or ciliated pseudostratified columnar epithelium	Malignant potential
2	15–20	Appear as multiple bronchiole-type structures Multiple small cysts	0.5–2	Ciliated cuboidal or columnar epithelium	50% associated with other anomalies
3	5–10	Solid cysts Involve entire lobe or lung	<0.5	Non-ciliated cuboidal epithelium	Often cause mass effect in the thorax
4	10	Confined to one lobe Peripheral location	Up to 7	Type I alveolar (flat) and type II (rounded) epithelial cells	Strong malignant potential

carcinoma (BAC) is made in relatively young patients (10 to 42 years), and a spectrum of malignancy has been described.[211,212] This is the only CPAM type that is associated with malignancy, and the young age of affected patients suggests that the CPAM lesion is the cause of the malignancy.[213] It is hypothesized that type 1 CPAM lesions serve as precursors to BAC, sharing similar differentiation profile, and high frequency of K-ras mutations.[214]

Type 2 CPAMs are most commonly associated with other anomalies, in 50% of cases, which include severe bilateral renal dysplasia or agenesis, agenesis of other genitourinary structures, sirenomelia, extralobar pulmonary sequestration, and diaphragmatic hernia.[215,216] Conotruncal cardiac malformations have also been described in association with type 1 and type 2 CPAMs, and ventricular septal defects have been diagnosed in fetuses and infants with all CPAM types.[217]

Type 3 CPAM infants and children may present with mild respiratory distress, or more severe symptoms if pneumonia occurs, or if the lesion ruptures, causing pneumothorax. However, type 4 CPAMs can also be detected by incidental imaging. These lesions are lined with type I alveolar (flat) and type II (rounded) epithelial cells. The presence of substantial portions of cells representing the more proximal areas of the lung should raise suspicion for pleuropulmonary blastoma (PPB), a distinction that is important (because of malignancy associated with PPB; see later discussion), but one that can be difficult.

The approach to diagnose CPAMs in utero has led to a different classification system, based on the natural history of these fetal lesions (see later discussion). Macrocystic CPAMs are defined when a lesion contains cysts that are greater than or equal to 5 mm (types 1 and 2 CPAMs), and microcystic CPAMs are defined as



• **Fig. 44.9** Illustration and chest x-ray at birth in a neonate with type 1 congenital pulmonary airway malformation (CPAM). Large cysts ranging from 1 to 10 cm are present in the lesion. If large, these lesions can lead to mediastinal shift and compress adjacent lobes of the lung. Some of these cysts may have a tortuous connection to the airway. Arterial supply to CPAM is commonly from the pulmonary arterial tree and venous drainage is to the pulmonary venous system. (Courtesy Dr. Satyan Lakshminrusimha, UC Davis Health System, Sacramento, CA.)

a solid lesion, with cysts less than 5 mm (type 3 CPAMs).[218,219] Some lesions categorized by these criteria on fetal ultrasound have been diagnosed as BPS after postnatal resection.[220]

Fetal Diagnosis and Natural History

Fetal diagnosis of a CPAM is often made when mediastinal shift because of mass effect from the lesion is identified and a cystic, intermediate, or solid mass is detected. This may occur during routine fetal ultrasonography or when the mother is referred for evaluation of polyhydramnios (thought to occur when the mass compresses the esophagus and thus limits fetal swallowing).[221] A systemic vascular supply identified on imaging is more consistent with a diagnosis of BPS.[222] Fetal MRI can be used to help distinguish a CPAM from other pulmonary pathology, including BPS and congenital diaphragmatic hernias (CDHs).[223] In a recent population base study of Western Europe, CPAM have an estimated prevalence near 1 in 10,000 live births.[224] Generally, growth of CPAM lesions increases until about 28 weeks' gestation, after which time it plateaus and the lesion regresses in size while the fetus continues to grow.[225]

Fetal and neonatal problems that arise as the result of a CPAM include the development of nonimmune hydrops and lung hypoplasia, which may be due to compression of the otherwise normally developing lung. Up to one-third of fetuses with large CPAM develop hydrops. Factors associated with the development of nonimmune hydrops include an everted ipsilateral hemidiaphragm, predominantly cystic lesions, lesions that exist into the third trimester, and lesions with a CPAM volume ratio (CVR) greater than 1.6.[226] Maternal steroids have been demonstrated to improve hydrops.[227] In a series evaluating fetal macrocystic and microcystic lesions, generally the fetuses with microcystic lesions had poorer outcomes, with intrauterine demise or early neonatal death.[218] Compared with normal lung, CPAM lesions have unregulated growth, with increased proliferation and decreased apoptosis, although they are relatively hypovascular.[228,229] In fetal CPAM tissue, increased Platelet-Derived Growth Factor subunit B (PDGF-β), decreased fatty acid binding protein-7 expression, and dysregulation of Ras, PI3K-AKT-mTOR signaling have been detected.[230–232]

Prenatal Management

Initial ultrasound evaluation of the fetus with possible CPAM should include assessment of lesion size with relevant indices, vascular supply, degree of mediastinal shift, ipsilateral diaphragmatic conformation (normal, flat, or everted), polyhydramnios, placentomegaly, and the presence of any other fluid collections (ascites, integumentary edema, pleural or pericardial effusion) indicating the development of fetal hydrops. Additional prenatal evaluation of a fetus with a known CPAM should include a full fetal survey to identify other anomalies, a karyotype, and an echocardiogram, to evaluate for congenital heart disease and to assess cardiac inflow patterns and outputs. These cardiac indices may help identify impending hydrops, mandating closer ultrasound follow-up and repeated echocardiographic measurements. Most referral centers recommend at least weekly follow-up ultrasound examinations evaluating lesion size and monitoring for development of hydrops until 28 to 29 weeks' gestation, at which point regression of the lesion should be occurring. Thereafter, ultrasound evaluations can be spread out to every 2 weeks. Some referral centers have advocated twice-weekly surveillance if the CVR is greater than 1.6, until 28 weeks' gestation.[221] In multiple studies, between 35% and 40% of fetal lung masses decreased in size or resolved by delivery.[220,225]

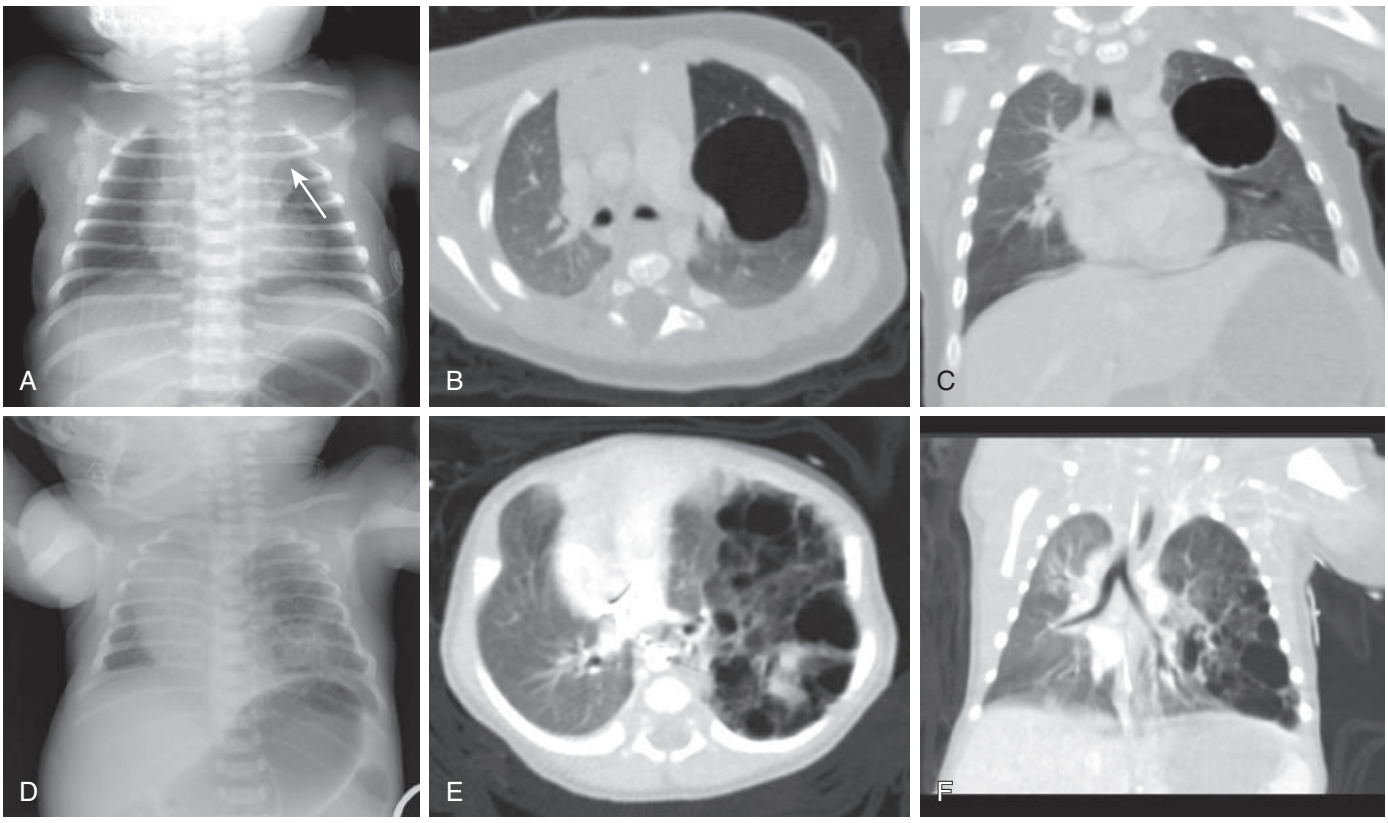
The diagnosis of hydrops in a fetus with CPAM portends a poor prognosis, with either fetal demise or preterm delivery of a compromised infant.[233] Thus impending or definite hydrops is an indication for fetal intervention. The specific intervention depends on the type of lesion. In fetuses with a macrocystic CPAM, a growing, dominant cyst, and impending or definite hydrops, placement of a thoracoamniotic shunt results in a decrease in cyst size and hemodynamic improvement, with relatively good neonatal survival.[234]

However, shunt placement at less than 21 weeks' gestation may be associated with chest wall deformity, which can compromise later respiratory function, so other interventions may need to be taken at that early gestation.[235]Additionally, shunt displacement is a complication and can lead to refractory tension pneumothorax requiring early surgical intervention.[236] Fetal thoracentesis is rarely effective as definitive treatment, because cyst fluid invariably reaccumulates, but it might serve as a temporizing measure until further treatment can be undertaken.[222] Sclerotherapy for patients with hydrops secondary to type II or III is unclear in efficacy and being further studied.[237] For fetuses with impending hydrops, heart failure, and a microcystic CPAM (or failed decompression of a large cyst), open fetal resection (lobectomy or pneumonectomy) improves the chances of survival, although mortality remains high (10 of 23 in one series), and non-survivors tended to be more premature.[238] This selected approach to fetal resection has resulted in resolution of hydrops and mediastinal shift in survivors.[221] For fetuses greater than 32 weeks' gestation, or in the case where fetal surgery is contraindicated because of preterm labor, an EXIT-to-resection procedure may provide a chance for survival. EXIT procedures have also been undertaken in later-gestation fetuses with persistent large lesions (mean CVR 2.2) despite other fetal interventions, although the impact of this strategy on survival and other outcomes is unknown.[238]

Before undertaking any fetal intervention, prenatal glucocorticoid therapy to accelerate fetal lung maturation should be administered. This practice, utilizing betamethasone (12 mg intramuscularly every 24 hours × 2 doses), led to the observation that fetuses with microcystic and hydrops resolved their hydrops after betamethasone exposure.[239] In this series, three fetuses with mild-to-moderate hydrops treated at 21 to 26 weeks' gestation avoided further fetal interventions, were delivered at term, and survived. This observation led to subsequent studies comparing the use of steroids with open fetal surgery in fetuses with macrocystic CPAMs that demonstrated the effectiveness of steroids in not only halting further growth of a CPAM but also causing regression.[227,240] Since this discovery, steroids have become the first line of treatment in fetuses with large microcystic CPAM lesions.

Postnatal Management

In fetuses that do not develop hydrops, the overall prognosis is good and probably depends on the type of lesion and the presence of other anomalies. Generally, the prognosis of a type 1 CPAM is good, particularly if fetal intervention is not required. Following resection, there is usually resolution of any symptoms associated with the lesion (Fig. 44.10A–C). Type 2 CPAMs have a worse prognosis, because of the association with additional serious malformations (Fig. 44.10D–F). Type 3 CPAMs are thought



• **Fig. 44.10** Congenital pulmonary airway malformation. (A) Chest radiograph of an asymptomatic child with a prenatally diagnosed congenital pulmonary airway malformation (CPAM) in the left upper lobe (*arrow*). (B, C) Axial and coronal CT images demonstrate a single 3.1 × 2.7-cm cystic lesion in the left upper lobe consistent with a type 1 CPAM. The child underwent uncomplicated left upper lobectomy at 3 months of age. (D) Chest radiograph of an infant with a prenatally diagnosed CPAM that had rapid enlargement of the cystic component, resulting in mediastinal shift and acute respiratory compromise, is shown. (E, F) Axial and coronal CT images show a multicystic mass with cysts ranging from a few millimeters to 1.5 cm consistent with a type 2 CPAM. Patient was found to have a multicystic lesion confined to the left upper lobe with compressive atelectasis of the left lower lobe and underwent left upper lobectomy. (Courtesy Dr. Chirag V. Patel, UC Davis Health System, Sacramento, CA.)

to be uniformly lethal from their earliest descriptions and can be associated with lung hypoplasia.[208] For fetuses with large microcystic CPAMs, fetal resection, if indicated, may mitigate hypoplasia. However, case series have described substantial spontaneous regression of microcystic CPAMs, with an increased rate of in utero resolution compared with macrocystic lesions.[220] In a large series from a single referral center, neonatal survival was 98%.[238] Five infants underwent fetal procedures, with one death, and there were two neonatal deaths. At a different center, there was one postnatal death out of 75 births. Thirteen fetuses underwent fetal treatment including four open fetal resections, three thoracentesis, and six EXIT procedures.[241] Fig. 44.9 illustrates postnatal imaging with associated management of select infants.

Approximately 75% of newborns with a prenatally diagnosed CPAM are asymptomatic at birth.[242] The remainder may present with respiratory distress with or without pulmonary hypertension, which can be severe enough to require ECMO support.[225] The likelihood of respiratory distress is best predicted by a CVR of greater than 0.84, and the severity of the respiratory distress usually increases as the size of the CPAM increases.[243] For newborns with prenatally diagnosed CPAMs, lesions may be detected on chest radiograph, as solid masses, or fluid or air-filled cysts. Mediastinal shift, mass effect, or areas of air trapping due to airway obstruction may be appreciated. Some centers use ultrasound as an adjunctive modality in the neonatal period, but many surgeons prefer a CT scan as the definitive imaging study, which also can detect lesions that are no longer present on fetal ultrasound.[244] CT scans can also determine if there are multiple bronchopulmonary malformations, which may also require resection, and the use of intravenous contrast allows for definition of the vascular supply, helping to differentiate CPAMs from BPS. For asymptomatic newborns, surgeons will often defer this study until several months of age, because surgical resection is also deferred. However, there is some evidence that early elective surgical correction has similar outcomes as delayed operation, and can be recommended for patients with prenatal diagnosis.[245]

The usual surgical approach is lobectomy for the majority of lesions confined to a single lobe. More diffuse lesions might require bilobectomy or pneumonectomy. These surgical approaches are likely to result in the removal of normal lung but may also decrease the risk of air leak and infection after resection, and compensatory lung growth does occur after lobectomy.[246] Additional postoperative complications may include prolonged pleural effusion, pneumothorax, pneumonia, and wound infection.[247] The need for resection in asymptomatic newborns remains controversial, and there is no consensus on this topic.[248] Those who argue for watchful waiting cite the risks of surgery and anesthesia, the overtreatment of "nondisease," and a lack of evidence of the associated malignancy risk as factors for not resecting asymptomatic lesions. Those who argue for intervention say that resection prevents complications of disease, including the risk of malignancy, the psychological benefit of removing the lesion, and the improvement in lung volume after resection. Canadian Association of Pediatric Surgeons currently recommend elective resection of asymptomatic CPAM lesions as a level 3 evidence. Systemic review from the American Pediatric Surgical Association evidence-based practice committee provided level 4 evidence and grade D recommendations regarding observational strategy for CPAM and optimal timing for CPAM resection.[249] There remains widely varied recommendations for resection and management of asymptomatic lesions.

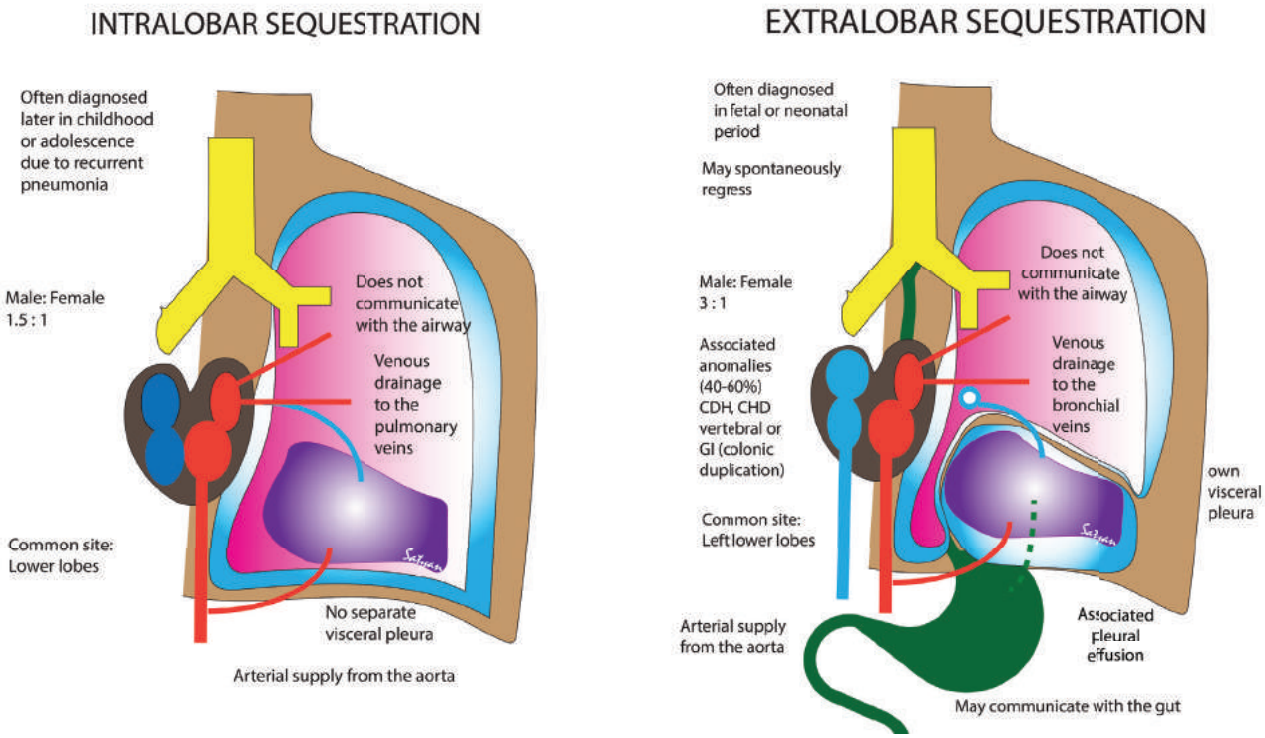
Generally, children who have undergone resection for CPAMs are healthy. Pulmonary function data demonstrate normal total lung capacity, forced vital capacity, forced expiratory volume or maximal work capacity, and no correlation between age at lobectomy and pulmonary function.[250] There are some reports of reactive airway disease and lower respiratory tract infection, and, in more severely affected children, chronic lung disease and pulmonary hypertension.[251] With these possible complications, poor tolerance for respiratory syncytial virus infection is expected in 6 to 12 months following resection; prophylaxis with palivizumab is recommended in first year of life.[252]

Bronchopulmonary Sequestration

BPS is a rare congenital malformation consisting of nonfunctional lung parenchyma that does not have a normal connection to the tracheobronchial tree. The lesions have a systemic arterial supply. Sequestrations are categorized into *extralobar* sequestrations, which are lesions that have their own pleural investment and systemic (80% of the time) venous drainage (and are therefore separate from the lung), and *intralobar* sequestrations, which are integral to the lung pleura and drain via the pulmonary venous system (Fig. 44.11). Intralobar lesions usually require lobectomy for removal because they are invested within the lung. The origin of an intralobar sequestration is somewhat controversial. Some experts believe it is not a congenital lung lesion but is rather always acquired after lung infection and injury, because inflammation, fibrosis, and cystic degeneration are its primary pathologic features.[253] In a large pathologic series, intralobar lesions account for ~75% of BPS and is usually diagnosed prior to 20 years of age with recurrent lower respiratory tract infection.[253] Others believe that intralobar sequestration can be congenital in origin but is relatively rare in that setting when compared to extrapulmonary sequestration.[208,254]

Extralobar sequestrations probably originate as an independent bud from the foregut that derives its blood supply from splanchnic vessels.[255] Usually the connection to the foregut is lost during development, although some lesions have persistent connections to the esophagus or the stomach (also referred to as bronchopulmonary foregut malformations). As accessory lobes, they occur within both the thorax and abdomen. Hybrid lesions with features of both a CPAM and a sequestration can occur.[256] Extralobar sequestrations are situated on the left side in 65% of cases, with the most common location between the lower lobe and the hemidiaphragm in approximately 75% of cases.[257] Extrapulmonary BPS can also occur in the abdomen, the neck, the mediastinum, and the diaphragm itself.[257] It has male predominance, and commonly occurs in association with other anomalies, including foregut duplication cysts, bronchogenic cysts, CPAMs, pericardial defects, and ectopic pancreas. In addition, approximately 5% of children with a CDH will have extralobar sequestration.[258] Histologically, these lesions appear as normal lung, except with dilated airway structures and, commonly, lymphangiectasia. A normal-appearing bronchus is present about 50% of the time. Associated pleural effusions are not uncommon and may be secondary to torsion of the vascular pedicle with resultant venous obstruction and elevated pressure or lymphatic abnormalities.[259]

Fetal diagnosis of intrathoracic BPS is suspected when there is an echogenic mass, which may also contain cysts. Depending on its size, the mass may be associated with some degree of mediastinal shift.[260] It can be difficult to distinguish BPS from a CPAM, but when a systemic arterial supply is identified, the diagnosis of BPS

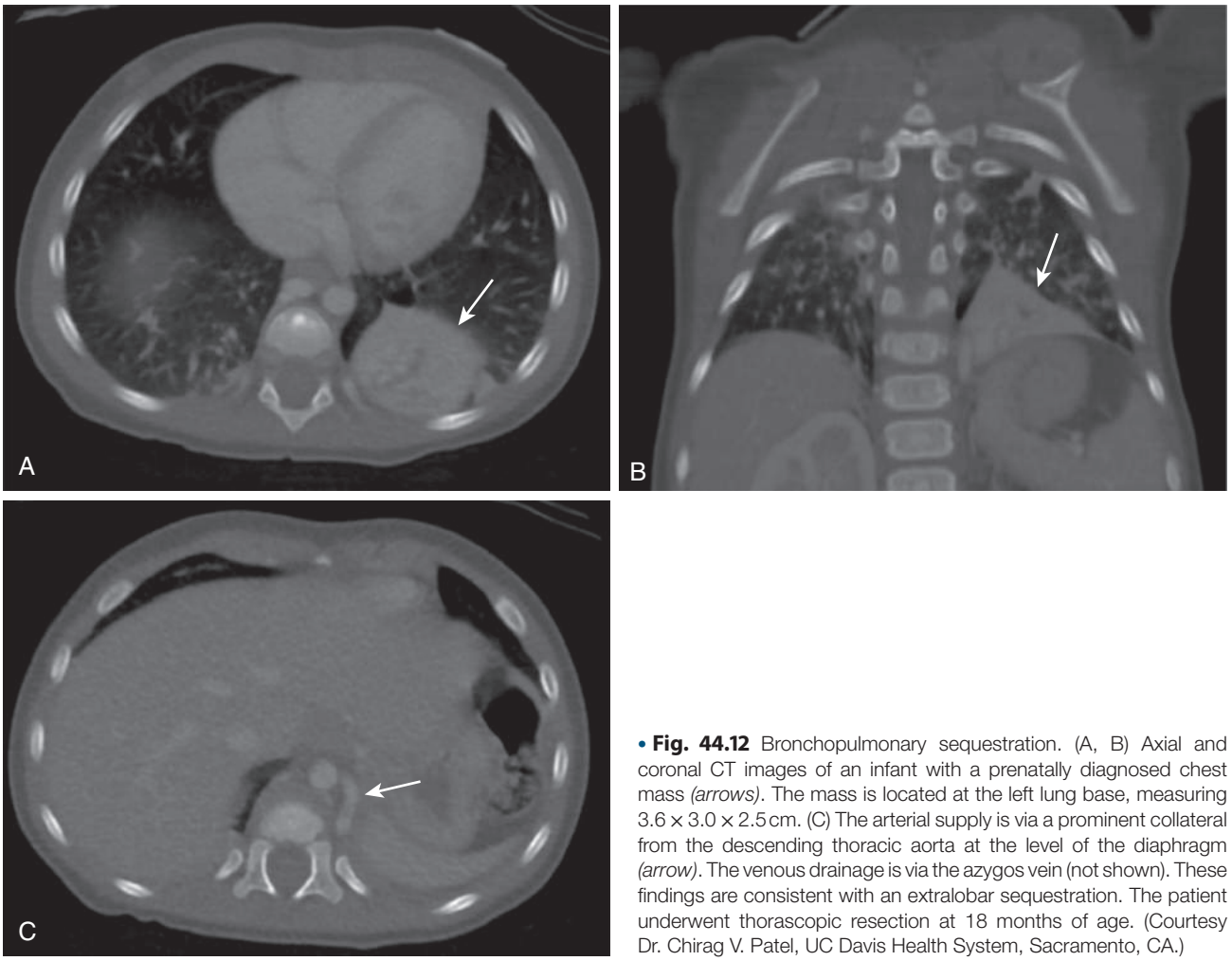


• **Fig. 44.11** Differences between intralobar and extralobar sequestration. Intralobar sequestrations are most common but less often present during neonatal period. Arterial and venous supply and relation with pleural reflection are shown. (Copyright Dr. Satyan Lakshminrusimha, UC Davis Health System, Sacramento, CA.)

is highly likely. If there is also an associated ipsilateral pleural effusion, the diagnosis is almost certainly BPS. If the diagnosis is still in doubt, fetal MRI can be used to help distinguish between the two.[260] Infradiaphragmatic masses also need to be distinguished from neuroblastoma, other tumors (such as lymphangioma), and adrenal hemorrhage.[261] As with CPAM, BPSs often regress spontaneously over time, and if there are no associated anomalies, the prognosis for the fetus is good.[252] Close ultrasound follow-up is prudent, however, until regression is documented. When pleural effusion occurs, there is a risk of development of tension hydrothorax. In these cases, repeated fetal thoracentesis or placement of a thoracoamniotic shunt can avert or resolve hydrops fetalis, which may improve the chances for survival.[236,259,262]

On postnatal imaging, BPS may be present as a radiographic density on plain film. The presence of linear or cystic lucencies within the radiopaque density suggests a persistent communication between an extralobar sequestration and the GI tract.[263] An upper GI study can demonstrate communication with the GI tract and is indicated for surgical planning if feeding difficulties are present. Ultrasound can also be useful in demonstrating the lesion (most easily seen if it is located at the lung base), and Doppler studies can identify a systemic feeding vessel and venous drainage. The use of contrast-enhanced CT provides the best visualization of the parenchymal abnormalities but has variable sensitivity for delineation of the vascular supply, although the lesion itself can often be demonstrated even when it has resolved by fetal imaging (Fig. 44.12A–C). Magnetic resonance with angiography can also be useful in demonstrating the lesion and its blood supply. Conventional angiography can identify the vasculature, but this has been replaced by the imaging modalities discussed above.

Symptomatic BPSs are usually identified in the first 6 months of life, with respiratory distress or feeding difficulties. Less commonly, recurrent infection, congestive heart failure (because of a high output state), or pulmonary hemorrhage is present. Distress at birth can be severe, particularly with large lesions complicated by a pleural effusion or hydrops fetalis. For neonates that present with symptoms in the first week of life, early resection is indicated.[252] Because the lesion is completely separate from lung, sequestrectomy is not a complex operation and can be done thoracoscopically. However, the feeding vessels can be very large in more severe cases and thoracotomy may be considered. The primary risk associated with an unresected BPS is recurrent infection, although this risk is not well quantified, as there are probably adults with persistent, small, asymptomatic lesions. Consequently, as with CPAM, there is controversy as to whether fetal lesions that are not identified on plain film should be further investigated in asymptomatic infants and, in general, whether asymptomatic lesions should be resected. However, since sequestrectomy can now be accomplished thoracoscopically with rapid recovery and short hospitalization, most lesions are surgically resected.[264] In addition, this approach may preserve rib architecture and limit later chest wall deformity.[265] Early resection may limit infectious complications associated with unresected lesions, because secondary changes such as emphysema might be averted. Some have attempted coil embolization of the feeding vessels with hope of complete or partial involution of the lesion.[266] However, coil embolization has also resulted in partial vascular occlusion and incomplete regression, transient lower limb ischemia due to distal migration of embolic material, sepsis, and other blood vessel complications.[267] Because of this, thoracoscopic resection is the current standard of care.



• **Fig. 44.12** Bronchopulmonary sequestration. (A, B) Axial and coronal CT images of an infant with a prenatally diagnosed chest mass *(arrows)*. The mass is located at the left lung base, measuring 3.6 × 3.0 × 2.5 cm. (C) The arterial supply is via a prominent collateral from the descending thoracic aorta at the level of the diaphragm *(arrow)*. The venous drainage is via the azygos vein (not shown). These findings are consistent with an extralobar sequestration. The patient underwent thoracoscopic resection at 18 months of age. (Courtesy Dr. Chirag V. Patel, UC Davis Health System, Sacramento, CA.)

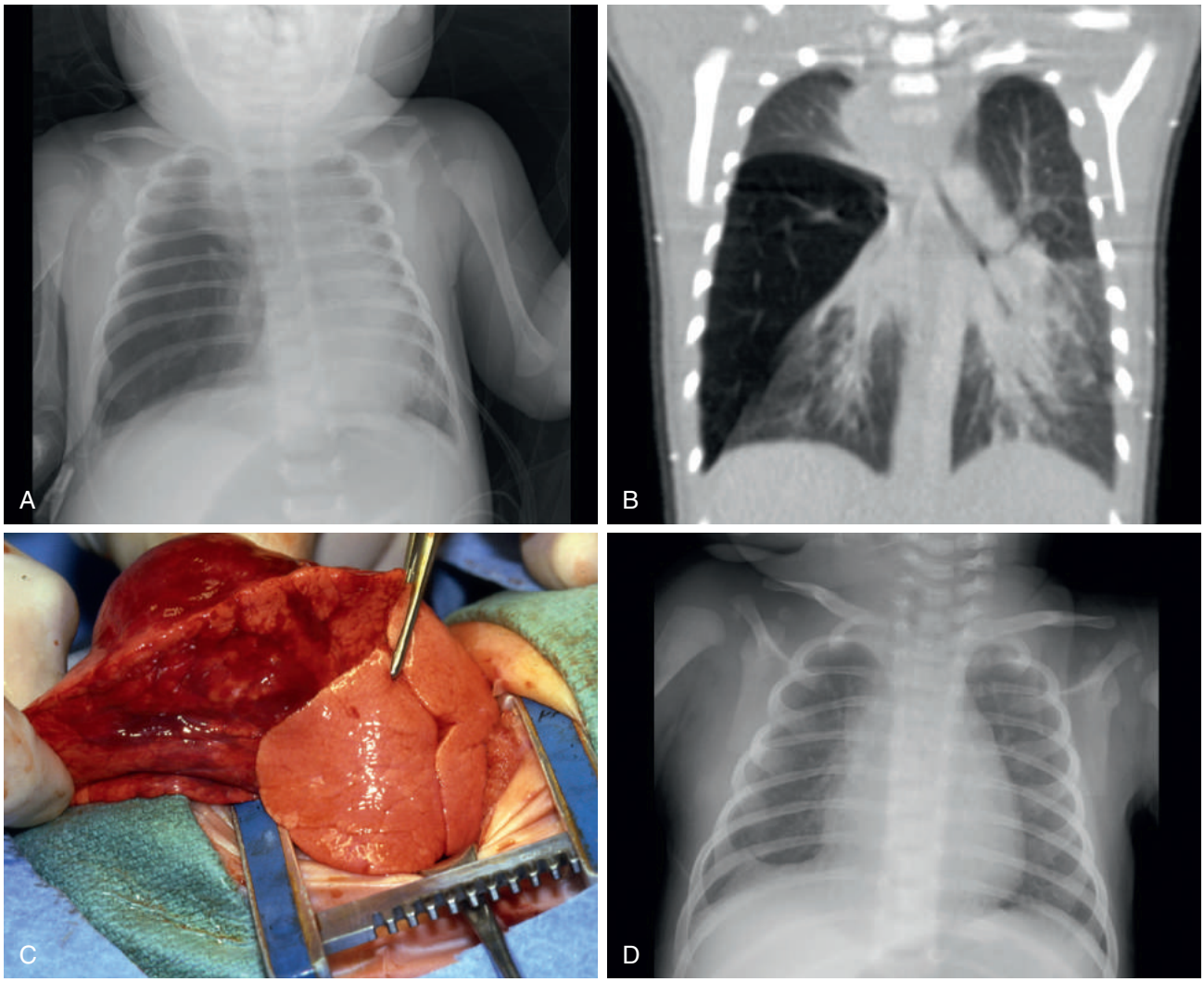
Other Cystic Lesions

Additional cystic lung lesions include CLE, PPB, and acquired cysts (pneumatoceles or emphysema, in association with infection or BPD, respectively). Extrapulmonary cystic lesions include foregut cysts (bronchogenic or enteric duplication cysts) and neuroenteric cysts, which are less common than the other lesions and usually present later in infancy or childhood.[144]

Congenital Lobar Emphysema

CLE occurs in 1 in 20,000 to 30,000 live births.[268] Because there is no evidence of lung destruction (true emphysematous changes), the common term CLE is a misnomer with this lesion, which is also known as *congenital lobar over inflation*.[144] CLE is thought to arise from an obstructed lobar bronchus, which can either be intrinsic (including malacia) or extrinsic in origin. While the cause varies, the fundamental mechanism is that air can pass into the affected bronchus but is unable to leave, causing air trapping and lobar overexpansion. The upper lobes are most commonly affected, with the left upper lobe the single most commonly affected lobe.[268] Lesions occupying multiple lobes are infrequent. Although the affected lobe is larger than usual, the number of alveoli in the involved area is within normal limits. The exception to this is the subset of these lesions with *polyalveolar lobe*, which was present in 27% of CLE in one series and has an overlapping clinical presentation with CLE.[268] In polyalveolosis, the total number of alveoli is increased severalfold from normal, but the conducting airways are normal in size and number. This form of lung hyperplasia is consistent with pathophysiology associated with fetal airway obstruction.[144]

CLE can be detected on fetal ultrasound, although it is difficult to make the correct diagnosis. Clinical reports have described the appearance of the lesion by ultrasound as cystic and/or echogenic, with mediastinal shift present, and subsequent regression with advancing gestation.[268] As expected, these lesions are suspected to be CPAM or BPS, based on these findings. Fetal MRI can be helpful in characterizing the lesion, although it is not diagnostic.[269] The postnatal clinical presentation and histology (after resection) distinguish CLE from the other lesions. The majority of children with CLE present with respiratory distress, cyanosis, or recurrent pulmonary infections in the first 6 months of life, up to 82% in one series.[270] The severity of symptoms depends on the size of the affected lobe, the compression of the surrounding tissue, and the extent of mediastinal shift. Chest radiographs demonstrate a hyperinflated lung (transitioning from fluid filled to air filled over the initial postnatal days), with compression of other areas of the lung and mediastinal shift (Fig. 44.13A). These findings are generally diagnostic. In children with less severe presentation, bronchoscopy or CT scan can be helpful in management decisions, because some surgeons will elect to manage



• **Fig. 44.13** Congenital lobar emphysema. (A) Chest radiograph showing significant hyperinflation of the right lung with mediastinal shift to the left. (B) Coronal chest CT image demonstrating hyperaeration of the right middle lobe consistent with congenital right-sided lobar emphysema. (C) Intraoperative photo showing the right middle lobe characteristically "popping out" of the thoracotomy incision prior to resection. (D) Chest radiograph 3 days after right middle lobe resection showing normalization of lung volume on the right with resolution of mediastinal shift. (A, B, and D, courtesy Dr. Chirag V. Patel, UC Davis Health System, Sacramento, CA; C, courtesy Dr. Clifford C. Marr, Sutter Medical Center, Sacramento, CA.)

these patients expectantly, with resolution of symptoms in some cases (Fig. 44.13B).[269] Thoracoscopic lobectomy is the mainstay of treatment.[271] After resection, prognosis is generally good, with compensatory lung growth present on the affected side (Fig. 44.13C and D).[272] Airway obstruction continues to be a feature of the disease on pulmonary function tests, although these findings could be consistent with either compensatory lung growth exceeding airway growth (dysanapsis) or intrinsic, diffuse airway abnormality.

Pleuropulmonary Blastoma

PPB is a rare but malignant lesion arising from the lung or the pleura. Lesions can be predominantly cystic (type I), mixed cystic and solid (type II), or predominantly solid type (III) and can occur in association with other congenital lung lesions. The average age of diagnosis of type I, II, and III PPBs is 8 months, 35 months, and 41 months, respectively, and overall survival of type I PPBs is 91%, while survival in type II and IIII patients is 71% and 53%, respectively.[273] The diagnosis and resection of this lesion are essential, because of the risk of metastasis, recurrence, and associated malignancies.[274] Although these lesions tend to present later in childhood than CPAMs, there is overlap in the timing of presentation, and PPB can be detected on fetal ultrasound; thus the consideration of this diagnosis in the perinatal period is relevant for counseling and surveillance, even if a newborn is not symptomatic.[274]

Symptomatic infants usually present with respiratory distress. Before resection, most lesions are thought to be CPAMs, based on their appearance on fetal ultrasound or postnatal CT scan. The treatment of choice for PPBs is surgical resection. Many studies have failed to show statistical benefit for chemotherapy in patients with type I lesion.[273,275] Type II and type III lesions should undergo both systemic chemotherapy and surgical resection, with unclear benefit for radiation therapy.[276,277] Resected lesions contain

cuboidal or columnar epithelial cells with underlying rhabdomyosarcoma cells (or other sarcomas). The malignant cells may not be widespread in the lesion, making the diagnosis of PPB challenging even by histology.[278] Because of the difficulty in distinguishing CPAMs from PPBs, careful histologic evaluation of prophylactically resected CPAMs is recommended, as those with stellate and spindle cells should be followed closely.[278] Furthermore, PPBs may require a more extensive resection than CPAMs, so this distinction is important in determining surgical approach as well.[213] PPB can be associated with a DICER1 gene mutation in 50% to 75% of instances, so genetic testing is necessary; DICER1 mutations can also be associated with cystic nephroma, ovarian tumors, and thyroid pathology.[276,277] In addition to DICER1, kindreds also demonstrated multiple malignancies, suggesting that familial surveillance for disease might be indicated.[276,277]

Postinfectious Pneumatoceles

Pneumatoceles are thin-walled, air-containing cystic structures resulting from alveolar and bronchiolar necrosis. In our experience, the most common infection associated with pneumatoceles in the newborn (maybe because of its higher frequency of infection) is *Staphylococcus aureus* pneumonia.[279] Other infections seen in the NICU that are associated with development of pneumatocele include pneumonia caused by *Klebsiella pneumoniae, Streptococcus pneumoniae, and Enterobacter*.[280] Pneumatoceles are often present on initial chest radiograph documenting the infiltrate, although they can also occur later in the process (Fig. 44.14). Given the thin wall, pneumatoceles can rupture, resulting in a pneumothorax that may be under tension or compress the lung tissue through mass effect, resulting in worsening respiratory status.[279,280] Acutely, urgent interventions may be needed to improve ventilation and oxygenation in the setting of rupture. Placement of chest drains into the pneumatoceles in the absence of rupture, either percutaneously or under direct visualization via VATS, can be considered; however, the majority of these cysts are regressive and resolve spontaneously.[279,280] For patients with chronic ventilator dependence, in particular former premature infants with BPD, resection of the affected lobe could be considered.

Hyperinflation and Emphysema in Chronic Lung Disease

Similar to postinfectious pneumatoceles, frank cysts or hyperinflated lung may develop in association with chronic lung disease of prematurity (like BPD) or with certain developmental lung abnormalities. This cystic hyperinflation can cause compression of more functional areas of the lung, with consequent respiratory compromise.[281] While this very severe chronic lung disease complication of prematurity rarely occurs today, affected infants can acutely decompensate or remain ventilator dependent despite maximal medical therapy. Evaluation of six infants with BPD and decompensated lobar hyperinflation in one series revealed extensive lobar bronchomalacia, with almost complete collapse of the affected airway through the expiratory phase or the entire respiratory cycle.[282] Lobectomy results in acute improvement, although ultimately only half of infants who are so treated survived.

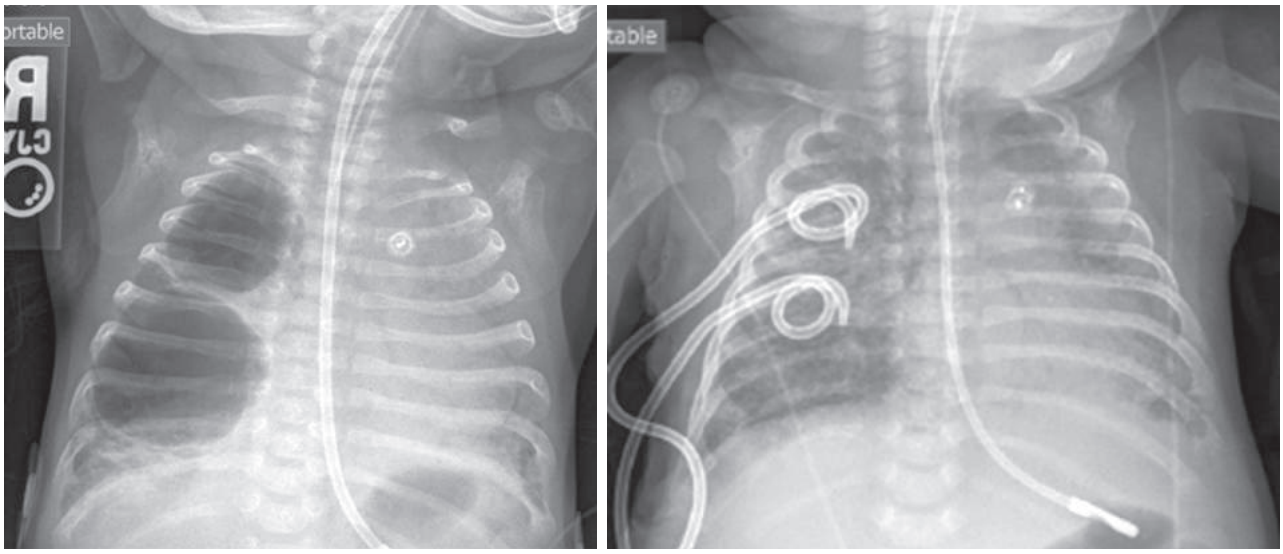
Miscellaneous Cysts

Lymphatic, lymphangiomatous, mesothelial, and parenchymal cysts can be detected in the thorax, so these lesions may need to be included in the differential diagnosis of cystic lesions.[144]

Disorders of the Diaphragm

Congenital Diaphragmatic Hernia

CDH is a disorder of pulmonary alveolar and vascular hypoplasia that results from failure of formation of the diaphragm.[283] This may be due to primary deficiency of the embryonic pleuroperitoneal fold, which is one of the critical primordial diaphragmatic structures that must fuse at 6 to 8 weeks' gestation to form an intact diaphragm.[284] Failure of this event results in herniation of abdominal contents into the hemithorax, and the subsequent

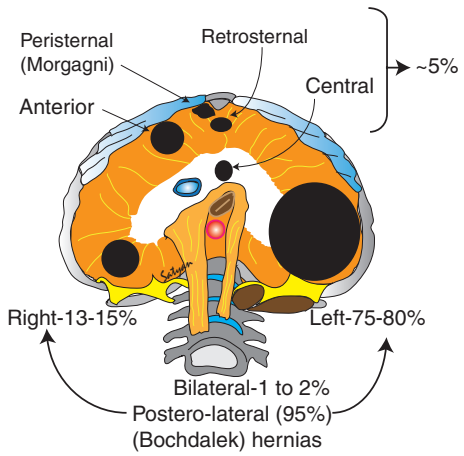


• **Fig. 44.14** Postinfectious pneumatoceles. Chest radiographs from a former preterm infant with pneumatoceles secondary to *Staphylococcus aureus* pneumonia. Cysts are compressing the lung and causing mediastinal shift *(left)*. Placement of draining thoracostomy tubes decompressed the cysts and decreased mediastinal shift, but the infant ultimately succumbed to respiratory failure secondary to bronchopulmonary dysplasia. (Copyright Dr. Satyan Lakshminrusimha, UC Davis Health System, Sacramento, CA.)

arrest of preacinar airway branching at 10 to 14 weeks' gestation is consistent with this early developmental defect. Pulmonary hypoplasia is bilateral, although the lung ipsilateral to the hernia is most affected. Airway diameter is substantially decreased, but increase in airway muscle occurs as a later postnatal event.[285] Although acinar alveolar counts are normal, overall alveolar hypoplasia is present due to the branching defect (<10 million vs. 150 million alveoli in normal term births).[286]

Because of the interdependence of lung and vascular growth, both alveolar and capillary surface areas are decreased.[287] Vascular branching is impaired, with a decreased diameter of the vessels and increased muscle mass that is inversely related to the degree of lung hypoplasia.[287] Some morphometric reports have demonstrated abnormal distal extension of the muscular media to the intra-acinar arteries, whereas others have not demonstrated abnormal distal muscularization.[287,288] The mechanism of developmental lung and vascular hypoplasia is unknown but may include decreased static transthoracic pressure (secondary to open communication with the peritoneal cavity) and decreased phasic pressure alterations (secondary to impaired fetal breathing movements). Compensatory alveolar growth does occur in survivors, although it is more pronounced in the contralateral lung, and relative perfusion to the ipsilateral lung can be persistently diminished.[289,290] These findings are consistent with evidence of greater injury present in the more hypoplastic and vulnerable ipsilateral lung, compared with the contralateral lung in survivors of CDH, with consequent impairment of lung growth.[289]

Ninety-five percent of CDHs are posterolateral (Bochdalek) hernias.[284] The remaining 5% are either anterior, retrosternal, or peristernal (Morgagni). CDH is more common on the left side (75% to 80% of cases) than the right, probably because of slightly later fusion of the left-sided structures. Bilateral hernias account for 1% to 2% of cases (Fig. 44.15). Morgagni hernias are much less frequent in occurrence and usually are not associated with substantial lung hypoplasia, although they may be associated with pericardial, sternal, and abdominal wall defects as part of the pentalogy of Cantrell spectrum. There is a predominance of males to females in CDH (1.4 to 1.6:1 ratio), and the occurrence of CDH (including stillbirths) is about 1 in 4000 births.[284,291] Additional



• **Fig. 44.15** Location and incidence of diaphragmatic defects associated with congenital diaphragmatic hernias (CDH). (Modified from Chandrasekharan PK, Rawat M, Madappa R, et al. Congenital diaphragmatic hernia —a review. *Matern Health Neonatol Perinatol*. 2017;3:6; copyright Dr. Satyan Lakshminrusimha, UC Davis Health System, Sacramento, CA.)

anomalies occur in about 40% of affected infants and fetuses.[283,291] Musculoskeletal (including ribs, vertebrae, and digits) and cardiac anomalies are most common, although the patterns of malformation differ in association with right-sided CDH compared to left-sided CDH.[292] CDH can be associated with aneuploidy (most frequently trisomy 18), and it can present in autosomal recessive (e.g., Fryns syndrome), sex-linked (e.g., Simpson–Golabi–Behmel syndrome), and autosomal dominant (e.g., Cornelia de Lange syndrome) disorders.[292] With the exception of these disorders, recurrence rate is quoted at 1% to 2%, and more recent genetic studies have identified micro-deletions in affected infants through use of microarray technology.[284] It is unclear whether or not infants with isolated CDH (no other anomalies found by prenatal and postnatal investigation) are at increased risk for these minor chromosomal aberrations, because large-scale investigations have taken place with very limited positive findings. Single gene mutations have been identified in animal models and some humans as causal in CDH, and certain areas of the genome may be critical regions, wherein other causal genes might be found.[284,293]

In recent population-based studies, overall survival among liveborn affected infants ranged from 57% to 73%.[294,295] This is slightly improved from a 52% to 61% survival rate just a decade ago.[291,294] Those patients with left-sided diaphragmatic hernias fare better than those with right-sided hernias, with survival rates being 73% to 69%, respectively; however, this difference is not statistically significant. While the survival rate is similar, those patients with right-sided CDH have higher rates of pulmonary morbidity, including an increased need for tracheostomy, long-term vasodilatory therapy, and greater likelihood of requiring supplemental oxygen at the time of hospital discharge.[295] Survival of liveborn infants with isolated (no additional anomalies) CDH has been higher (63% to 77%) than survival in liveborn infants with other anomalies or chromosomal aberrations (19% to 43%).[291,296] Survival is low (<10%) in affected infants with chromosomal abnormalities, and there is a risk of intrauterine fetal demise in both isolated (2%) and non-isolated (11%) CDH. Although individual referral centers have reported survival rates ranging from 75% to 93%, there have been studies documenting a hidden mortality, demonstrating that a proportion of liveborn neonates die within hours of birth and before arrival at a surgical center.[297,298] In a study from Ontario, Canada, survival decreased from 67% to 58% for multicenter compared to population-based data, after accounting for infants who never reached a referral center.[298]

Both population-based and center-based studies have identified some risk factors for mortality for liveborn infants undergoing full resuscitative measures and ongoing neonatal care. All of these are probably related to the severity of the CDH, with respect to the degree of lung and vascular hypoplasia. For instance, prenatal diagnosis has been associated with increased mortality and may be due to the identification of more severe CDH (because mediastinal shift will be more pronounced and present earlier in gestation; see later discussion).[283,291,296] A multicenter, retrospective Children's Hospital Neonatal Database analysis of 677 children with CDH identified six variables independently associated with mortality and hospital length of stay greater than 109 days: infants that were small for gestational age, those with major birth anomalies, 5-minute Apgar scores less than or equal to 3, acidosis at the time of referral, those requiring ECMO, and those with bacteremia.[299] Other postnatal factors that are associated with increased mortality include prematurity (<37 weeks) and air leak, conditions associated with immature lung development, and/or increased risk for lung injury.[300]

Interestingly, two studies have shown somewhat conflicting data regarding the timing of term delivery and survival. For infants receiving ECMO support, late-term (40 to 41 weeks' gestation) infants had somewhat better survival compared with early-term (38 to 39 weeks' gestation) infants, although the relationship was not statistically significant, and fewer ECMO-related complications were seen in the late-term group.[301] However, overall survival was slightly better for early-term (37 to 38 weeks' gestation) versus late-term (39 to 41 weeks' gestation) infants in another study that included infants not receiving ECMO support.[301] However, among 928 infants born following spontaneous vaginal delivery in the US with isolated CDH, mortality was lowest at 40 weeks gestation.[302] Based on these results, we recommend delivery as close to the due date as possible in infants with CDH.

Other data show that infants not born at a tertiary center but subsequently transferred to a tertiary center have higher survival rates than those that are born at a tertiary center, which may reflect the hidden mortality associated with more severe, prenatally diagnosed CDH.[303,304] For those infants who do undergo surgical repair, the need for a prosthetic patch is associated with subsequent mortality, indicating a larger diaphragmatic defect and probably more severe lung hypoplasia.[283]

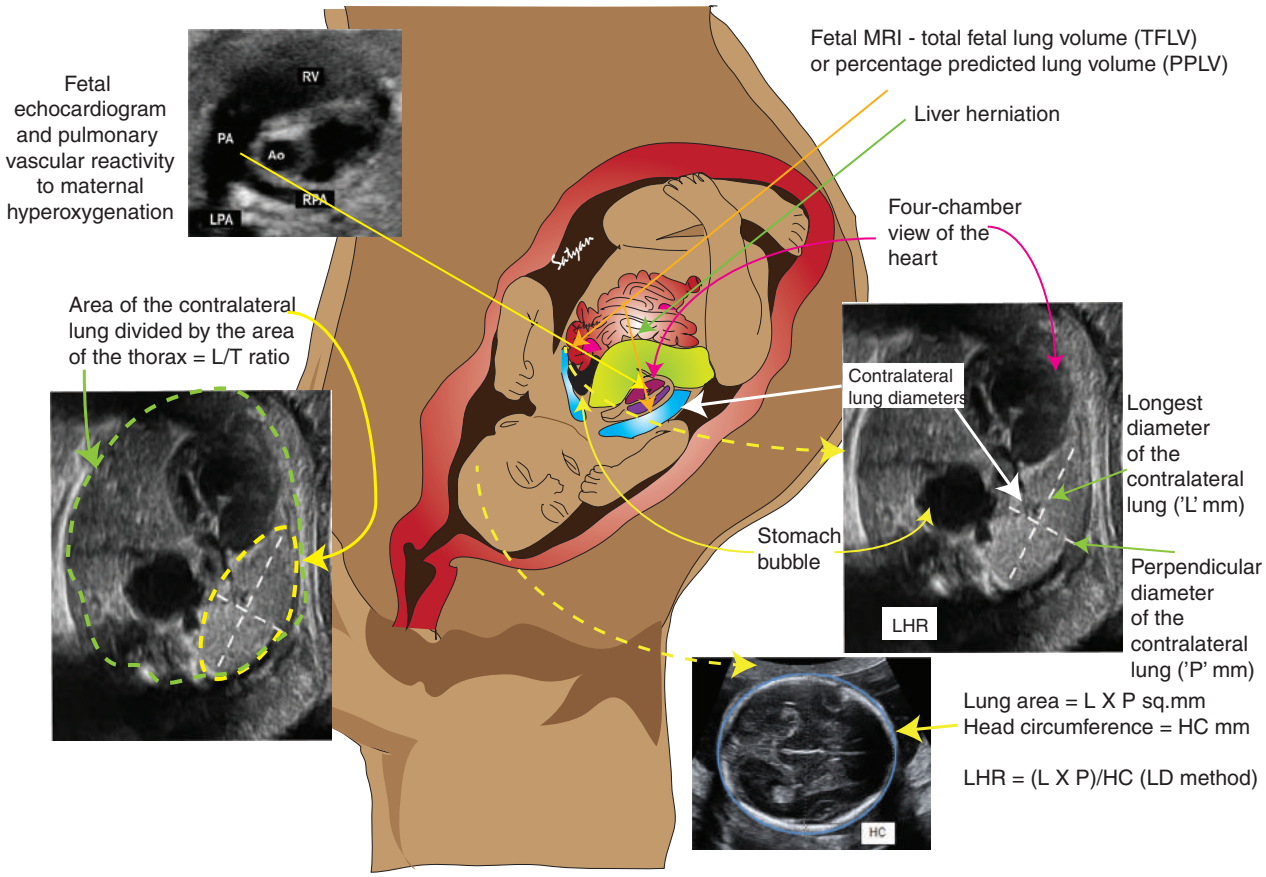
Prenatal Diagnosis and Management

CDH is usually suspected on prenatal ultrasound when mediastinal shift away from the side of the hernia is appreciated. Prenatal detection rates are higher with left-sided than right-sided hernias, and bilateral hernias can be difficult to discern because of the distorted anatomy. CDH in fetuses with additional anomalies are also detected at a higher rate than CDH in isolated cases.[291] Fetal MRI can be helpful in determining the diagnosis, if the anatomy is difficult to identify. A prenatal diagnosis of CDH mandates careful evaluation for other anomalies, including a fetal echocardiogram, because of the high rate of additional anomalies and their association with lower survival rates. Some of this evaluation may be limited by the anatomic distortion caused by the hernia. A karyotype and other genetic analysis (as indicated) are also recommended. Additional prognostic information regarding the severity of the hernia can be gathered during prenatal evaluation by ultrasound and MRI, although the prognostic ability of these measures is likely center dependent, as survival varies to some extent across centers. One useful discriminator of CDH severity is herniation of the liver into the hemithorax.[283,300] Liver herniation can occur with both right-sided and left-sided CDH. In left CDH, the left lobe of the liver herniates in the thorax; thus, the course of the hepatic vasculature is distorted and indicative of liver herniation. Herniation of the liver is also predictive, with one study showing a significantly higher survival rate in fetuses without liver herniation (74%) versus those with herniation (45%).[305] Further research looking at the predictive nature of prenatal ultrasound in the assessment of liver herniation has demonstrated that sonographic measurements (liver-to-thorax ratio and stomach position) are predictive of survival outcomes in those with isolated left-sided CDH.[306] The prenatal liver-to-thorax ratio can also be predictive of the need for ECMO.

Other prenatal discriminators of severity that have been used include stomach herniation and polyhydramnios (a later finding, secondary to gastrointestinal obstruction or esophageal dysmotility). Several measures have been developed as intrauterine estimate of lung size (Fig. 44.16) The lung-to-head ratio (LHR) is widely used for this purpose.[307] It is the perpendicular area of the lung contralateral to the hernia at the level of the cardiac atria divided by the biparietal diameter. It has been used predominantly in mid-gestation (22 to 27 weeks' gestation), with an LHR $\leq$ 1.0 combined with liver herniation accepted as the most severe group of CDH. LHR has been studied in left CDH, although it has also been extrapolated to right CDH and probably has prognostic ability; LHR thresholds for severity could be lower in right CDH, since the normal right lung is larger than the left lung.[308] Investigators have developed nomograms for normal LHR over 12 to 32 weeks, which has led to an observed-to-expected (O/E) LHR measurement, employing the mean LHR at any gestational age as the "expected LHR," which increases with advancing gestational age because of a more rapid increase in lung area compared with head circumference.[309] It follows that the LHR $\leq$ 1.0 will represent a higher O/E LHR earlier in gestation than it does later in gestation, and the O/E LHR will be higher with right CDH than left CDH for a given LHR and gestational age.[310] In a large database of 354 fetuses with isolated CDH (including 25 fetuses with right CDH), the O/E LHR was predictive of survival, independent of liver herniation.[311] Subsequent analyses of this data have stratified fetuses with and without liver herniation, because survival does differ between these groups for a given O/E LHR range.[309] In these analyses, survival was less than 20% for fetuses with isolated left CDH and O/E LHR less than or equal to 25%. Fetuses with a higher O/E LHR of 26% to 35% survived in greater numbers: 30% if liver was herniated and almost 60% if liver was not herniated. The lower bound of the 95% confidence interval for O/E LHR in unaffected fetuses is 60%.[311] Decreasing O/E LHR was also related to increasing time on assisted ventilation, prolonged hospitalization, and an increased risk for prosthetic patch repair.[310] In the recently concluded trial of fetal endoluminal tracheal occlusion (FETO), stratification was performed using O/E LHR and these measurements correlated well with survival to discharge, 6-month survival, and 6-month survival without oxygen supplementation.[312]

Fetal MRI techniques have also been pursued for prognostic information in CDH. Liver herniation can be determined by fetal MRI, and at some centers it is the preferred technique for this determination. A number of different nomograms to determine lung volume as a percent of normal (based on estimated fetal size or gestational age) have been developed. Büsing et al. evaluated seven published nomograms for estimation of relative fetal lung volume in 68 fetuses with isolated left CDH evaluated at their center.[313] They generated receiver operating characteristic curves for each of the seven equations and found high (0.800 to 0.900) area under the curve (AUC) for prediction of survival, regardless of technique used. In this dataset, prediction of need for ECMO was not as strong (AUC 0.653 to 0.739), although individual centers have found that relative fetal lung volume is a very useful predictor of the need for subsequent ECMO support.[314]

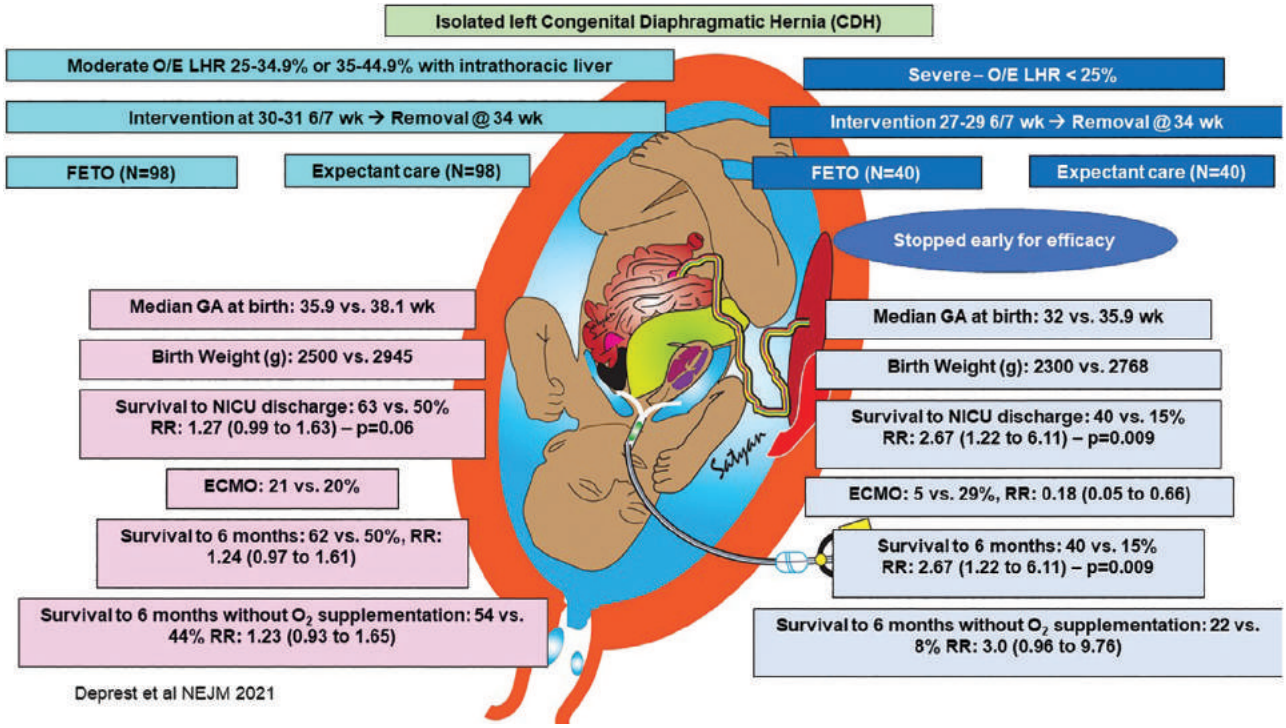
Early attempts at fetal intervention for CDH focused on an anatomic repair of the diaphragmatic defect; however, this approach was quickly abandoned after survival outcomes did not improve.[315] Fetal intervention has since shifted its focus to enhancing intrauterine lung growth via endoscopic temporary balloon tracheal occlusion. Only fetuses likely to be the most severely affected newborns are candidates if additional anomalies are not found after careful investigation. A randomized controlled trial evaluating this technique was terminated prematurely because of unexpectedly high survival in the control group, with no difference in survival between tracheal occlusion and standard care infants.[315] The tracheal occlusion procedure was complicated



• **Fig. 44.16** Antenatal diagnosis and assessment of lung volume of congenital diaphragmatic hernia (CDH). Lung volume can be assessed by ultrasound by estimating lung to thoracic (L/T) ratio and lung area-to-head circumference ratio (LHR) and observed to expected LHR (O/E LHR). Fetal MRI can be used to estimate total fetal lung volume (FTLV) or percentage predicted lung volume (PPLV). (Modified from Chandrasekharan PK, Rawat M, Madappa R, et al. Congenital diaphragmatic hernia —a review. *Matern Health Neonatol Perinatol*. 2017;3:6; copyright Dr. Satyan Lakshminrusimha, UC Davis Health System, Sacramento, CA.)

by premature delivery (mean gestational age 30.8 weeks), often stemming from premature rupture of the membranes, and this may have compromised survival in the intervention group. With development of smaller instruments, this technique can now be accomplished as a fully endoscopic procedure, and the incorporation of a second procedure to remove the tracheal balloon (i.e., plug–unplug) has also decreased the need for delivery by EXIT procedure, allowing for vaginal delivery in the majority of cases.[315] The European FETO task group has been performing minimally invasive tracheal occlusion in fetuses with LHR less than or equal to 1.0 with promising results demonstrating an improved survival to discharge and a later mean gestational age at the time of delivery (33.5 weeks).[316] This was attributed to the less invasive nature of the procedure. With operator experience, the procedure has been accomplished more rapidly, and shorter procedures were associated with lower rates of premature membrane rupture. Elective prenatal removal of the intratracheal balloon (i.e., unplug) was accomplished at a median of 34 weeks in 70% of the cases. Survival to neonatal discharge was 47% (98 of 210); 6 deaths were fetal and 10 deaths occurred secondary to difficulty with removal of the intratracheal balloon. From their historical experience, the investigators cite an expected survival of only 24%. In a subset of these fetuses, and some expectantly managed with CDH, Cannie et al. evaluated changes in relative lung volume over gestation by fetal MRI.[317] They found that relative lung volume was largely unchanged in fetuses without intervention, and it tended to increase more consistently in fetuses with CDH when tracheal occlusion was undertaken at greater than or equal to 29 weeks' gestation ($n = 8$). Most recently, a small randomized controlled trial comparing FETO to standard postnatal management found that 50% of fetuses treated with tracheal occlusion survived to 6 months of life compared with 4.8% in the postnatal treatment group.[318]

Two recent trials published in 2021 by Deprest et al. evaluated infants with isolated left CDH with moderate (O/E LHR of 25% to 34.9% or 35% to 44.9% with intrathoracic liver)[319] or severe lung hypoplasia (O/E LHR < 25%) (Fig. 44.17).[312] Infants with moderate hypoplasia underwent FETO intervention at 30 to 31 6/7 weeks' gestation followed by removal of the balloon at 34 weeks. Gestational age at birth was lower with FETO and survival to NICU discharge and 6-month survival were not statistically significant. The FETO intervention in left CDH with severe pulmonary hypoplasia was performed at 27 to 29 6/7 weeks' gestation followed by balloon removal at 34 weeks. 40% of intervention cases delivered prior to 34 weeks' gestation (vs. 0% in control arm). However, there was a significant increase in survival to NICU discharge, 6-month survival, and 6-month survival without supplemental oxygen.



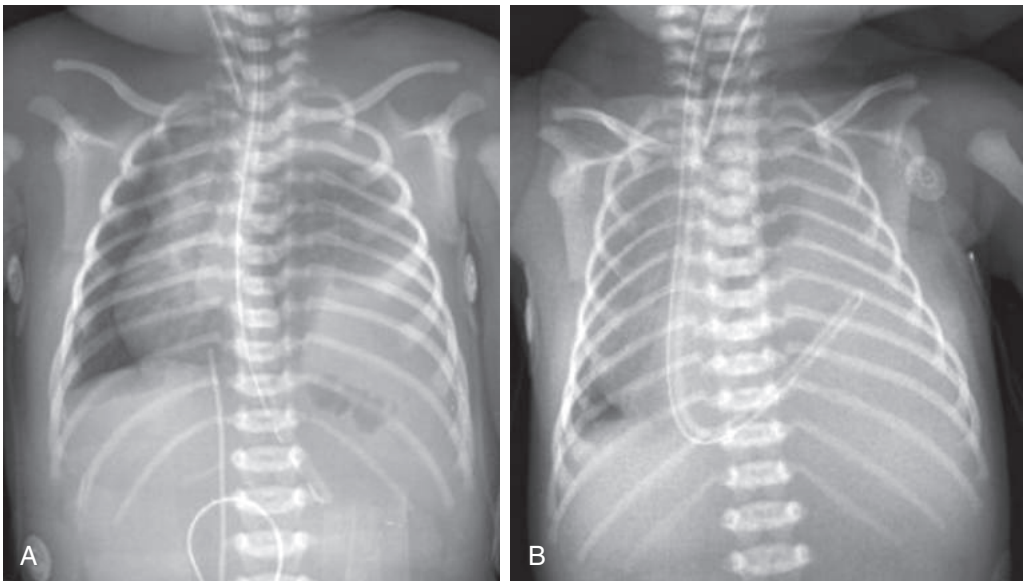
• **Fig. 44.17** Graphic abstract of two trials evaluating fetal endoscopic tracheal occlusion (FETO) therapy by Deprest et al. *NEJM* 2021. Moderate pulmonary hypoplasia is shown on the left side and severe hypoplasia on the right side. *O/E LHR*, observed to expected lung area-to-head circumference ratio; *GA*, gestational age; *ECMO*, extracorporeal membrane oxygenation; *RR*, relative risk. (Copyright Dr. Satyan Lakshminrusimha, UC Davis Health System, Sacramento, CA.)

Postnatal Diagnosis and Management

Most newborns with CDH will present immediately or within several hours of birth with respiratory distress, cyanosis, decreased breath sounds on the hernia side, and a scaphoid abdomen. An occasional infant will not have symptoms until several days or months of age and often will have feeding intolerance and mild respiratory distress.[300] An initial chest radiograph will show a smaller lung on the hernia side, with bowel gas in the chest and shift of mediastinal structures (Fig. 44.18A,B). The findings of a small lung, with no mediastinal shift and usually without concern for herniated bowel, should raise suspicion for other diagnoses, which may require an alternate surgical approach or may not require surgery at all (see later discussion).

Delivery Room Management: Newborns with a fetal diagnosis of CDH require a resuscitation team capable of intubation and low umbilical venous line placement along with a respiratory therapist at delivery (Fig. 44.19). A discussion with the obstetric team regarding optimal cord management is important prior to delivery. Delayed cord clamping is feasible in CDH and has been associated with some physiological benefits in animal models.[320,321] However, clinical trials are needed before this is implemented in wide practice. Mask ventilation should be avoided to prevent additional air entering the stomach. Most infants with CDH and respiratory distress need endotracheal intubation and mechanical ventilation, and a Replogle (Covidien—Medtronic, Minneapolis MN) orogastric tube for continuous gastric suction to minimize accumulation of thoracic intraintestinal air and reduce lung compression. The optimal initial oxygen concentration for resuscitation of an infant with CDH in the delivery room is not known. However, routine use of 100% oxygen is probably not needed and use of ~21 to 50% oxygen[322] and titrating to target preductal oxygen saturation as recommended by the neonatal resuscitation recommendations may be adequate.[323]

Hypoplastic lungs with small alveoli have poor compliance, and thus ventilation is severely reduced.[324] Combined with the restrictive pulmonary vascular bed and consequent pulmonary hypertension, this leads to severely impaired oxygenation. Because these physiologic challenges cannot be overcome without lung growth, most high-volume centers use a gentle ventilation strategy. This strategy attempts to achieve adequate oxygen delivery and preserve the potential for lung growth while minimizing oxygen toxicity and ventilator-induced lung injury (barotrauma and volutrauma). Actual targets for ventilation and oxygenation vary somewhat, but consistency in care within a center is important.[325] The ventilation target is often an arterial partial pressure of carbon dioxide ($PaCO_2$) of 50 to 65 mmHg (permissive hypercapnia). For oxygenation, a more liberal strategy allows for a preductal (right upper extremity) and post-ductal (descending aorta or lower extremity) arterial saturation (SaO_2) differential, with oxygenation targets based on preductal oxygen saturation ($SpO_{2)}$ (permissive oxygenation). Oxygenation targets are generally preductal SpO_2 between 85% and 95% for infants on supplemental oxygen. Ventilator pressures are limited, with either positive inflation pressure (PIP) targeted at less than or equal to 25 to 28 cmH_2O or mean airway pressure on high-frequency ventilation at less than or equal to 15 cmH_2O. Aggressive weaning strategies are used to achieve gentle ventilation goals and to minimize lung injury. Lung recruitment with surfactant and high PEEP or mean airway pressure are not effective strategies to achieve persistent improvements

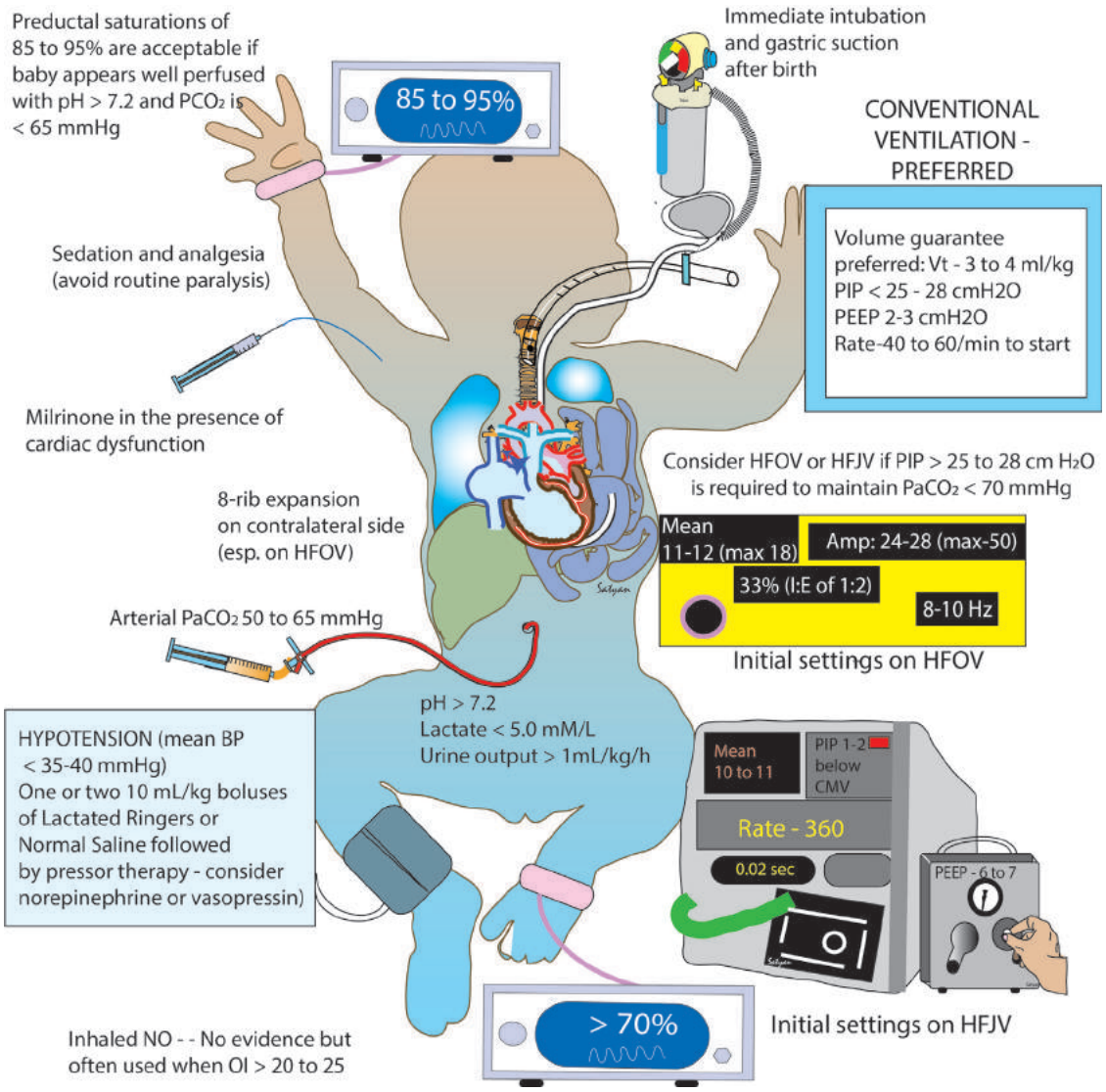


• **Fig. 44.18** Left congenital diaphragmatic hernia. (A) Radiograph of an infant with congenital diaphragmatic hernia (CDH) without liver or stomach herniated into thorax. He subsequently underwent primary repair of the diaphragmatic defect, was extubated within several days of surgery, and was discharged to home without supplemental oxygen. (B) Radiograph of an infant with severe CDH with liver and stomach herniated into thorax. He subsequently required extracorporeal membrane oxygenation (ECMO) support, underwent prosthetic patch repair for diaphragmatic aplasia, and succumbed to respiratory failure and pulmonary hypertension after decannulation from ECMO support. (Copyright Dr. Satyan Lakshminrusimha, UC Davis Health System, Sacramento, CA.)

in oxygenation in CDH, and increases in PIP to achieve transient improvements in oxygenation lead to further increases in ventilator support, with worsening compliance because of lung injury and edema.[325] There are also some centers that advocate low PEEP and high ventilator rates (which are physiologic in lung hypoplasia). With high ventilator rates, gas trapping and auto-PEEP may be an important factor, so limitation of the PEEP set on the ventilator is important.[304] In addition, low PEEP (2 to 3 cmH_2O) is associated with higher lung compliance in infants with CDH than PEEP of 4 to 6 cmH_2O.[326] Routine use of high-frequency oscillatory ventilation (HFOV) with relatively high mean airway pressure for infants with CDH did not result in any improvement in outcomes compared to conventional ventilation in a randomized controlled trial.[327] However, to this day there are no trials evaluating low mean airway pressure strategy on HFOV or other modalities of high-frequency ventilation such as jet ventilation.

Employment of gentle ventilation strategies has been associated with improved survival at individual centers;[325] however, regardless of actual targets for ventilation and oxygenation and preferential mode of ventilation, it is likely that other aspects of care, such as infant stimulation and positioning and sedation and feeding practices, affect survival and other outcomes. Although there are a number of ancillary treatments employed in infants with CDH (prenatal glucocorticoids, surfactant replacement therapy, inhaled nitric oxide, and other pulmonary vasodilator therapies), there are no studies documenting broad efficacy of these treatments in term infants with CDH. In fact, in one randomized trial, the use of inhaled nitric oxide was associated with higher ECMO use compared to placebo in CDH.[328] In addition, the use of prostaglandin infusion to maintain ductal patency and improve right heart function in cases of prolonged pulmonary hypertension has been advocated.[325] Two randomized trials to evaluate milrinone/placebo and inhaled nitric oxide/sildenafil in CDH are currently recruiting subjects.[329,330] The one ancillary treatment that has shown benefit is the use of ECMO, which improved survival to hospital discharge in the United Kingdom Collaborative study, although the effect dissipated at follow-up with later deaths among initial survivors.[331]

ECMO can be used to rescue newborns with CDH, particularly those with lung injury evident by pneumothorax, and it may be used in the setting of gentle ventilation (where pneumothorax is a less frequent occurrence), to avoid prolonged high ventilator pressures or fraction of inspired oxygen (FiO_2).[332] Both veno-venous (VV-ECMO) and veno-arterial (VA-ECMO) approaches have been utilized in infants with CDH. Although CDH has become the most common indication for neonatal ECMO (other than cardiac defects), its efficacy is difficult to assess given the absence of clinical trials. Among infants eligible for ECMO (birth weight > 1.8 kg, gestational age > 32 weeks without major cardiac or chromosomal abnormality), approximately 29% of patients with CDH need ECMO. However, those with CDH who go onto ECMO have a 40% to 50% survival rate based on observational data.[300] Because ECMO is a temporary therapy designed to allow for resolution of a reversible process, it is unlikely that all infants with CDH will benefit from ECMO support; some will have lethal pulmonary hypoplasia. Thibeault and Haney described persistent pulmonary hypertensive changes in vessels of infants with CDH dying after ECMO support, and others have described recurrent pulmonary hypertension.[333,334] The results of ECMO in CDH greatly depend on patient selection criteria, which varies widely among institutions. The primary indication for ECMO should be failure of conventional therapy. This failure can be characterized by persistent preductal SpO_2 less than or equal to low 80 s, hypotension resistant to fluid/inotropic support, peak inspiratory pressures less than 30 cmH_2O, or worsening metabolic acidosis.[332]



• **Fig. 44.19** Management guidelines for congenital diaphragmatic hernia following admission to the NICU. *HFOV*, high-frequency oscillatory ventilation; *Vt*, tidal volume; *PIP*, peak inflation pressure; *PEEP*, peak end expiratory pressure; *HFJV*, high-frequency jet ventilation; *$PaCO_2$*, arterial partial pressure of carbon dioxide; *NO*, nitric oxide; *PPHN*, persistent pulmonary hypertension of the newborn; *OI*, oxygenation index = mean airway pressure in cmH_2O X oxygen concentration as % ÷ PaO_2 in mmHg). (Modified from Chandrasekharan PK, Rawat M, Madappa R, et al. Congenital diaphragmatic hernia —a review. *Matern Health Neonatol Perinatol*. 2017;3:6; copyright Dr. Satyan Lakshminrusimha, UC Davis Health System, Sacramento, CA.)

Survival following ECMO is also related to degree of pulmonary hypoplasia as reflected by the lowest $PaCO_2$ in the first 24 hours after birth.[335] If the lowest $PaCO_2$ during the first 24 hours is > 70 to 80 mmHg, mortality is higher. For those who do go on ECMO, a duration of ECMO support greater than 2 weeks and the use of renal replacement therapy for renal insufficiency have been identified as independent predictors of mortality. Survival among both of these groups was approximately 20% (2 of 11 for prolonged ECMO and 4 of 18 for renal insufficiency).[336] Longer ECMO runs, renal complications, and multiple complications were also independently associated with mortality in infants with CDH in an analysis from the Extracorporeal Life Support Organization database.[337] Thus, failure to decannulate an infant at less than 10 to 14 days of support is probably an indicator of severe lung and vascular hypoplasia. In these severely affected infants, it often takes longer than 4 weeks to resolve pulmonary hypertension, and therefore awaiting resolution of pulmonary hypertension in these cases before decannulation would require prolonged ECMO support.[251] Prolonged ECMO runs are prone to mechanical complications, which may further prolong the ECMO run or be irrecoverable. Thus, it is unclear to what degree prolonging ECMO support could increase survival in CDH, unless there is still evidence of reversibility in the infant's condition. In the case of availability of a specific therapy, such as perfluorocarbon partial liquid ventilation, wherein lung growth might be induced, the benefits of continuing ECMO support might become evident.[338] This therapy, however, is still controversial, with recent clinical studies showing that perfluorocarbon ventilation does not improve pulmonary vascular remodeling and CDH-associated pulmonary hypertension.[338] Otherwise, strategies to limit the duration of ECMO support in CDH could be employed, accepting ventilator settings at decannulation that are significantly higher than what might otherwise be acceptable for newborns coming off ECMO support. These strategies could include prevention of complete lung collapse with

aggressive pulmonary toilet, because re-recruitment of lung volume can be difficult in these infants (which may be related to small-caliber airways that have been damaged by pre-ECMO support regimens). Also, for infants cannulated pre-repair, performance of the CDH repair post-ECMO decannulation could further limit time on ECMO support. Surgical repair of the diaphragmatic defect while on ECMO is associated with higher mortality, even after adjusting for other markers of CDH severity.[339] This phenomenon may be related to hematologic complications from the repair (which are independently associated with decreased survival).[337] However, some centers advocate CDH repair as soon as possible after stabilization on ECMO support. In addition, some centers utilize an "EXIT-to-ECMO" approach for fetuses with high-risk criteria based on fetal evaluation (low relative lung volume).[340] These fetuses are intubated and ventilated during an EXIT procedure. If they meet certain criteria for adequate gas exchange, they are delivered for conventional management. If they do not meet these criteria, they are cannulated and placed on ECMO support. Using this strategy, Kunisaki et al. reported a 71% (10 of 14) survival with 11 infants going directly to ECMO support and 7 of 11 surviving. Most infants required prolonged mechanical ventilation and hospitalization.[340]

Repair of the diaphragmatic defect usually occurs after some degree of stabilization of cardiopulmonary status, either with conventional therapy or ECMO support. Prior studies demonstrated that lung compliance improves before surgery after a short period (several days) of stabilization in infants with CDH, and that compliance worsens with early surgery in almost all infants.[341] Keller et al. studied infants with severe CDH (liver herniation and LHR < 1.4) and found that, although compliance did not improve before surgery after a period of stabilization, compliance did improve within 24 hours after surgery.[342] In low-risk infants that do not require ECMO, the Congenital Diaphragmatic Hernia Study Group found that the timing of surgery did not seem to affect survival and that those who underwent surgery at age 0 to 3 days, 4 to 7 days, or greater than 8 days had similar mortality rates when adjusted for known risk factors.[343] Thus, the rationale for delayed surgery is sound with respect to lung function, but specific clinical parameters to guide the timing of elective CDH repair remain unknown. Some centers advocate very delayed surgery, while awaiting complete resolution of pulmonary hypertension.[344] However, this approach can be problematic, because pulmonary hypertension may require weeks for resolution.[345] Subsequent failure to reduce the hernia contents is likely to delay establishment of enteral nutrition, with a consequent increased risk of infection and complications from parenteral nutrition. The achievement of even modest reduction in FiO_2 before surgery allows for a transient increase, if needed, after surgery, and even in cases where FiO_2 remains high, the modest improvement in lung function that occurs with surgery may help with further recovery for the infant in the most severe cases. Another issue with respect to timing of surgery arises when an infant also has congenital heart disease that requires neonatal surgery. Most centers will undertake CDH repair and then proceed to the cardiac surgery once the infant meets reasonable hemodynamic criteria for that surgical intervention.[346] In newborns who might require urgent cardiac surgery, survival is unlikely unless the lung hypoplasia and pulmonary hypertension are very mild. In a large series of 280 infants with CDH and congenital heart disease, overall survival was 41% but only 5% for infants with single ventricle physiology and 18% (2 of 11) for infants with total anomalous pulmonary venous return.[347]

Surgical repair of CDH involves reduction of the hernia contents and closure of the diaphragmatic defect. The surgical approach traditionally has been via laparotomy (subcostal incision on the side of the hernia), to abrogate the detrimental effect of thoracotomy on lung function. However, some surgeons will preferentially do a thoracotomy for a right CDH repair. With the advance in endoscopic technology, thoracoscopic and laparoscopic repairs are being performed in selected patients.[348] These patients include infants who have achieved low ventilator settings and FiO_2 before surgery. Despite both thoracoscopic and laparoscopic repairs being feasible, there are questions about the safety and efficacy of these approaches, with minimally invasive techniques being linked to higher rates of reoccurrence, as well as intraoperative acidosis and hypercapnia, particularly with thoracoscopic repair.[349] Additional randomized controlled trials are needed to determine the efficacy of minimally invasive repairs and to define the specific patient populations who are most appropriate for a minimally invasive repair.

Regardless of the surgical approach, when the diaphragmatic defect cannot be closed primarily, a prosthetic patch is used to bridge the gap. There is significant variability among surgeons in the need for patch repair, however. Clinical series have demonstrated that it is more common in the case of liver herniation and right-sided CDH (which may also be an association with herniated liver).[350,351] Patch repair can be accomplished with use of a polytetrafluoroethylene (PTFE/Gore-Tex, W.L Gore & Associates, Inc., Flagstaff, AZ) or a bioabsorbable intestinal mucosa (Surgisis, Cook Medical Inc., Bloomington, IN) material. Polypropylene (Marlex, Bard Davol, Warwick, RI) and other materials have been used sporadically.[348] Use of an abdominal silo and/or prosthesis to close the abdominal wall is sometimes necessary and may decrease the need for a prosthetic diaphragm.[348] Temporary abdominal closures with skin or vacuum-assisted closures have also been used, most commonly in children who are repaired while on ECMO.[352] Some surgeons advocate construction of a latissimus dorsi flap for initial CDH repair when primary closure of the diaphragmatic defect cannot be accomplished.[348] This technique allows for some potential for diaphragmatic function in the innervated flap, which usually remains abnormal at late follow-up even with primary repair.[348] However, the technique is time consuming and may not be tolerated in patients during the acute neonatal period, so others have reserved this technique only for hernia recurrence.[353] Generally, use of a prosthetic patch for diaphragmatic closure is associated with increased risk of hernia recurrence, although the actual risk varies widely and may be dependent on multiple factors, including surgical technique and the type of patch used.[354] Recurrent herniation may be associated with small bowel obstruction. It is also associated with persistent chest wall deformity, which might be due to the severity of the underlying disease or the complication of re-herniation (requiring multiple surgical procedures). Some surgeons have moved toward use of composite patches (more than one material), which may decrease the risk of recurrence by allowing for both durability and accommodation of rapid growth in infancy.[348]

Another area of variable practice is related to the intraoperative placement of a thoracostomy tube. Some of this controversy is related to the application of negative pressure, because that creates additional transpulmonary pressure and potential for barotrauma and lowers lung compliance.[326] However, negative pressure drainage is not necessary. Because the ipsilateral thorax will fill with fluid after reduction of the hernia contents as a result of the hypoplastic lung, placement of an anterior thoracostomy tube in a supine infant will prevent the accumulation of excess pleural fluid (which can occur with chylothorax). Chylous pleural effusion will cause respiratory compromise. However, it may be difficult

to ascertain the cause of this deterioration because mediastinal structures remain shifted for some period of time postoperatively and may be modestly more exaggerated with tension hydrothorax. Chylothorax is not uncommon after CDH repair. In a recent meta-analysis, patients with patch repair have 2.5 times higher risk of developing chylothorax.[355] Both surgical trauma and underlying hemodynamics due to right heart failure may contribute to the cause of this problem in infants with CDH (see Chylothorax earlier, for diagnosis and management).

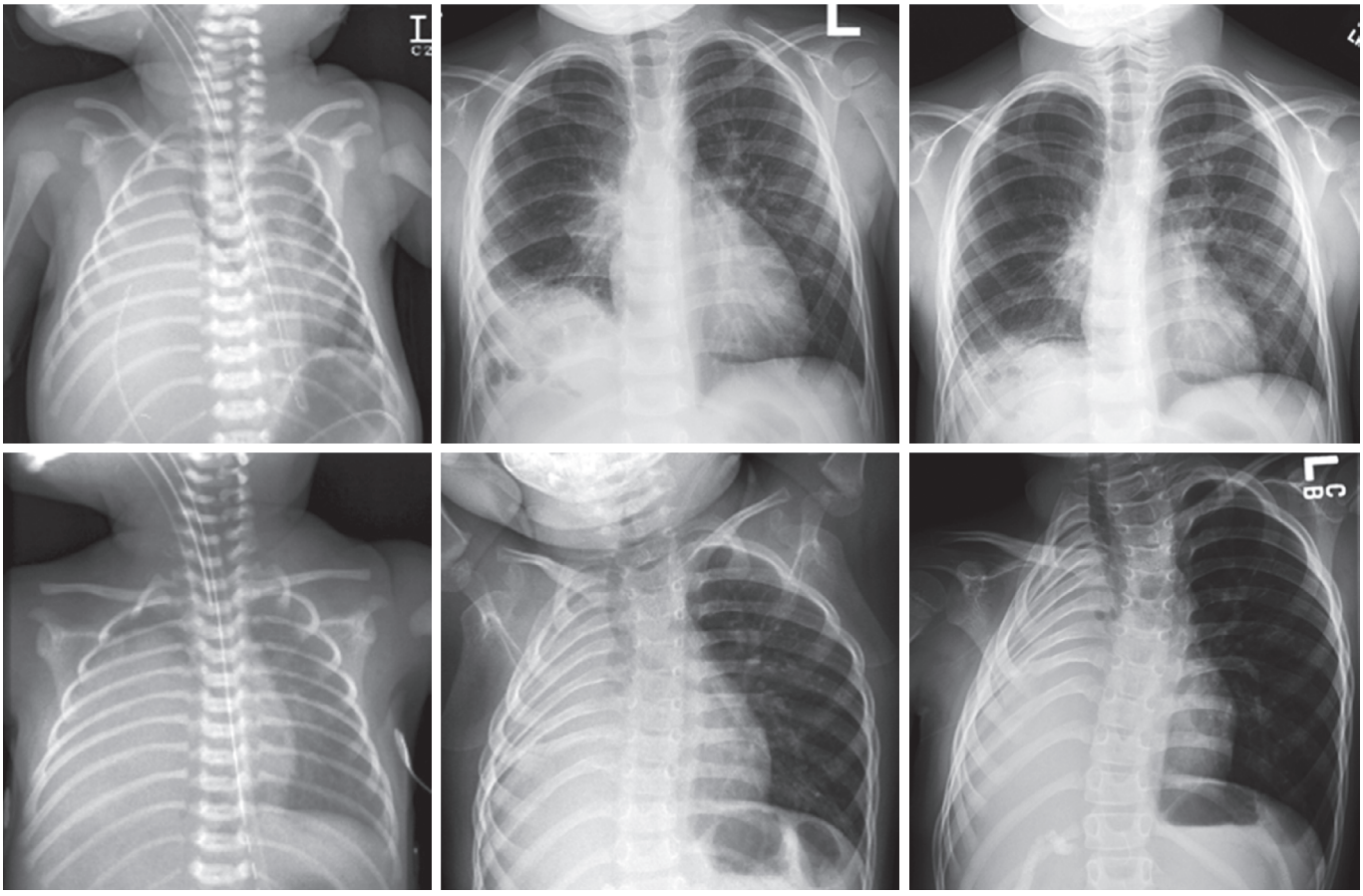
Long-Term Morbidity

Survivors of CDH have substantial pulmonary and gastrointestinal morbidity at follow-up.[354] Early gastroesophageal reflux following repair occurs in up to 40% of CDH patients and is more common in patients who undergo patch closure or have an intrathoracic stomach.[356] In addition, failure to thrive is common with many patients requiring supplemental enteral feedings via gastrostomy tube. Although low lung volumes and restrictive lung disease may be seen in the first months of life, obstructive lung disease is the most common finding in early childhood and at later follow-up.[357] Children requiring prosthetic patch repair and prolonged mechanical ventilation are most likely to have later morbidity, but the relative contribution of anatomic abnormalities, physiologic derangements, and secondary injury to these outcomes is unknown. Developmental delay and hearing loss also occur and require careful follow-up for early identification and intervention.[354]

Hepatopulmonary Fusion

Hepatopulmonary fusion probably represents a severe form of CDH. It has been described variably in the literature as both CDH and severe eventration.[358] All reported cases have occurred on the right side, although there is a report of a late-presenting left CDH with fusion of liver and lung tissue.[359] It is not clear if this is the same entity that other authors have described, because these infants usually present in extremis, with respiratory failure and pulmonary hypertension.[358,360]

The embryology of this defect is speculative, and the distinction between severe CDH versus eventration is probably not critical. However, the diaphragm is not intact, it is usually moderately to severely hypoplastic, and there is fusion of hepatic and pulmonary tissue without pleura or liver capsule present, no dissectable plane, and a fibrous membrane adherent to the liver and the lung.[361] In cases where hepatopulmonary fusion is determined or suspected, there is a lack of mediastinal shift, consistent with severe right lung hypoplasia rather than a mass effect from a large right CDH with herniated liver[362,363] (Fig. 44.20).



• **Fig. 44.20** Congenital diaphragmatic hernia and hepatopulmonary fusion. Chest radiographs in two infants with respiratory distress at admission to the intensive care nursery and at 3 and 5 years of age (*left to right*). *Top row*, Infant with right congenital diaphragmatic hernia with liver herniated into thorax and diaphragmatic aplasia. Note mediastinal shift into left hemithorax, with subsequent improvement in aeration and then normalization of right lung volume. *Bottom row*, Infant with hepatopulmonary fusion. Surgical repair required resection of hypoplastic nubbin of lung. Note lack of mediastinal shift at presentation, compensatory growth of left lung with trachea deviated to the right, and scoliosis.

The suspicion of this diagnosis is important because the surgical approach may differ. The need to separate the liver and the lung (usually requiring ligation or resection of part of the hypoplastic lung because of the risk of air leak and hemorrhage) will require a thoracotomy. MRI has been utilized preoperatively to help make this distinction and found enhanced lung tissue adherent and conforming to the dome of the liver in a case of hepatopulmonary fusion, suggestive of the anatomy subsequently encountered at the time of surgical repair.[362] The goal of surgery is to separate the thoracic and abdominal cavities, often requiring placement of a prosthetic patch. If these children survive, later growth of the right lung is much more restricted than it is in children with severe right CDH. Whether this is due to more pronounced ipsilateral lung hypoplasia, less pronounced contralateral lung hypoplasia, or the need for resection at the time of surgical repair is unclear. However, chest wall deformity and scoliosis are significant problems in these children.

Congenital Eventration of the Diaphragm

Congenital eventration of the diaphragm occurs when the hemidiaphragm is partially or completely replaced by fibroelastic tissue, leading to a thinned, pliable portion of the diaphragm. Unlike CDH, the diaphragm is intact with normal insertion points. However, it is elevated, and thus intra-abdominal organs are present in the thorax but still confined to below the diaphragm. It can therefore be difficult to distinguish eventration from a diaphragmatic hernia with a sac. In recent series, the right side was affected more often (~75% compared to ~25%), and bilateral eventration was very rare.[363,364] Overall, diaphragm eventration is rare, occurring in 0.02 to 0.07 out of 1000 births, and affects males in 60% to 80% of cases.[365] Approximately 18% of children with eventration have associated anomalies, with the most common being hypoplastic lungs, congenital heart disease, and pectus excavatum.[363] Eventration accounts for 5% to 7% of all diaphragm diseases.[365]

The peak age of diagnosis is 0 to 3 months, with a mean age of around 10 months.[363] With diaphragmatic eventration, assessment of hemidiaphragm movement is critical. In instances of paradoxical movement, the abnormally elevated diaphragm muscle can result in collapse of the affected alveoli and negatively affect lung ventilation and/or lung development.[364] In this instance, surgical repair may result in improved outcomes; when paradoxical motion is absent, repair may not be beneficial.[364] As many as 50% of children with congenital diaphragm eventration are without symptoms.[363]

The most common symptoms in infants < 12 months[363]:

- Rapid breathing
- Vomiting
- Difficulty breathing

The most common symptoms in children > 12 months[363]:

- Recurrent respiratory infections
- Chest tightness
- Cough or expectoration

Diagnosis is usually made by fluoroscopy of the chest, ultrasound of the diaphragm, or dynamic contrast-enhanced MRI. Small eventrations that are asymptomatic can be observed. Surgical intervention for symptomatic eventration involves plication of the diaphragm to eliminate the paradoxical movement, decrease lung compression, and strengthen the respiratory action of the respiratory muscles.[366] The diaphragm can be approached via the abdomen or chest using either open or laparoscopic/thoracoscopic techniques, with no difference in recurrence rates between the types.[365,367] Results are generally good, with severe lung hypoplasia being uncommon and surgery resulting in improvement in symptoms with low rates of recurrence. However, infants with associated conditions, particularly neuromuscular disorders, may not survive.

Diaphragmatic Paresis

Diaphragmatic paresis occurs most commonly secondary to vaginal birth trauma and is associated with phrenic nerve injury from ipsilateral brachial plexus palsy. While overall rare, the exact incidence is unknown.[368] As mentioned, it is associated with brachial plexus palsy, but of those patients who have brachial plexus palsy, only 2% of them develop phrenic nerve injury and diaphragm paresis. The most notable risk factors for diaphragm paresis secondary to birth trauma are macrosomia, shoulder dystocia, and breech presentation.[369] Diaphragm paresis has also been reported after surgical trauma, most commonly cardiac surgery.[368,370]

Paresis of the diaphragm leads to an elevated hemidiaphragm on chest radiograph and paradoxical movement evident by ultrasound or fluoroscopy (Fig. 44.21A,B). It generally presents as symptomatic respiratory distress, difficulty weaning from ventilator, and even respiratory insufficiency.[368,371] Phrenic nerve conduction studies may be used to make the definitive diagnosis.[369]

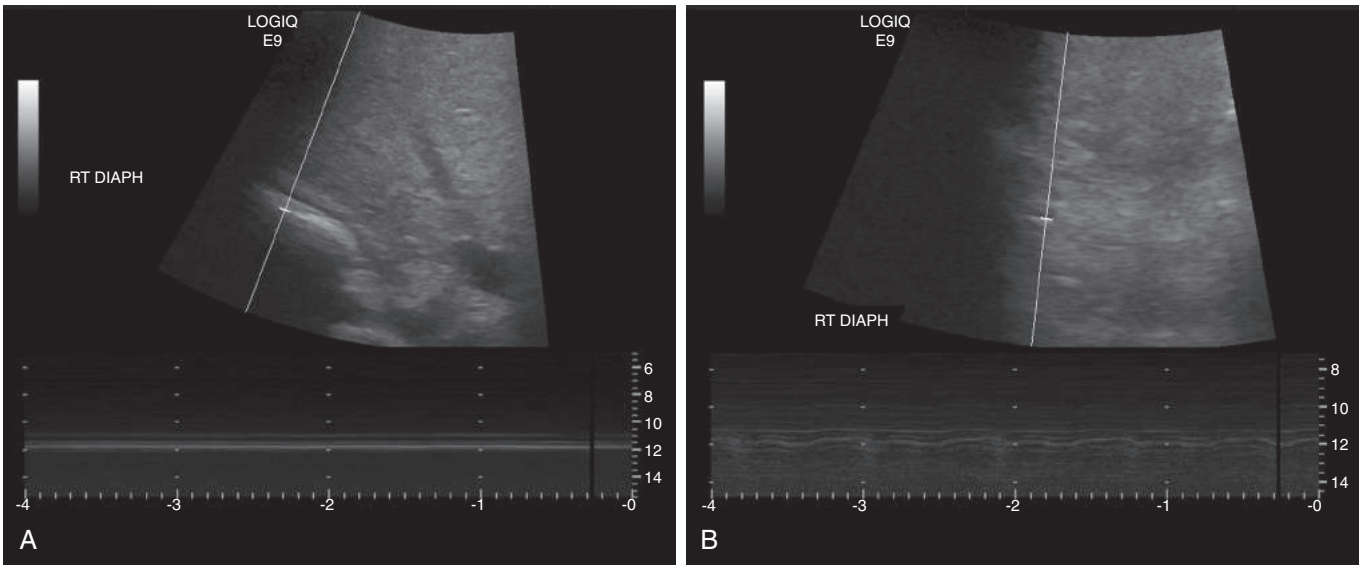
Management of diaphragmatic paresis secondary to phrenic nerve injury remains controversial. These injuries can recover on their own over time so a conservative approach using prolonged mechanical ventilation is an option.[368] Many observe a waiting period of 2 to 4 weeks to allow for possible improvement followed by diaphragmatic plication if persistently symptomatic.[372] Surgical repair entails diaphragmatic plication in an approach similar to that for diaphragm eventration. Repair may reduce need for mechanical ventilation and other modes of respiratory support. Plication of the diaphragm can be done via thoracic or abdominal approach and by open or minimally invasive techniques with the end result being fixation of the redundant folds of diaphragm tissue and an overall flattening of the diaphragm.

Neonatal Scimitar Syndrome

Scimitar syndrome is a rare constellation of cardiopulmonary anomalies, consisting of anomalous pulmonary venous drainage from the right lung to the inferior vena cava. Additional anomalies, including hypoplasia of the left heart or aorta, hypoplasia of the right lung, anomalous arterial supply to the right lung with or without pulmonary sequestration, pulmonary hypertension, dextroposition of the heart, and atrial septal defect (ASD), may also be detected.[373]

Scimitar syndrome may be suspected on fetal ultrasound, because of findings of cardiac dextroposition with a small right pulmonary artery.[374] The presentation of scimitar syndrome in the first days after birth is usually severe, with tachypnea, pulmonary hypertension, respiratory distress, and early heart failure.[375] Some infants will present later with failure to thrive. Chest radiograph reveals an elevated right hemidiaphragm with the mediastinum shifted to the right, consistent with primary lung hypoplasia. The classic finding of the scimitar vein is often not appreciated.

Surgical approaches are varied as they depend on the anatomic and pathologic features present in each specific case. Regarding pulmonary status, and because these infants have a normally developed diaphragm, the surgical approach will depend on hemodynamic effects of the anomalous venous return and the sequestration (if present). Thus, careful hemodynamic and vascular assessment are critical and may be accomplished through cardiac



• **Fig. 44.21** Diaphragmatic paresis. M mode ultrasound images of a newborn with tachypnea and moderate respiratory distress. (A) Absence of right diaphragmatic excursions is consistent with complete diaphragmatic paralysis. (B) Left diaphragm has low-amplitude excursions with normal inspiratory and expiratory motion. (Courtesy Dr. Chirag V. Patel, UC Davis Health System, Sacramento, CA.)

catheterization and angiography or, in some cases, by magnetic resonance angiography. The classic operation involves a long intra-atrial baffle being constructed from the entry point of the scimitar vein into the inferior vena cava to the left atrium through an ASD, which requires cardiopulmonary bypass. Pulmonary vein stenosis can complicate the hemodynamic status and this classic operative technique, and medical or surgical treatment for additional cardiac lesions may need to be addressed. Another technique involves anastomosing the scimitar vein directly to the left atrium through a right thoracotomy which avoids baffles, an extracorporeal circuit, and also addresses any stenosis present.[376] Some infants will require pneumonectomy, others ligation or coil embolization of systemic feeding vessels, and others no intervention because the hypoplastic lung may have very little blood flow.

Acknowledgments

The authors would like to acknowledge Dr. Benjamin A. Keller and Dr. Shinjiro Hirose for their writing of the 10th edition of this chapter.

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45 Developmental Biology of the Heart

ELLEN DEES AND H. SCOTT BALDWIN

KEY POINTS

- The heart forms from cardiac mesoderm as a symmetric linear tube with connections to the primitive arterial and venous systems. The heart tube is formed by 3 weeks' gestation in the human.
- The heart tube loops, establishing laterality, and undergoes septation into four chambers with connections to the systemic and pulmonary arteries and veins. The heart is fully formed by 9 weeks' gestation.
- External populations of cells migrate into the developing heart, making important contributions to the cardiac valves, coronary arteries, and cardiac chambers.
- Congenital heart malformations arise when these processes are altered or incomplete.

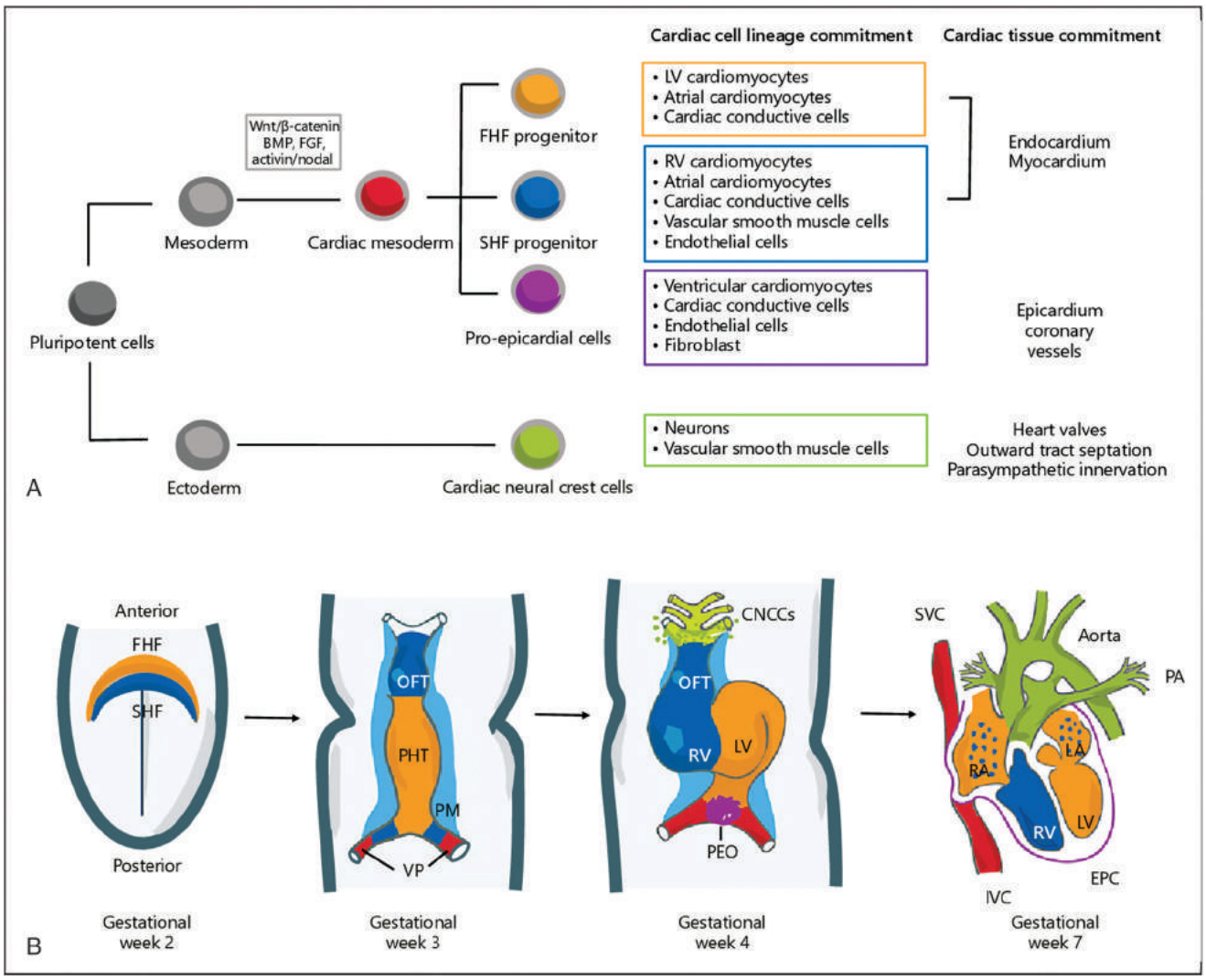
Overview of Cardiac Developmental Anatomy

This chapter is a review of embryonic and fetal cardiac anatomy, including the cell types that contribute to the developing heart, and genetic regulation of heart development. We will follow the heart from its stages as a linear tube with inflow and outflow segments, through cardiac looping and septation into a four-chambered heart with separate pulmonary and systemic circulations. Understanding these processes is of interest to clinical neonatologists caring for neonates and infants with normal and abnormal cardiac physiology.

Heart development begins at gastrulation as a field of cells; cardiac mesoderm (Fig. 45.1A) is specified and moves ventrally and midline within the embryo, fusing into a tube. This occurs in the human embryo by 3 weeks, post-fertilization, or approximately week 5 after the last menstrual period (Fig. 45.1B). At the posterior/dorsal end of the tube is the venous pole, or sinus venosus, connecting to the systemic and placental venous system. At the anterior/ventral end of the tube is the arterial pole, or outflow tract, which connects to the developing aorta. The heart tube itself is divided into an atrial segment, adjacent to the venous pole, and atrioventricular (AV) canal, and a ventricular segment adjacent to the arterial pole. Each of these segments is histologically and functionally distinct from the beginning—atrial and ventricular myocytes have distinct characteristics even when isolated from the primitive mesoderm. By the time the heart tube is formed, differentiating cardiomyocytes have generated functional contractile units mature enough to begin spontaneous contractions. These are initially peristaltic in nature, moving blood in one direction from the venous inlet to the arterial outlet. This is initially clear fluid, but red blood cells begin to enter the circulation from blood islands in the yolk sac soon after the heart starts to beat.[1] For the first 6 weeks, the yolk sac remains the exclusive source of hematopoietic cells, until the liver (and to a lesser extent spleen) takes over. The bone marrow gradually becomes populated with hematopoietic cells beginning in the second trimester of pregnancy, and by birth is the major source of blood cells.[1] Development of a circulatory system early is critical to maintaining nutrition and oxygen delivery to developing tissues, which are at this point beyond the ability to depend on simple diffusion of nutrients.

From the primitive heart tube state, the heart undergoes significant growth and morphologic alterations. The symmetry of the heart tube is lost and well-defined left and right structures acquire their functions as the heart undergoes a dramatic change in shape, via a process known as cardiac looping. This occurs in the human embryo during weeks 4 to 5 of gestation (see Fig. 45.1B). From this stage, the heart realigns and septates the inflow and outflow segments, septates the atria and ventricles, and forms the atrioventricular and semilunar valves in processes that will be detailed in this chapter. The "final product" will be a mature heart, formed by gestational week 7 (or 9 weeks after the last menstrual period; Fig. 45.1B) with the following structures:

1. A **venous pole or sinus venosus** *(red)* that is now connected to systemic veins from the upper body (superior vena cava), lower body (inferior vena cava [IVC]), liver, and coronary circulation, all of which pass into the **right atrium (RA)**.
2. A separate **left atrium (LA)**, connected to the venous system separately by ingrowth of **pulmonary veins** from the lungs.
3. An **atrioventricular canal** that begins as a single unrestricted opening, and septates into two atrioventricular valves. The **tricuspid valve** opens from the right atrium into the right ventricle, and the **mitral valve** opens from the left atrium to the left ventricle.
4. A **primitive ventricle** that has expanded and septated into two distinct and separate chambers, a **right and left ventricle**. By processes known as compaction and trabeculation, the working myocardium of each ventricle becomes highly organized and adapts to the unique requirement of a pulmonary (RV) or systemic ventricle (LV).
5. An **arterial pole or bulbus cordis** that forms the two separate outflow tracts of the heart. The distal part of the bulbus cordis is the **arterial trunk**, which undergoes septation in a spiral fashion creating an anterior **pulmonary artery** connecting



• **Fig. 45.1** Cell types and morphology in heart development. (A) Pluripotent cells from the bilayer disc commit to mesoderm and ectoderm lineages as shown; endoderm is also specified and gives rise to the gut (not shown). Ectoderm gives rise to neural tissues, including neural crest cells *(green)*. Cardiac mesoderm forms three populations within the heart as shown: first heart field *(orange)*, second heart field *(blue)*, and proepicardium *(purple)*. The eventual cell types and tissues formed from each are as shown. (B) Schematic of cardiac morphology at the cardiac crescent, heart tube, looping heart, and mature heart are shown. Colors match the lineage markers described above. (From Tan CMJ, Lewandowski AJ. The transitional heart: from early embryonic and fetal development to neonatal life. *Fetal Diagn Ther*. 2020;47:373–386.)

to the lungs and a posterior **aorta** connecting to the body. The proximal part of the bulbus cordis is the **conus**, which is retained by the right ventricle. At the junction between conus and the pulmonary artery, the **pulmonary valve** forms. The **aortic valve** forms at the junction between the left ventricle and the aorta.

6. Paired **dorsal aortae** connected to the **aortic sac** that have extensively remodeled into a single leftward **aortic arch** and **descending aorta**. This connects proximally to the **coronary arteries**, the head and neck arteries, and to a temporary structure important in fetal life, the **ductus arteriosus**.
7. Networks of coronary arteries that have formed within the myocardium of the heart. These connect to the aorta as the right and left coronary arteries.
8. Networks of myocytes that have differentiated into the conduction system of the heart, including the sinoatrial node, the atrioventricular node, and the His-Purkinje system of the ventricles.

Cell Types Within the Heart and Their Origins

The mature heart has three cell layers: the endocardium, a single cell thickness epithelial lining; the myocardium, made up of myocytes that perform the contractile work of the heart; and the epicardium, a single cell thickness epithelium covering the external surface of the heart. Beyond this, the heart resides within a pericardial sac, similar in composition and function to the pleura covering the lungs.

The heart proper has its embryonic origin from a field of lateral plate mesodermal cells referred to as the cardiogenic mesoderm (Fig. 45.1A and B; *orange/red*). The primitive heart tube arises from the primary heart field (Fig. 45.1A and B, *orange*) and has two layers, an endocardium and a myocardium, both arising from cardiac mesoderm. A subset of cells from the endocardium undergoes an epithelial-to-mesenchymal transition to form the endocardial cushions. These cells will be vital to proper valve development and to complete septation of the atria and ventricles. The myocardium primarily remains muscle, but a subpopulation of these cells also differentiate into Purkinje fibers of the conduction system.

The outflow tracts of the heart are arguably the most complex genetically and morphologically, and thus most prone to developmental defects. The secondary heart field is a population of cardiac mesoderm adjacent to the original heart field that migrates into the heart during looping (Fig. 45.1A and B, *blue*). The secondary heart field contributes significantly to the right ventricle and outflow region of the heart, to the atrial and ventricular septa, and to parts of the atria.[2–5]

Neural crest cells are an important population that also migrate into the developing outflow tract, interacting with the secondary heart field myocardium (Fig. 45.1A and B, *green*). Neural cells originate from the ectoderm in the anterior rhombencephalon and migrate as a sheet through the pharyngeal region and into the aortic arches, truncus, and proximal conus.[6–10] Here they interact with endocardial cushion cells to septate the great arteries and close the conal septum.[11] These neural crest cells are also important to the development of the nearby parathyroid, thyroid, and thymus glands. They also innervate the heart and form much of the smooth muscle of the proximal aorta.[6]

Another external population of cells that make important contributions to the mature heart is a cluster of cells dorsal and inferior to the heart tube known as the proepicardial organ (Fig. 45.1A and B, *purple*).[12,13] The origin of these cells is a subject of debate; one leading theory is that they are derived from liver primordium.[12] These cells expand as an epithelial sheet covering the surface of the heart to form the epicardium. From the epicardium, subgroups of cells delaminate and migrate into the myocardium beneath in a process known as epithelial to mesenchymal transformation. These cells differentiate into vascular smooth muscle, vascular endothelial cells of the coronary arteries, and cardiac fibroblasts, which make up a sizable population of cells residing within the myocardium, between myofibers. A recent study using multiple lineage markers in tandem showed that only about 4% of the coronary endothelium comes from the pro-epicardium, while the contribution to fibroblast and smooth muscle population is much higher. This study identified a population of circulating endothelial progenitors as an important source of cardiac endothelium.[14]

Thus in addition to cardiomyocytes arising from at least two populations of mesoderm, the heart is composed of cells from epithelial and neural crest origins that migrate in with spatial and temporal precision during cardiac development. The next several sections will highlight the steps of this process.

Gastrulation and the Cardiac Crescent

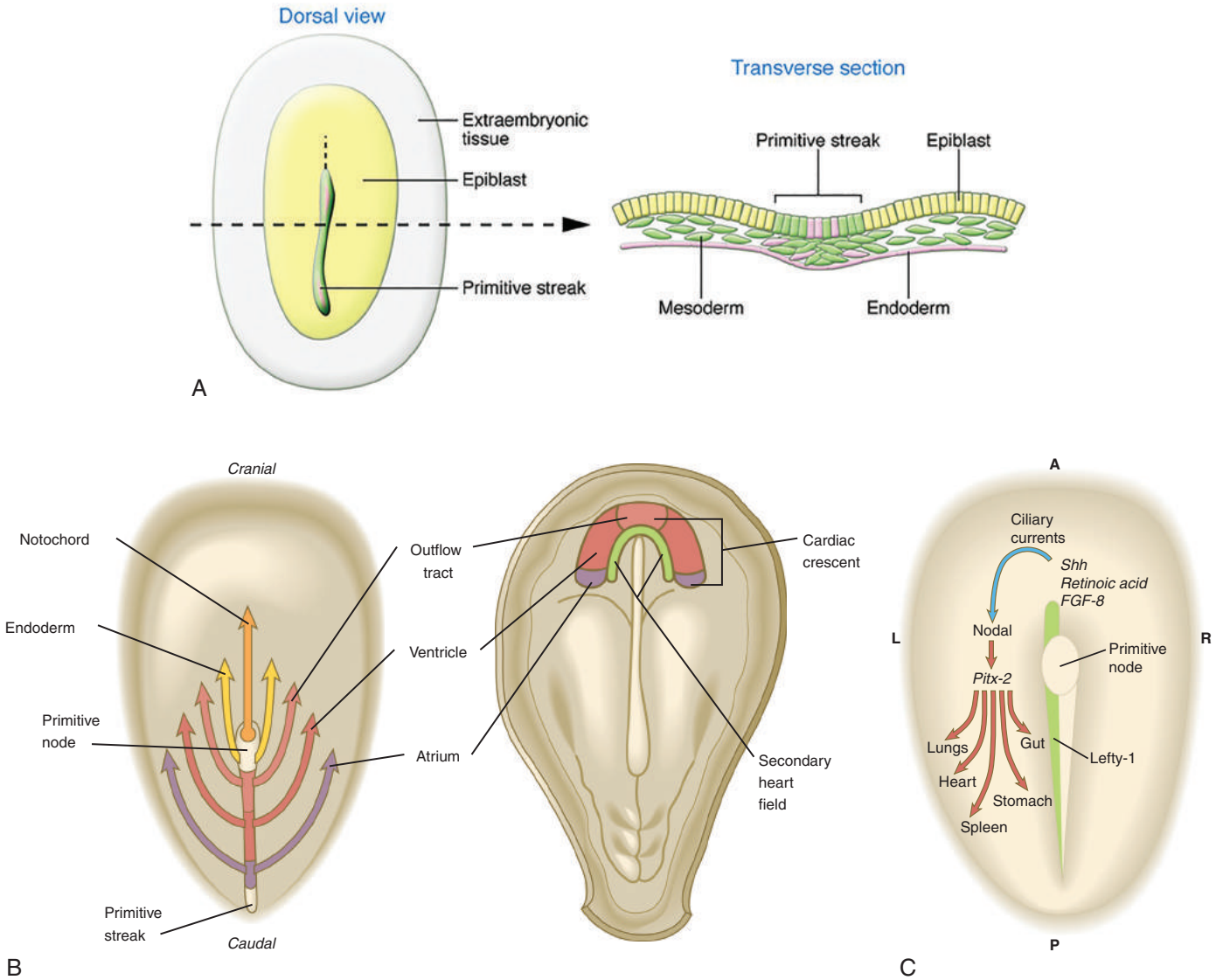
Much of what is known about the early stages of embryonic development comes from studies of avian, zebrafish, and mouse models extended, with some acknowledged gaps, to human development.[15–27] In its earliest stages, the embryo exists as a bilayer disc of two epithelial sheets of cells suspended between two fluid-filled cavities, the yolk sac and the amniotic cavity (Fig. 45.2A). The ventral layer (facing the yolk sac) is the hypoblast, which will eventually be relegated to extraembryonic structures. The dorsal layer (facing the amniotic cavity) is the epiblast, which will form all three embryonic germ layers: the ectoderm (nervous system and skin), mesoderm (heart, skeleton, muscle, and connective tissue), and endoderm (gut). Mesoderm and embryonic endoderm separate from the primitive ectoderm by a process known as gastrulation. Gastrulation begins as a groove that forms in the epiblast starting at the tail end (caudal) and gradually extending cranially to an endpoint known as the prechordal plate (Fig. 45.2A). The groove is known as the primitive streak, and its leading edge is the primitive node. Along the primitive streak, epiblast cells delaminate from the epithelial sheet and invade the potential space between the epiblast and hypoblast, forming a crescent of cells cranial to the prechordal plate and extending along both sides (Fig. 45.2B). Genetic and fate mapping studies also show that the relative position of cells during migration is key to their ultimate position within the heart. Specifically, the apex of the crescent is formed from cells that have migrated through the primitive streak closest to the primitive node, and these are precursors of the outflow tract myocardium. The cells on either side of this region have migrated through the mid-portion of the streak and are ventricular myocyte precursors. The most lateral and caudal cells are those which have migrated through the most posterior part of the streak and will become atrial myocytes (Fig. 45.2B). Finally, the secondary heart field mesoderm lies medial and extends along the entire crescent such that it is continuous with both the outflow and inflow segments of the crescent (Fig. 45.2B, green). Fate-mapping studies using live-cell tracking and time-lapse imaging demonstrate that these cells move as a cohort (tissue motion) rather than individually migrating; this is a way to maintain cells' relative positions within a tissue, and eventually, an organ.[19]

Genetic Control of Gastrulation

The earliest known marker of the cardiomyocyte lineage is the transcription factor Mesp1, expressed in the mesoderm at the onset of gastrulation.[28,29] Mesp1 acts as a switch between pluripotency (phenotypically linked to mesenchymal, migratory, and proliferative characteristics) and differentiation.[28,30]

At the primitive node, growth factors of the wingless integrated or Wnt family are expressed which block differentiation and promote proliferation and migration.[31] Surrounding structures, including the neural tube and endoderm, secrete inhibitory factors forming the boundaries of the crescent-shaped heart field.[32,33]

Thus as the migrating cells make their way into the cardiac crescent, they exit the region of Wnt expression and enter a field of active Wnt inhibition. The cardiac mesoderm then begins to differentiate into cardiomyocytes under the control of a different class of growth factors, bone morphogenetic proteins (BMP).[34] Early markers of the differentiated cardiomyocyte phenotype include transcription factors Islet 1, TBX5, GATA4, and Nkx 2.5. The mechanisms for the shifts in gene expression are complex and involve many epigenetic factors including modification of chromatin structure in differentiating cells and enhancer and repressor regulatory elements.[35] Factors such as GATA4 function both to



• **Fig. 45.2** Gastrulation and the formation of the three germ layers. (A) Schematic view of gastrulation, showing the primitive streak as it appears from a dorsal view and in transverse section. In the transverse section, the process of epithelial to mesenchymal transformation (EMT) can be appreciated, as mesoderm and endodermal cells (shown in *green* and *pink*, respectively) delaminate from the epiblast *(yellow)* and migrate to form new structures. (B) Formation of the cardiac crescent, with primary and secondary heart fields. In the primary heart field, the cell position at migration determines its fate within the crescent and heart tube as shown. (C) Genes regulating left-right asymmetry during gastrulation, with ciliary currents creating a gradient of Nodal expression to the left side of the embryo, initiating a cascade of other signaling molecules as shown. (A from Acloque H, Adams MS, Fishwick K, et al. Epithelial-mesenchymal transitions: the importance of changing cell state in development and disease. *J Clin Invest*. 2009;119(6):1438–1449; B, C from Carlson BM. *Human Embryology and Developmental Biology*. 5th ed. Philadelphia: Elsevier; 2014:105.)

modify chromatin structure, for example, cause "unwinding" of specific segments of DNA rendering it accessible to transcription, and to bind to certain gene promoters to enhance transcription directly.[35] Furthermore, Gata4 recruits TBX5 to muscle-specific gene promoters in the primary heart field; in mice TBX5 deletion causes abnormal development of the left ventricle. In humans, TBX5 haploinsufficiency causes atrial septal defect (ASD), conduction defects and limb anomalies, but can also be associated with hypoplastic left heart syndrome.[36,37] Similarly, both NKX 2.5 and Islet1 function by binding to regulatory elements of cardiac-specific genes, including chromatic structure and transcription enhancer elements; there is additional complexity in this system from the interdependency and to some extent redundancy of GATA4, NKZ2.5, and TBX5.[35,38]

The basic left-right asymmetry of the embryo is set during gastrulation by concentration gradients of the factors sonic hedgehog (shh) and fibroblast growth factor 8 (see Fig. 45.2C). This gradient is created by ciliary motion at the primitive node and causes a cascade of downstream genes to be activated, including nodal, lefty, and pitx-2.[39–41] All of these are markers of left-sidedness,

whereas as far as currently understood right-sidedness is specified entirely by the absence of these factors. When the strict left/right gradient of gene expression is altered, the result is abnormalities of left/right structures in the heart, lungs, and/or gut known as heterotaxy (see section below and Fig. 45.4).

Looping and Laterality of the Heart Tube

When initially formed the cardiac crescent is a symmetric structure, and it next fuses into an initially symmetric heart tube. This primitive heart tube forms as the cardiac crescent moves ventrally and medially, such that the two arms of the crescent fuse into a tube (Fig. 45.3A).[42] The developing foregut dorsally and the neural tube cranially help to push the heart tube into its new position. As fusion of the heart fields occurs, the heart begins to beat. In a human fetus, this happens at approximately 23 days' gestation or early week 6 after the last menstrual period.

The heart tube next begins to undergo a dramatic change in shape known as looping. Here the heart tube loses its symmetry, and distinct left and right morphology can be identified (Fig. 45.3B). Morphologically, cardiac looping involves some degree of differential growth, with a higher proliferation of myocytes along the outer curvature than the inner.[43] The primary contribution to the morphologic change during looping, however, is a substantial migration of cells from the developing secondary heart field.[3,44–46] Of note, this migration of secondary heart field cells occurs both at the arterial and venous poles of the heart. All of this serves to elongate the outflow tract and enlarge the ventricular segment. Recent detailed lineage tracing and studies in chick heart tubes confirm that the entire right ventricle arises from the secondary rather than the primary heart field.[47] The atria and sinus venosus also incorporate secondary heart field cells, and this portion of the heart tube is shifted cranially from its original position. Overall, the heart tube increases fivefold in length during looping.[48] In addition to cell migration and proliferation, there are mechanical forces literally pulling, twisting, and realigning structures of the primitive heart tube. This is thought to involve the cytoskeleton, including non-muscle myosin,[49] the motor protein dynein,[50–52] microtubules,[53] and non-muscle actin bundles.[54]

Genetic Control of the Secondary Heart Field and Cardiac Looping

Genetically, the secondary heart field is characterized by transcription factors Tbx1, islet 1, Mef2c, Tbx20, and growth factors Fgf 8 and Fgf 10. Knockout of any of these genes in mice causes outflow tract defects.[55–62] Interestingly, a second wave of Mesp1 expression late in gastrulation signals the migration of the secondary heart field cells into the developing heart.[63] TBX1 expression in the secondary heart field coordinates this migration at both the arterial and venous segments of the heart tube.[64] As the secondary heart field cells move into the outflow tract of the heart, they stop expressing many of their original genetic markers and express more general myocardial markers such as Hand1, Nkx2-5, Tbx5, Gata4, and Mef2.[21,45,46,56,65,66] These are factors already well-studied in the primary heart field to contribute to myogenesis.[38,67–75] Defects in the expression of these genes in the secondary heart field can lead to right ventricular hypoplasia and outflow tract abnormalities, including tetralogy of Fallot.[65,66]

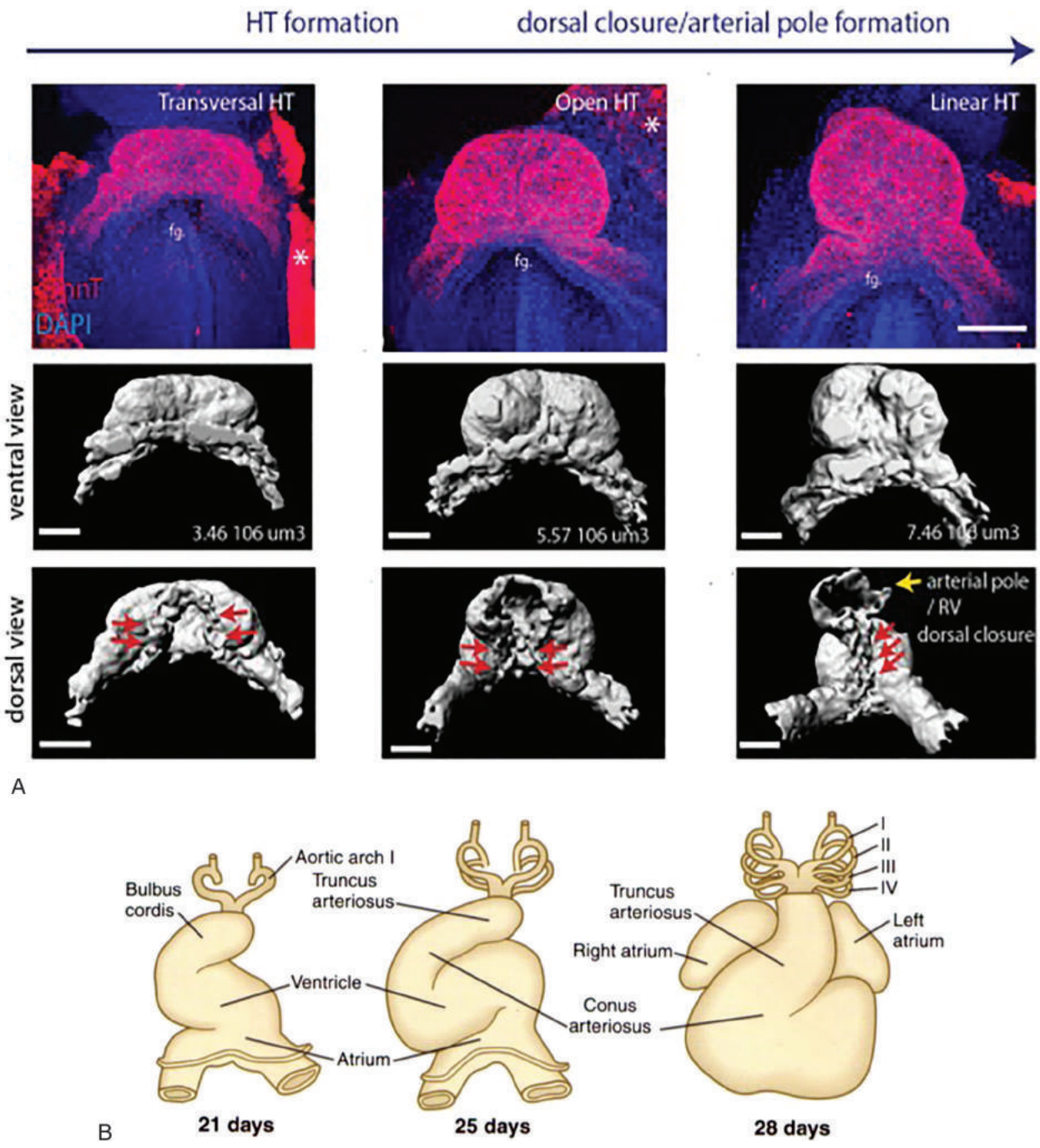
Clinical Abnormalities in Cardiac Looping and Laterality

Normal looping occurs to the right (D-looping), that is, the sinus venosus and atria move toward the left and posterior, and the ventricles and bulbus cordis move to the right and anterior. Importantly, the venous pole also moves cranially such that it lines up with the arterial pole along the horizontal body axis (Fig. 45.3B, 28 days). The bulbus cordis remains to the right of the primitive ventricle. This repositioning is critical for proper atrioventricular and ventriculoarterial connections. Also with looping, an outer and an inner curvature of the heart is established. The anterior outer curvature is an area of rapid myocardial growth and expansion to form the ventricular chambers. The posterior inner curvature undergoes slower growth and acts rather like a fulcrum for the looping process. The inner curvature myocardium contributes to the atrioventricular canal and is critical to the formation of the endocardial cushions.

Sometimes, looping can occur to the left, with the venous pole and atria moving rightward and the ventricles and arterial pole moving leftward. This is referred to as L-looping (Fig. 45.4A shows normal looping; compare to L-looping in Fig. 45.4B). Cardiac connections are conventionally defined by (1) atrial situs (solitus, right atrium on the right side vs. inversus, right atrium on the left side); (2) ventricular looping (D or L); and (3) great artery relationships (S, normal with aorta to the right and posterior to the pulmonary artery; D, malposed with aorta to the right but anterior; or L, malposed with aorta to the left and anterior). Normal cardiac connections then are defined as (S,D,S).

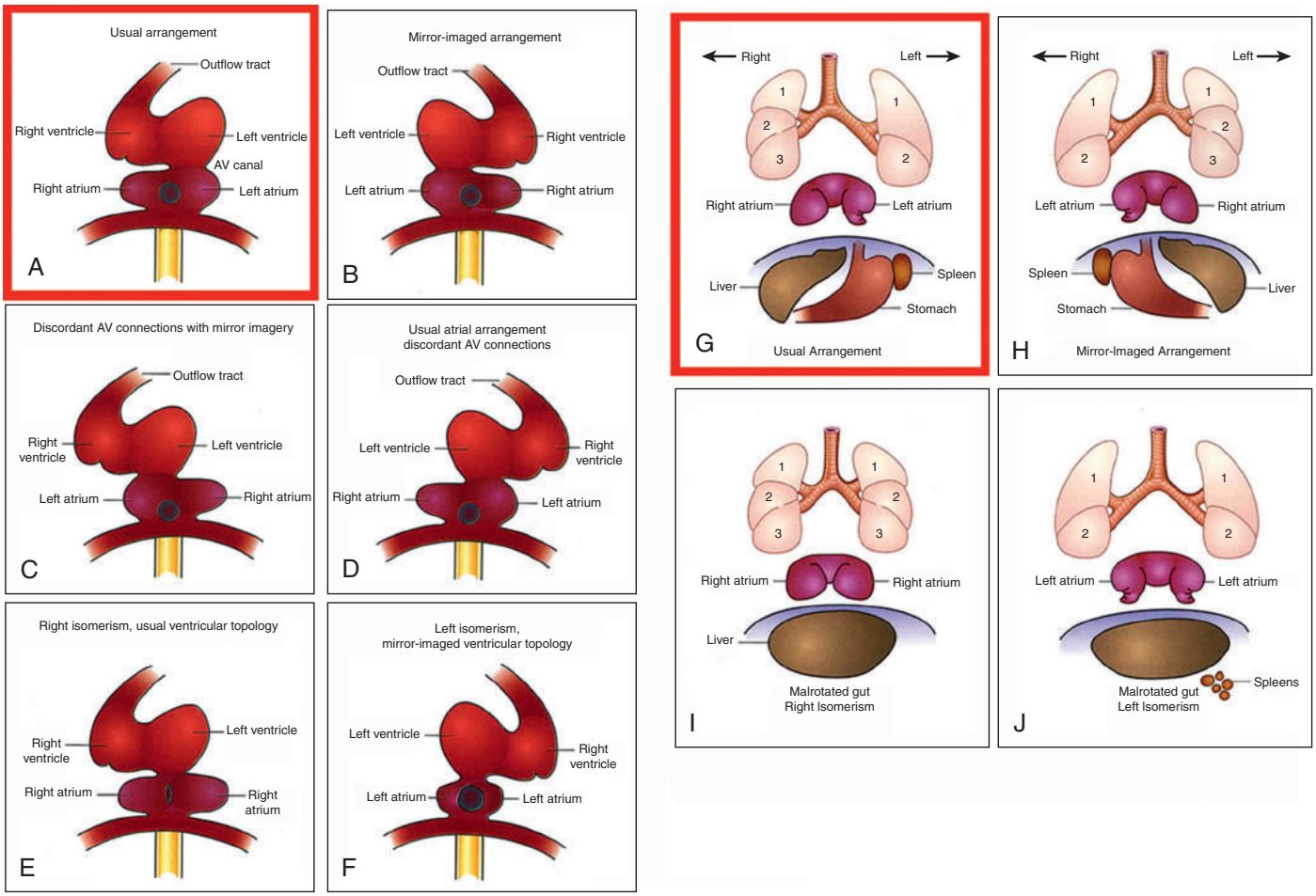
If there is complete mirror imagery, the cardiac connections and physiology are normal, but the heart is on the right side of the body (dextrocardia) and atrio-ventricular and ventriculo-arterial connections are reversed, or (I,L,L). Sometimes there is both atrioventricular and ventriculoarterial discordance, an entity referred to as congenitally corrected transposition. Here the atrial situs may be normal, ventricles looped to the left, with the bulbus cordis to the left of the primitive ventricle (Fig. 45.4D). When the great arteries septate, there is usually L malposition of the great arteries, so the aorta is to the left of the pulmonary artery (S,L,L). The resulting circulation is: right atrium to left ventricle to pulmonary artery; left atrium to right ventricle to aorta. In isolation, then, this defect allows for normal circulation—although with the right ventricle, which is structured to pump to the low-pressure pulmonary circulation, as the systemic ventricle. Alternately, this physiology can occur with atrial situs inversus, D looping of the ventricles, and D malposition of the great arteries, (I,D,D; Fig. 45.4C). Often however there are additional cardiac defects ranging from a ventricular septal defect (VSD) with pulmonary valve stenosis to more complex defects involving hypoplasia of one of the ventricles or hypoplasia, aplasia, or malformation of one or more valves.

L-looping may also be associated with more global defects known as heterotaxy syndromes. Heterotaxy syndromes are a heterogeneous group of congenital defects, with the commonality being abnormal left-right asymmetry of the embryo.[76] In heterotaxy, left-right assignments along the body axis are randomized. Thus it is possible to have mirror image asymmetry, or bilateral symmetry, with either two right sides or two left sides. This is often termed right or left isomerism (Fig. 45.4E, F and I, J). This can affect the abdomen, lungs, and heart. In the abdomen, the gut, liver, and spleen may be reversed from normal (abdominal situs inversus). There may be malrotation of the bowel and anomalies of



• **Fig. 45.3** Fusion of the heart tube and looping. (A) Mouse embryos immunostained for cardiac troponin T *(red)*, a marker of cardiac muscle, and Dapi, a marker of cell nuclei. Shown are early fusion into a transverse oriented tube, further fusion into an open tube, and final fusion into the linear heart tube. Beneath each is a 3D rendering from cardiac troponin T signal reconstruction showing ventral and dorsal views. (B) Schematic of the stages of heart tube looping. (A adapted from Ivanovitch K, Temino S, Torres M. Live imaging of heart tube development in mouse reveals alternating phases of cardiac differentiation and morphogenesis. *Elife*. 2017;6:e306686; B from Carlson BM. *Human Embryology and Developmental Biology*. 4th ed. Philadelphia: Elsevier; 2009:457.)

the biliary tree. The spleen, being a normally left-sided structure, can be duplicated in left isomerism (polysplenia), and absent in right isomerism (asplenia). Lung abnormalities in heterotaxy manifest in the bronchial and lung anatomy as shown in Fig. 45.4. In the heart, heterotaxy causes randomization of atrial sidedness. As discussed above, the atria may be normal (situs solitus) or left-right reversed (situs inversus); sometimes it is unclear (situs ambiguous; including bilateral right or bilateral left atria). Clinically, the atria are most practically defined by their venous return, systemic to the right atrium and pulmonary to the left. But in heterotaxy, this is not always possible, as the pulmonary or systemic veins may return to both atria. The pulmonary veins grow in from the



• **Fig. 45.4** Normal and abnormal looping patterns. (A) Diagram of normal cardiac D-looping with normal AV connections. (B-D) Several common abnormal looping patterns, including mirror image L-looping with normal AV connections (B); discordant AV connections with atrial situs inversus (C); and with normal atrial situs (D). Both (C) and (D) are usually associated with discordant ventriculoarterial connections, creating congenitally corrected transposition. (E, F) Right and left atrial isomerism. (F) Bilateral right-sidedness and bilateral left-sidedness generally found along with anomalies of the systemic and pulmonary veins, of cardiac septation, and of the inflow and outflow tracts. Right and left isomerization syndromes also go along with abnormal sidedness on other asymmetric organs of the chest and abdomen, or heterotaxy. (G) Normal visceral organ arrangement. (H) Mirror image, or situs inversus. (I) Right isomerism. (J) Left isomerism. (Adapted from Brown N, Anderson R. Symmetry and laterality in the human heart: developmental implications. In: Harvey RP, Rosenthal N, eds. *Heart Development*. Academic Press; 1999:449, 459.)

developing lungs normally joining the back wall of the left atrium. When the left-right cues are scrambled, the pulmonary veins often return ipsilaterally: left-sided veins to left-sided atrium and right-sided veins to right-sided atrium. Likewise, the systemic veins may return to either or both atria in heterotaxy—for example, a right superior vena cava to right atrium and left superior vena cava to left atrium. This is because the systemic veins start out as paired symmetric structures (see section on systemic veins), with the involution of certain structures occurring as part of the establishment of left-right asymmetry in the embryo. Finally, certain patterns of additional cardiac defects often accompany asplenia/right isomerism. These include failure of septation of the ventricles resulting in a single ventricle, pulmonary underdevelopment (stenosis or atresia of the pulmonary valve), bilateral superior vena cava, and anomalous pulmonary venous return. For polysplenia/left isomerism, commonly associated defects include endocardial cushion defects (see below), left-sided obstructive lesions such as coarctation of the aorta, and interruption of the inferior vena cava with azygous continuation to the superior vena cava.

Ventricular Inlet Septation: Endocardial Cushions

After cardiac looping, the orientation of the heart tube has changed drastically, but the progression of blood flow through it remains in essentially the same sequence: into the heart from the venous pole, through the common atrium into the common ventricle, and out the conotruncus to the aorta. Inflow and atrial segments are leftward, and the ventricle and outflow are to the right. If the

process arrests near this point, a heart may form with its entire inlet portion aligned over the leftward ventricle (a double inlet left ventricle) or its entire outlet portion aligned over the rightward ventricle (a double outlet right ventricle). Both entities are seen in humans. What are very rarely seen are a double inlet right ventricle and a double outlet left ventricle—even if there is L-looping and/or dextrocardia, this basic sequence is maintained.

During the next phase of heart development, the atrioventricular (inlet) and ventriculoarterial (outlet) structures will realign and septate, such that there is a valved inlet and a valved outlet for each ventricle. In human embryos, this occurs during weeks 6 to 7 of gestation.[77,78] These valves are formed primarily from the endocardial cushions. The endocardial cushions are also critical to complete ventricular and atrial septation, completing the portions of the septum adjacent to the AV valves (detailed further in sections on atrial and ventricular septation). Thus the endocardial cushions form the crux of the heart: a point in the center of the heart at which separate AV canals are to the left and right, and atrial and ventricular septa are aligned above and below.

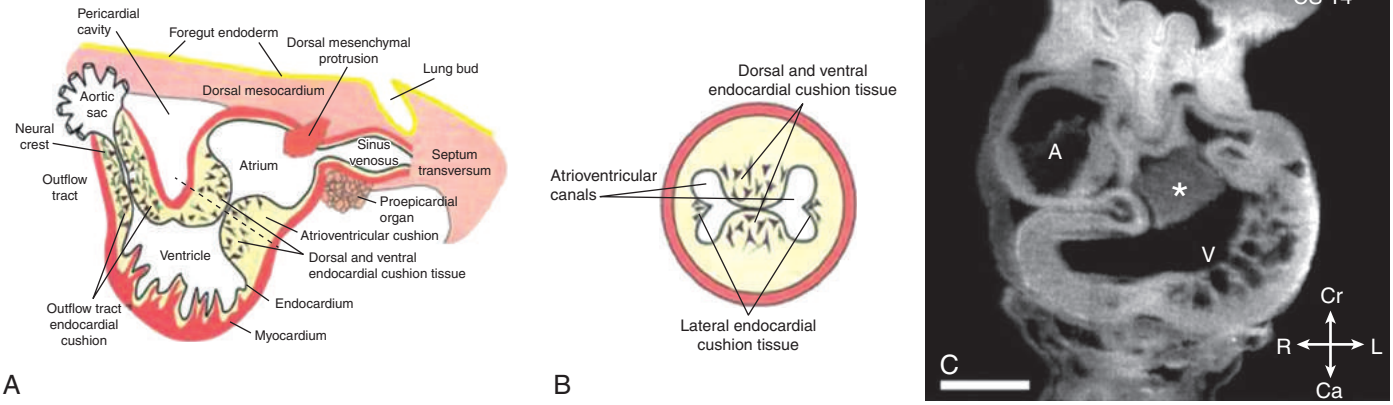
The endocardial cushions initially appear as swellings in the atrioventricular and conotruncal segments of the primitive heart by 6 weeks' gestation (Fig. 45.5). The swellings are caused as cells from the inner lining of the heart (endocardium) delaminate and migrate into the extracellular matrix in between the endocardium and the myocardium. The delaminating cells change phenotype in a process known as epithelial to mesenchymal transformation (EMT). The cells lose their epithelial, or sheet-like, characteristics and acquire a mesenchymal phenotype, losing junctions with neighboring cells, invading into the matrix, and proliferating faster.[18,79–81] In both the AV canal and in the conotruncus, four distinct cushions form, named by their anatomic locations. For the AV canal these are: superior (ventral), inferior (dorsal), left lateral, and right lateral (Fig. 45.5B). In the conotruncus they are: right superior, right dorsal, left inferior, and left ventral. The AV and conotruncal cushions are separate with one important exception: the left ventral conal cushion and the superior AV cushion are in continuity along the inner curvature of the heart (Fig. 45.5A). This proximity will persist in the fully septated heart as aortic-mitral valve continuity. The atrioventricular canal septates as the superior and inferior cushions grow and extend, finally fusing in the midline. This results in complete separation of the AV canal into left (mitral) and right (tricuspid) sides. The AV valves form from remnants of the cushions: a leftward mitral valve with two leaflets, and a rightward tricuspid valve with three.

Clinical Abnormalities of the AV Canal

Failure of complete fusion between the superior and inferior cushions in the midline results in a cleft within the anterior leaflet of the mitral valve. This is a mild form of an endocardial cushion defect, often associated with a primum atrial septal defect (see section on atrial septation) and known as a partial atrioventricular canal. The septal leaflet of the tricuspid valve also forms from the superior and inferior cushions and can also be affected, although this is less often clinically significant. More profound failure of proper AV cushion expansion and fusion results in a complete atrioventricular canal. Here the crux of the heart is unformed. There remains a common orifice overlying both ventricles, with defects in the adjacent atrial and ventricular septa. The common atrioventricular valve has a leaflet structure based on the unseptated AV canal, with four or sometimes five separate leaflets corresponding to the two lateral, the superior (referred to as anterior bridging), and inferior leaflets.

Ventricular Outflow Tract Septation: Endocardial Cushions and Neural Crest

While the AV cushions form a three-dimensional crux of the heart, the conotruncal cushions form a three-dimensional spiral, completing an almost 180-degree rotation. The outflow tract



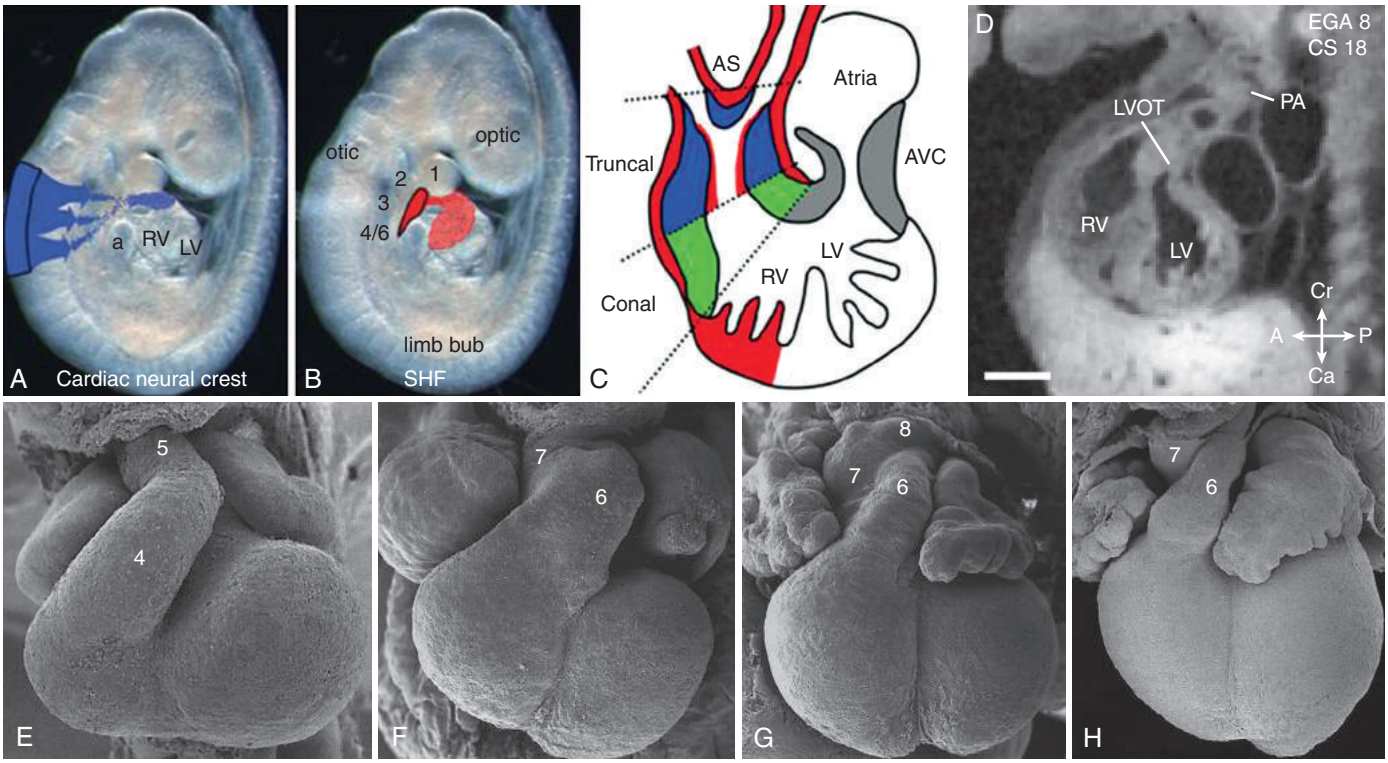
• **Fig. 45.5** Formation of the endocardial cushions. (A) Schematic diagram of cushion formation in the inflow and outflow tracts by epithelial to mesenchymal transition in the looped heart tube. Also shown are the neighboring tissues and cell populations contributing to the developing heart at this stage. (B) Schematic of the septating AV canal. (C) EFIC (episcopic fluorescence image capture) image of a sectioned paraffin-embedded human embryos at 6 6/7 weeks. Seen are atria (A), ventricles (V), and AV cushions (*). Scale bar = 0.515 mm. (A, B from Schoenwolf G, Bleyl S, Brauer P, Francis-Wested P, eds. *Larsen's Human Embryology*. Philadelphia: Elsevier; 2015; C from Dhanantwari P, Lee E, Krishnan A, et al. Human cardiac development in the first trimester: a high-resolution magnetic resonance imaging and episcopic fluorescence image capture atlas. *Circulation*. 2009;120:347.)

myocardium arises from the secondary heart field (Fig. 45.6B and C, red). During septation of the outflow tracts, the primitive outflow tract is separable into two segments: the truncus adjacent to the aortic sac and the conus adjacent to the ventricular myocardium (Fig. 45.6C, *blue* and *green*, respectively). The right superior and left inferior cushions are within the truncal region and will contribute to the aorticopulmonary septum. This region undergoes important interaction with the migrating neural crest cells (Fig. 45.6A, *blue*). The right dorsal and left ventral cushions are within the conus region and are important for pulmonary and aortic valve formation and for completion of septation between the ventricles at the level of the pulmonary and aortic valves. The interface between the two sets of cushions is a critical region for the formation and remodeling of the ventricular outflow tracts.[45,82,83]

Specifically, continuity between the superior AV cushion and left ventral conus cushion is maintained in the development of the left ventricular outflow tract. The conus is entirely maintained over the developing right ventricular outflow, creating a "neck" of tissue between the inflow and outflow segments.

Clinical Conotruncal Defects and DiGeorge Syndrome

Clinically, there are several defects seen in humans related to abnormal conotruncal development. These include truncus arteriosus, interrupted aortic arch, tetralogy of Fallot, double-outlet right ventricle, and conotruncal ventricular septal defects. In truncus arteriosus, the conotruncus completely fails to septate, leaving a single outflow tract aligned over both ventricles with an outlet VSD. In interrupted aortic arch, there is a posterior deviation of the conal septum, leading to a VSD and failure of proper arch development. In tetralogy of Fallot, there is an anterior deviation of the conal septum, with multi level pulmonary outflow tract obstruction, a VSD with aortic override, and hypertrophy of the right ventricle. In double-outlet right ventricle, there is the failure of a complete rotation of the conotruncal cushions such that there is malalignment of both great arteries over the anterior right ventricle, along with a ventricular septal defect.[84,85]



• **Fig. 45.6** Septation of the outflow tracts. Contribution of neural crest (A, *blue*) migrating into secondary heart field (B, *red*) in developing outflow tracts in the mouse embryo. (C) Schematic shows extent of these tissues, as well as the contribution from conal endocardial cushions *(green)*. *AS*, aortic sac; *AVC*, atrioventricular canal; *RV*, *LV*, right and left ventricles.) (D) EFIC (episcopic fluorescence image capture) image of an embryo at 8 weeks showing LV and developing LV outflow tract (LVOT) and the anterior RV and a portion of the pulmonary artery (PA). Scale bar = 1.35 mm. (E-H) Septation of the outflow tracts, as viewed externally by scanning electron microscopy. (E) Human embryo at 5 weeks' gestation; the conus and truncus of the common outflow tract are labeled 4 and 5, respectively. (F) Human embryo at 6 weeks' gestation. Separating pulmonary (6) and aortic (7) outflows are starting to become apparent. (G, H), Human embryo at 7 and 8 weeks' gestation, respectively, with increasingly distinct separate pulmonary and aortic outflow tracts. (A-C from Neeb Z, Lajiness JD, Bolanis E, et al. Cardiac outflow tract anomalies. *WIREs Dev Biol*. 2013;2:499–530; D from Dhanantwari P, Lee E, Krishnan A, et al. Human cardiac development in the first trimester: a high-resolution magnetic resonance imaging and episcopic fluorescence image capture atlas. *Circulation*. Jul 28 2009;120:347; E-H adapted from Steding G. *The Anatomy of the Human Embryo*. Basel: S. Karger AG; 2009.)

Human DiGeorge syndrome is characterized by cardiac conotruncal and arch defects along with developmental defects of the parathyroid, thyroid, and thymus glands.[6] A deletion of up to three megabases in chromosome 22 (22q11) has long been known to be associated with DiGeorge, based on kindred studies in families with the syndrome.[57,86–88] In humans, the defect is autosomal dominant, with variable penetration, meaning that one copy of the gene deletion is sufficient to cause disease and that the disease severity varied from family member to family member. Experimentally, chick models of neural crest ablation show a very high prevalence of conotruncal defects along with abnormal parathyroid, thyroid, and thymus development.[6,9,10,89–91] More recently, mouse models of DiGeorge syndrome implicated TBX1 as a single gene defect mimicking the human DiGeorge phenotype.[55,57,92] Homozygous mutations of TBX1 caused the obliteration of the third, fourth, and sixth pharyngeal arches with embryonic lethality and caused most of the extra-cardiac defects as well, along with an abnormal ear, jaw, and pharynx.[55] Heterozygous mutants, which are analogous to the human disease state, showed more variable disease.

Interestingly, isolated TBX1 mutations appear to be very rare as causes of clinical DiGeorge syndrome;[86,88,93] most patients have a larger megabase deletion. Since the link between TBX1 and DiGeorge syndrome was discovered, a better understanding has gradually emerged. As already discussed, TBX1 is a transcription critical for proper patterning and incorporation of the secondary heart field into the developing heart. Importantly, TBX1 expression is not found in neural crest cells but is critical for interactions between the neural crest and secondary heart field.[94–96] There are clearly other modifying genes important to the pathogenesis of conotruncal defects, notably Wnt factors,[97] the BMP inhibitor Smad7,[98] and retinoic acid signaling genes.[99] Interestingly, overexpression of TBX1 in mouse models are also associated with conotruncal defects suggesting that there are critical levels of TBX1 necessary for proper outflow tract development.[95,100]

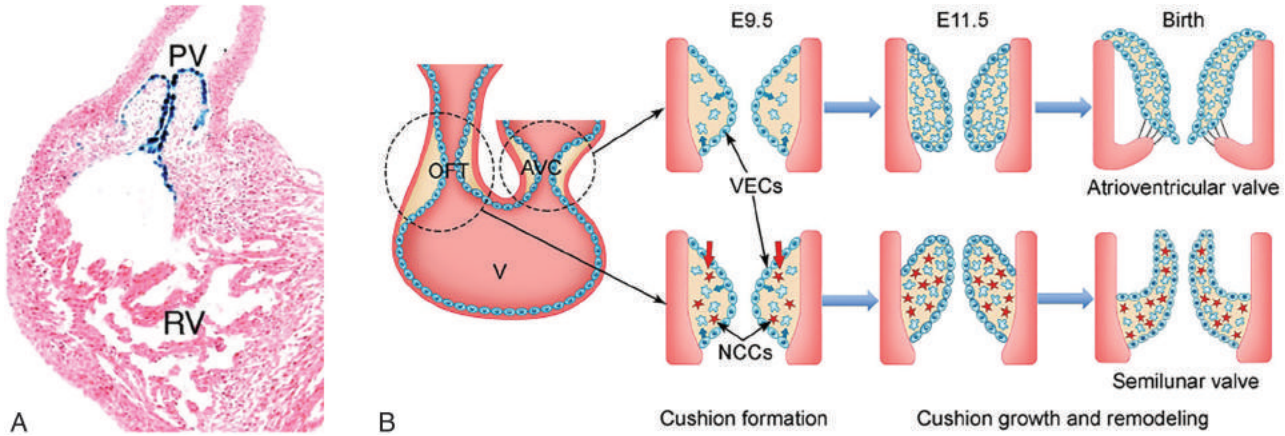
Separation of Aorta and Pulmonary Artery

Within the truncus segment, septation is also occurring during the remodeling of the conus. This normally occurs as the truncal cushions fuse in a spiral fashion. Starting at the level of the AV valves, the newly forming pulmonary artery is directly anterior to the aorta. Further along the outflow tract, the pulmonary artery and aorta are more left-right to one another, as the spiral extends. As the aorta arches to the left, the proximal transverse arch passes anterior to the pulmonary artery, completing a 180-degree turn of the spiral. This sequence is seen in Fig. 45.6E.[78] This rotation not only involves the truncal cushions but also the myocardial tube.[101,102]

If this 180-degree twist does not occur or is incomplete, the result is transposed great arteries. This is defined by the aortic valve being anterior to the pulmonary valve. The left-right orientation of the valves is most commonly preserved (aortic rightward), although it can vary within a 90-degree range from directly anterior-posterior to directly side by side. L-transposition, aortic leftward, is most often associated with L-looped ventricles or heterotaxy syndromes and is rare in isolated transposition. Transforming growth factor-β (TGF-β) signaling, important in left-right patterning and extracellular matrix remodeling and many other processes in normal development, appears to play an important role in this process both in mouse models and in human disease.[103–106]

Cardiac Valve Formation

Cardiac valve development begins with the endocardial cushions during week 7 of gestation, but the formation of mature atrioventricular (mitral and tricuspid) and semilunar valves (aortic and pulmonic) takes several weeks for completion.[18,20,77,78,80,107,108] As the endocardial cushions enlarge, they protrude into the lumen of the developing heart and outflow tracts, as shown in Fig. 45.7A. Here, subject to the constant flow of blood, the protrusions



• **Fig. 45.7** Cardiac valve formation and maturation. (A) Endothelial marker Nfatc1 expression marks the endocardial surface of the valves, with mesenchymal cushion tissue underlying. *PV*, pulmonary valve; *RV*, right ventricle. (B) Schematic of semilunar valve formation and remodeling in the outflow tract (OFT) and AV canal (AVC). Valve endocardial cells (VECs) undergoing epithelial to mesenchymal transformation and populating the cushions, as do migrating neural crest cells (NCCs). This is followed by growth, thinning, and elongation of the atrioventricular and semilunar valves. (A from Wu B, Wang Y, Lui W, et al. Nfatc1 coordinates valve endocardial cell lineage development required for heart valve formation, *Circ Res*. 2011;109:183–192; B from Wang Y, Fang Y, Lu P, et al. NOTCH signaling in aortic valve development and calcific aortic valve disease. *Front Cardiovasc Med*. 2021;8:682298.)

condense and elongate (Fig. 45.7B). Endocardial cells overlying the cushions proliferate slowly, and there is programmed cell death (apoptosis) of mesenchymal cells underneath and an influx of neural crest cells. The valves undergo extensive remodeling of the leaflets and of the extracellular matrix. In the case of the semilunar valves, this remodeling process of the endocardial cushions continues through the last trimester of pregnancy and into the neonatal period.[109,110]

Clinical Valve Abnormalities and Noonan Syndrome

Clinical valve disease is common in human disease and is generally related to incomplete growth and maturation processes of valve leaflets, leaving them immature, thickened, and unable to open and/or close properly. Noonan syndrome is characterized by cardiac malformations including a dysplastic pulmonary valve, systemic features of short stature, facial abnormalities, and a risk of myeloproliferative disease.[111] Linkage studies of families with Noonan syndrome suggest a candidate gene on chromosome 12, the PTPN11 gene.[112] The PTPN11 encodes for SHP-2, a signaling molecule important to growth factors, cytokines, and hormones,[112] although other causative alleles have also been identified.[111] Human mutations in the gene have been shown to cause over-activation of the SHP-2 protein.[111,113] This suggests a gain of function mechanism for the pathogenesis of Noonan syndrome, an interesting distinction from the more familiar concept of loss of functioning protein causing disease. Likewise, mutations in the Notch1 receptor and various components of the Notch signaling pathway have been associated with the formation of aortic valves and with multiple stages of cardiac development.[114,115]

Development of the Ventricles and Ventricular Septum

Most of the ventricular septum is muscular and made up of a protrusion of ventricular myocardium that starts at the apex of the primitive ventricle and extends into the ventricular cavity. The early septating ventricle, then, appears as a bilobed structure[77,78] (Fig. 45.8A). As the septum extends upward, the separation becomes nearly complete (Fig. 45.8B). There is controversy as to how the septum extends so quickly to form a wall between the left and right ventricles. Part of the answer seems to be rapid proliferation in these myocytes, which retain the ability to divide even as a working myocardium. This property is lost soon after birth, as the mature myocardium is for the most part incapable of proliferation. Small muscular ventricular septal defects are very common in newborns. One study screening asymptomatic newborns found an incidence of 53 per 1000 neonates with small muscular VSDs; only 1/10 of these had physical exam signs of their VSD. Nearly ninety percent of these small defects were closed by 10 months of age.[116] These data and others suggest continued low-level proliferation in human ventricles after birth.

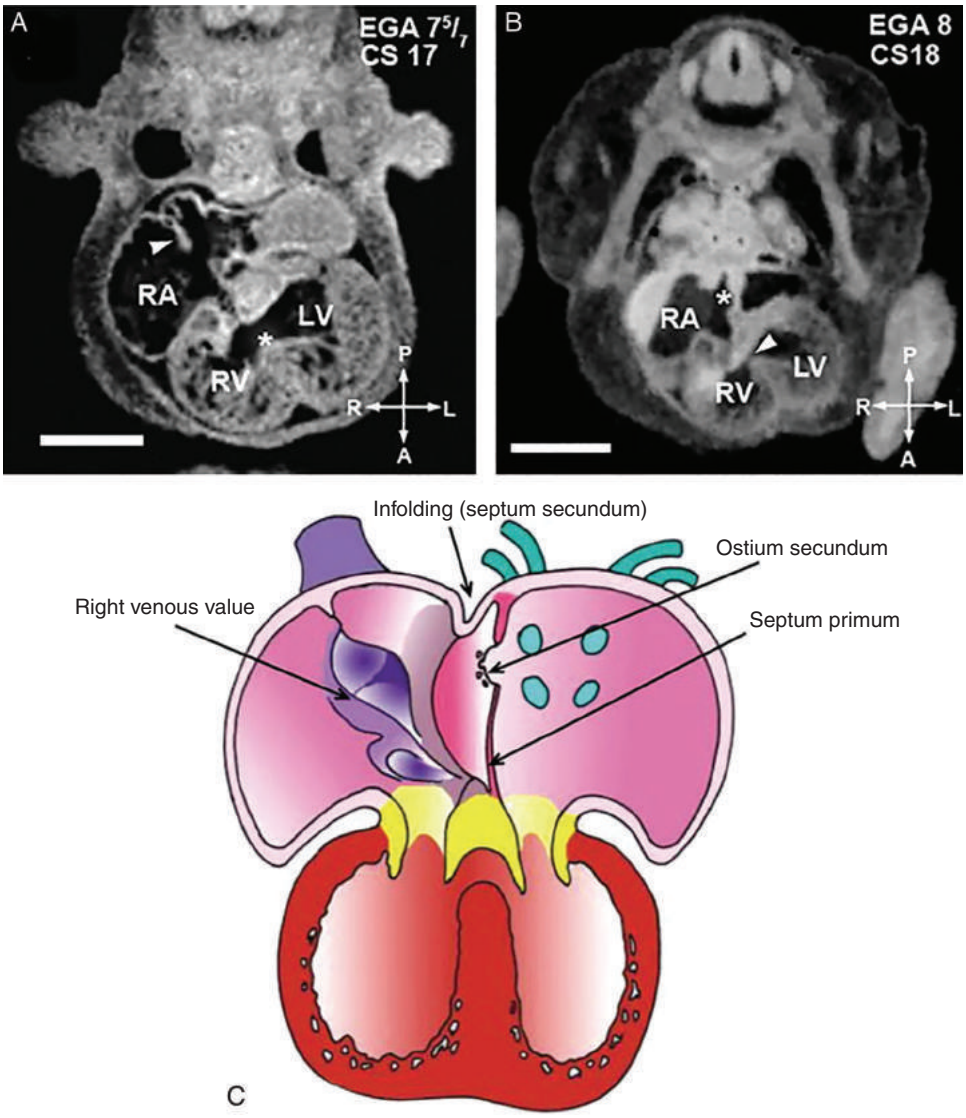
While most of the mass of the ventricular septum grows and extends from the ventricular apex, there are two important regions of the complete ventricular septum that are completed by endocardial cushions, as discussed in the above sections. First, the AV endocardial cushions form the posterior septum adjacent to the AV valves. Second, the conal cushions form the conal septum below the great arteries.

The left and right ventricles differ not only in their relative positions to one another but also in their basic muscular structure and in their function. Each ventricle can functionally be subdivided into inflow and outflow regions. The RV, as we have discussed, has a conal segment as well, and is therefore referred to as a tripartite ventricle. In the mature heart, the LV is a high-pressure system, connected to systemic pressures, while the RV is connected to the lower-pressure pulmonary circulation. The LV form follows its function, with concentric rings of myofibrils that contract with a slight twisting motion, which allows for an efficient ejection of blood. In the embryo, the myocardium thickens and arranges itself in processes known as trabeculation and compaction. Trabeculation refers to projections of muscular tissue into the lumen of the ventricle, such that the inner surface is no longer smooth-walled but ridged. The process of trabeculation is regulated by endocardial to myocardial signaling in the form of Tie2 and retinoic acid signaling.[117] Additional genetic factors shown to have a role in trabeculation include Notch 1, Neuregulin, and Bmp10.[32] Compaction refers to the alignment of myocytes from random and loosely packed, as they are in the immature myocardium, into bundles of tightly packed and well-coordinated myocytes working as a unit in the mature myocardium.[75] Non-compaction of the LV is recognized clinically as a "spongy" appearing myocardium and is increasingly recognized as a form of cardiomyopathy although can remain entirely asymptomatic. This entity is linked to genes regulating cell cycle regulators including the oncogene N-myc.[32]

Development of the Atria and Atrial Septum

Like the ventricular septum, the atrial septum begins as a ridge of muscular tissue, posterior and midline, which expands to divide the right side of the atrium containing the orifice of the sinus venous from the left side of the atrium containing the orifice of the common pulmonary vein. This structure is known as the septum primum, and it grows toward the fusing AV cushions (see Fig. 45.8A, *arrow*). As discussed above, the fusing AV cushions will form the inferior portion of the atrial septum (Fig. 45.8B, *asterisk*). Prior to septum primum and the cushions completing atrial septation, however, small perforations appear, enlarge, and coalesce within the primum septum (Fig. 45.8C). This is referred to as the ostium secundum. Soon afterward, a new crescent of atrial septum forms on the right atrial side of the septum primum and begins extending alongside the septum primum; this is referred to as the septum secundum. During fetal life, blood flow from the right atrium into the left prevents fusion of these two septa. The septum secundum is significantly more rigid than the septum primum, which flaps open to allow continued right to left flow. After birth and separation from the low resistance placenta, the left atrial pressure rises significantly and the atrial shunt reverses.

Failure of the cushions to form the inferior-most part of the atrial septum results in a primum atrial septal defect (Fig. 45.8C, *yellow*). Such defects do not close spontaneously and may be associated with other endocardial cushion defects, most commonly a cleft mitral valve. Finally, there is a small contribution to the atrial septum near the junctions of the superior and inferior vena cava (Fig. 45.8C, *purple*). Defects in this process result in a sinus venosus ASD, and there are superior or inferior types. Such defects again result from deficiency of atrial septal tissue and do not close spontaneously.



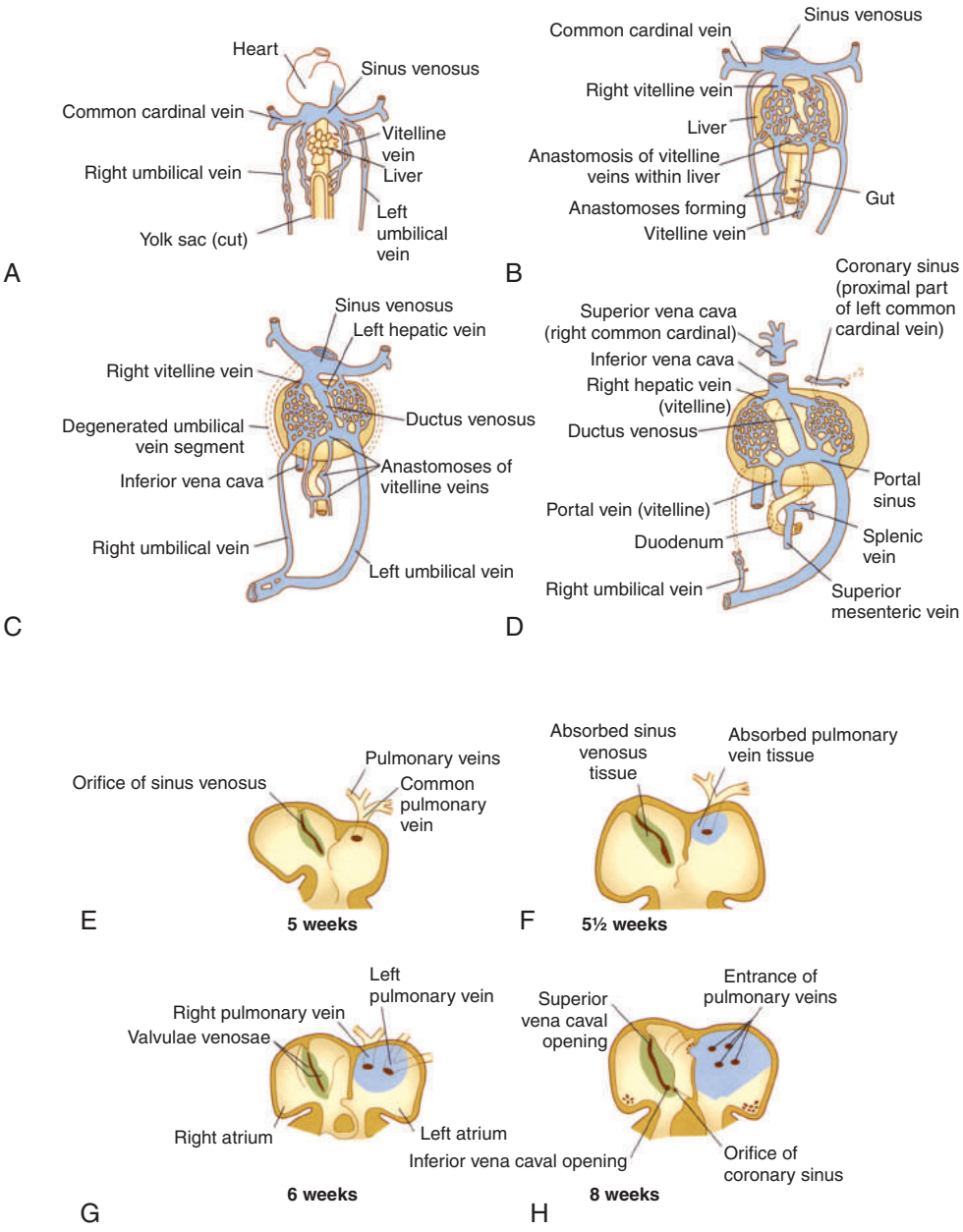
• **Fig. 45.8** Atrial and ventricular septation. (A, B) Human fetal MRI images in transverse plane showing four chambers of the heart (*LV*, Left ventricle; *RA*, right atrium; *RV*, right ventricle). Orientation as shown by the arrows: P (posterior), A (anterior), R (right), and L (left). (A) Fetus at 7 5/7 weeks' gestation. The atrial septum primum is marked by the arrowhead, with the foramen primum below allowing shunting of blood from RA to LA. Between the ventricles, the muscular intraventricular septum is forming but is not yet complete (*). Scale bar is 1.25 mm. (B) Fetus at 8 weeks' gestation. Here the atrial septum primum (*) is fused with the endocardial cushions inferiorly; not seen is the foramen ovale that allows continuous right to left atrial shunting in the fetus. The arrowhead shows a small residual ventricular septal defect in the inlet (posterior) septum, not yet closed by endocardial cushion tissue. Scale bar is 1.5 mm. (C) Schematic of atrial septation, structures as labeled and described in the text. (A, B adapted from Dhanantwari P, Lee E, Krishnan A, et al. Human cardiac development in the first trimester: a high-resolution magnetic resonance imaging and episcopic fluorescence image capture atlas. *Circulation*. 2009;120:345–346.) (C from Naqvi N, McCarthy KP, Ho SY. Anatomy of the atrial septum and interatrial communications. *J Thorac Dis*. 2018; 10:S2837-S2847.)

Systemic and Pulmonary Vein Development

The sinus venosus of the primitive heart tube is a symmetric structure and receives three sets of paired (left and right) systemic veins: the vitelline, umbilical, and common cardinal (Fig. 45.9A). This is the situation at 4 to 5 weeks' gestation.[118]

The vitelline veins return from the yolk sac, a structure that communicates with the primitive gut via the vitelline duct. The duct normally regresses completely, but an occasional remnant can be seen as a Meckel diverticulum.[1] Distally, the vitelline veins regress along with the duct and proximally lose their connection to the sinus venosus proximally by 6 weeks. The midportions of these vessels, however, expand within the liver to contribute to the hepatic and portal veins and contribute a small segment to the inferior vena cava.

The umbilical veins return from the placenta carrying oxygenated blood. These are the first to develop, by 3 weeks' gestation. As the liver develops, the umbilical veins develop connections to the



• **Fig. 45.9** Systemic and pulmonary vein development. (A–D) Stages of development of the systemic veins (weeks 3–7), and (E–H) stages of development of the pulmonary veins (weeks 5–8) as described in the text. (From Carlson BM. *Human Embryology and Developmental Biology*. 4th ed. Philadelphia: Mosby; 2009:452–453.)

liver venous plexus and the connection to the sinus venosus involutes. Outside the embryo within the umbilical cord, the left and right umbilical veins fuse into one; thus there is a single umbilical vein in the newborn. Within the embryo, the left and right segments remain separate, and the left umbilical vein becomes connected to the right hepatic veins via a new channel that forms, the ductus venosus (Fig. 45.9C and D). This circulation is formed by the seventh week of gestation. Again the umbilical veins are carrying oxygenated blood to the right atrium. This flow is primarily directed toward the atrial septum, and therefore primarily then the foramen ovale into the left atrium and systemic circulation.

The cardinal veins carry the venous return from the embryo proper. The left and right common cardinal veins are relatively short segments that connect to the sinus venosus. On each side, anterior and posterior cardinal veins join to form the common cardinal vein: the anterior carrying the venous return from the upper body, and the posterior from the lower body. The right anterior and right common cardinal vein will form the superior vena cava in the mature circulation. The left common cardinal vein and left segment of the sinus venosus form the coronary sinus, which receives venous return from the coronary system. These structures are pulled rightward as the IVC forms, such that the opening (os) of the coronary sinus ends up in the right atrium once atrial septation is complete. The inferior vena cava is more complex, and made up of five segments, coming from urogenital, mesenteric, and hepatic venous channels fusing together to the right of the spine as their leftward paired counterparts involute. As numerous vessels go into making the IVC, interruption of the IVC can

sometimes occur at various segments. This is most often in the setting of heterotaxy but can occur in isolation. When the IVC is interrupted, one of two major vessels that connect the lower and upper body venous systems generally enlarge to receive the flow and shunt it into the SVC. These are the rightward azygous vein, which runs from the suprarenal segment of the IVC to the SVC, and the leftward hemiazygos vein, which takes a more tortuous course from the leftward lumbar and renal veins up into the thoracic cavity, where it turns rightward to join the azygous vein. Thus an interrupted IVC generally has either azygous or hemiazygos continuation to the SVC.

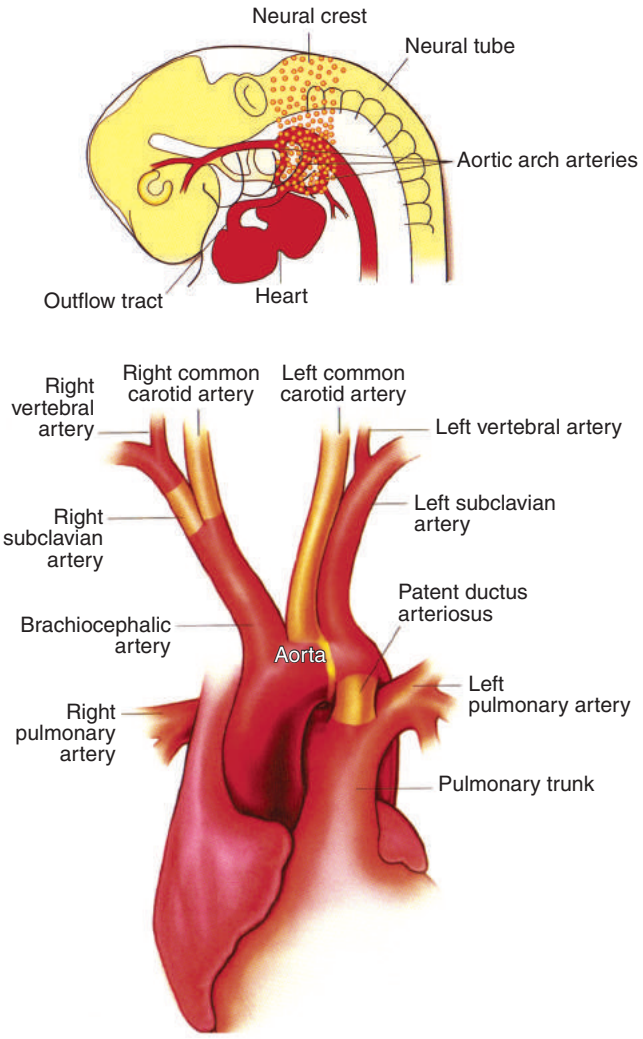
The pulmonary veins grow in progressively from the developing lung vasculature, first as a common pulmonary vein from both lungs. This vein fuses into the back of the left atrium.[119] As the atrium expands, the common pulmonary vein becomes increasingly absorbed into the back wall, such that at first two, then four of its distal branches eventually enter independently into the atrium, two from the left and two from the right lung (Fig. 45.9E–H). In the original atrial segment the heart tube is not part of the functioning atria in the mature heart, instead relegated to the right and left atrial appendages.

Aortic Arch Development

The primitive heart tube connects to the aortic sac, the precursor to the ascending aorta, and begins pumping to the developing systemic circulation of the embryo as soon as it is formed. The aortic sac connects to paired dorsal aortae posteriorly via an aortic root, which gives rise to a series of arches, the branchial arches (Fig. 45.10). It is along these arches that the neural crest migrates into the conotruncus and supports the development of the arch arteries. Again these are paired structures, and there is a total of six pairs, although not all are patent at the same time. Arches 1 and 2 mostly regress completely, although parts contribute to some of the arteries of the face. Arch 3 does not persist as an arch, but contributes to the formation of the carotid arteries, both left and right. The left limb of arch 4 remains in continuity with the aortic root. Together they make up the true aortic arch and its first branch, the brachiocephalic (innominate) artery. The right limb of arch 4 becomes part of the right subclavian artery, which retains its proximal connection with the brachiocephalic artery.[10] This arch remodeling has occurred by 7 weeks' gestation (Fig. 45.11). In some cases, either independently or as part of another developmental cardiac defect, the right limb of arch 4 remains patent and the left forms the left subclavian artery—this forms a right aortic arch with mirror-image branching. Arch 5 is small and never fully develops. Arch 6 forms with the developing pulmonary artery vasculature and makes up the proximal left and right pulmonary arteries and the ductus arteriosus.[9] The ductal arch persists throughout fetal life as another means of right to left shunt, allowing blood from the right ventricle to bypass the pulmonary circulation and cross over into the descending aorta.

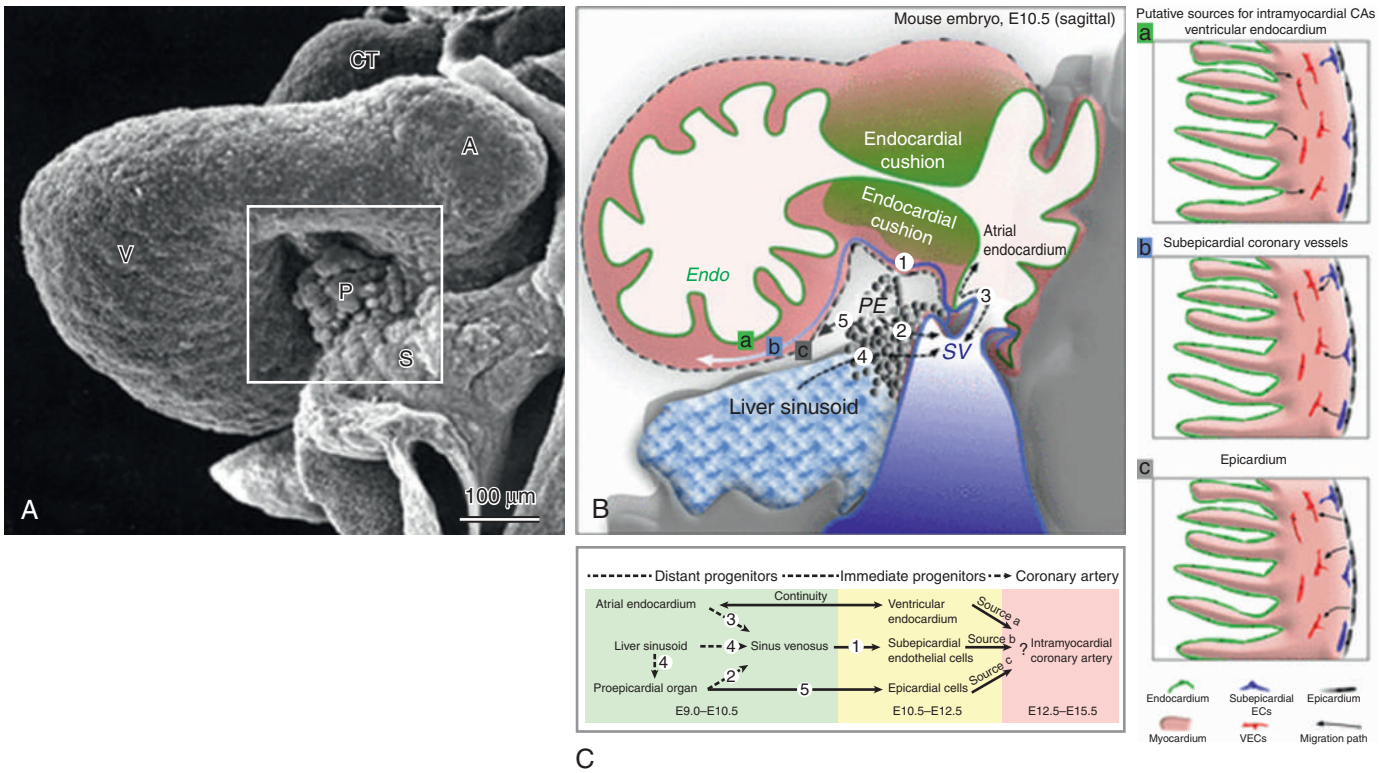
Coronary Arteries

The coronary arteries were for many years assumed to "sprout" from the ascending aorta and grow over the surface of the heart. This is now known to be incorrect. The coronary arteries form in situ within the myocardium and then connect to a coronary stem that forms within the aorta from peri-truncal capillaries.[120–124] The coronary vessels themselves form from several sources. One



• **Fig. 45.10** Aortic arch development. *Top*, Migration pattern of neural crest cells into pharyngeal arches 3, 4, and 6. *Bottom*, Normal aortic arch at term. Most of the pharyngeal arch segments have regressed. The third arch persists in part as the left and right common carotid arteries. The left fourth arch persists as the true aortic arch, while a portion of the right fourth arch makes up the proximal right subclavian artery. The sixth arch persists as the ductus arteriosus (ductal arch). (From Kirby ML. Contribution of neural crest to heart and vessel morphology. In: Harvey RP, Rosenthal N, eds. *Heart Development*. Academic Press; 1999:180–181.)

is the proepicardial organ (see Fig. 45.5A).[124–126] This is in close proximity to the sinus venosus, and there appears to be some movement of cells from one population to the other.[120] The proepicardial organ is a transient structure that spreads out over the surface of the heart as an epithelium, forming the epicardium. Early studies using lineage tracing methods in chick embryos suggested that the majority of coronary arteries, both endothelium and smooth muscle, were formed from the epicardium.[124,125] More recent studies have shown that while this is true for the smooth muscle component of the coronary arteries, the endothelium is more complex in origin. These studies have used detailed transgenic lineage tracing methods in mice to show important contributions from the endocardium[127] and sinus venosus.[128–130] The endocardium-derived cells invade the myocardium from the inner surface of the heart, migrating toward the epicardial



• **Fig. 45.11** Coronary vessel origins. (A) Scanning electron micrograph showing the developing proepicardial organ (P), Sinus venosus (S), atrium (A) ventricle (V), and conotruncus (CT). (B) Schematic of a mouse embryo at the same stage (E10.5) showing the various origins of the coronary plexus, including endocardium (endo, *green*), proepicardial organ (PE, *gray*), and sinus venosus (SV, *blue*). The panels at the right (*a,b,c*) correspond to magnified views as labeled in (B); *arrows* show migration paths of each cell type into the myocardium *(pink)*, using the same color coding as in (B); endocardium is in *green*, epicardium arises from the PE *(black)*, and subendocardial ECs are subendocardial endothelial cells arising from the sinus venosus *(blue)*. In *red* are the vascular endothelial cells (VECs) that form the coronary vessels. (C) Differentiation path of the various cell types with timing in the mouse embryo; numbers correspond to migration paths shown in (B). (A from Hiruma T, Hirakow R. Epicardial formation in embryonic chick heart: computer-aided reconstruction, scanning and transmission electron microscopic studies. *Am J Anat*. 1989;184132; B, C from Tian X, Pu WT, Zhou B. Cellular origin and developmental program of coronary angiogenesis. *Circ Res*. 2015;116:517.)

surface and differentiating into coronary artery endothelial cells. The cells from the sinus venosus migrate and expand within the subepicardial space, invading the myocardium as a distinct population from the epicardium. To add to the complexity of cell lineages, this process is not uniform throughout the ventricular myocardium. The coronaries within the ventricular septum appear to form differently from the ventricular free wall.[120,127] Regardless of ultimate origin, the coronary vessels form, grow and fuse with one another to form a working vasculature. This corresponds with the process of growth and trabeculation of the ventricles. With the increasing complexity and thickness of the myocardium, simple diffusion of nutrients from the blood inside the heart is no longer adequate. It is at this point, that the coronary artery plexus grows into the aorta just above the aortic valve. Signaling from the neural crest, epicardial-derived cells, and recently discovered intra-aortic cardiomyocytes all appear to play an important guiding role.[131–133] Occasionally this process can go awry, and a coronary artery (usually the left) can join into the pulmonary artery instead of the aorta. This does not damage the fetal myocardium given the low oxygen state of the fetus, with little difference in oxygen tension between the aorta and pulmonary artery. After birth, however, ischemia sets in rapidly, causing myocardial infarction in infancy.

Conduction System

The AV canal myocardium, part of the primitive heart tube, does not persist in the adult heart. There is no muscular connection between the mature atrial and ventricles. The process for isolation of the atrial and ventricular muscle appears to occur as the epicardium of the AV sulcus establishes continuity with the developing endocardial cushions beneath; this occurs all along the ventricular margin of the AV canal.[134] The only remaining connection that remains between atria and ventricles is within the conduction system, the AV node.[135–137] Occasional muscular bridges of tissue remain as accessory muscle connections, which can be clinically important as substrate for arrhythmias (specifically AV reciprocating tachycardia, a common form of supraventricular tachycardia).

Lineage tracing studies show that the cells of the conduction system differentiate in situ from myocytes.[138–142] There is evidence that the developing coronary vasculature is a source of signaling for this transdifferentiation to occur; the conduction system

tends to develop alongside coronary vessels.[139] Conduction system myocytes express different markers, junctions, and have different action potential profiles that allow for automaticity.[142–146] Under normal conditions, the automaticity of the remaining conduction system is suppressed as the dominant pacemaker of the heart forms, the sinoatrial node. Lineage tracing studies in early chick embryos showed that the SA node arises from a population of cardiac mesoderm outside of the primary and secondary heart fields that coalesce in the heart near the right sinus horn.[147] Activation mapping of embryonic chick heart conduction shows that the primitive SA node begins to function soon after cardiac looping (the site of earliest activation prior to this actually being in the left sinus horn).[147]

Conclusion

We have reviewed heart development, with attention to the morphology, cell biology, genetics, and physiology of the heart as it forms and remodels. The field of developmental biology of the heart continues to advance rapidly on all of these fronts. New technologies allow earlier and earlier visualization of the developing human heart, recently as early as week 6 gestation by cardiac MRI,[77] and week 10 to 11 by cardiac ultrasound.[148–155] Novel genetic techniques allow us to apply what is learned from animal models to humans, and to recreate human disease in animal models with greater and greater specificity. The common goals to be applied to human disease are early recognition of congenital heart malformations, prevention where feasible, and state-of-the-art intervention to allow an abnormal heart to function as normally as possible. Currently, such intervention is surgical and cardiac catheterization based and continues to evolve. In the future, gene therapy, cardiac stem cell grafting, and in vitro tissue engineering will likely be added to the therapeutic potential for patients with congenital heart malformations.

Suggested Readings

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46

Cardiovascular Compromise in the Newborn Infant

SHAHAB NOORI AND ISTVAN SERI

KEY POINTS

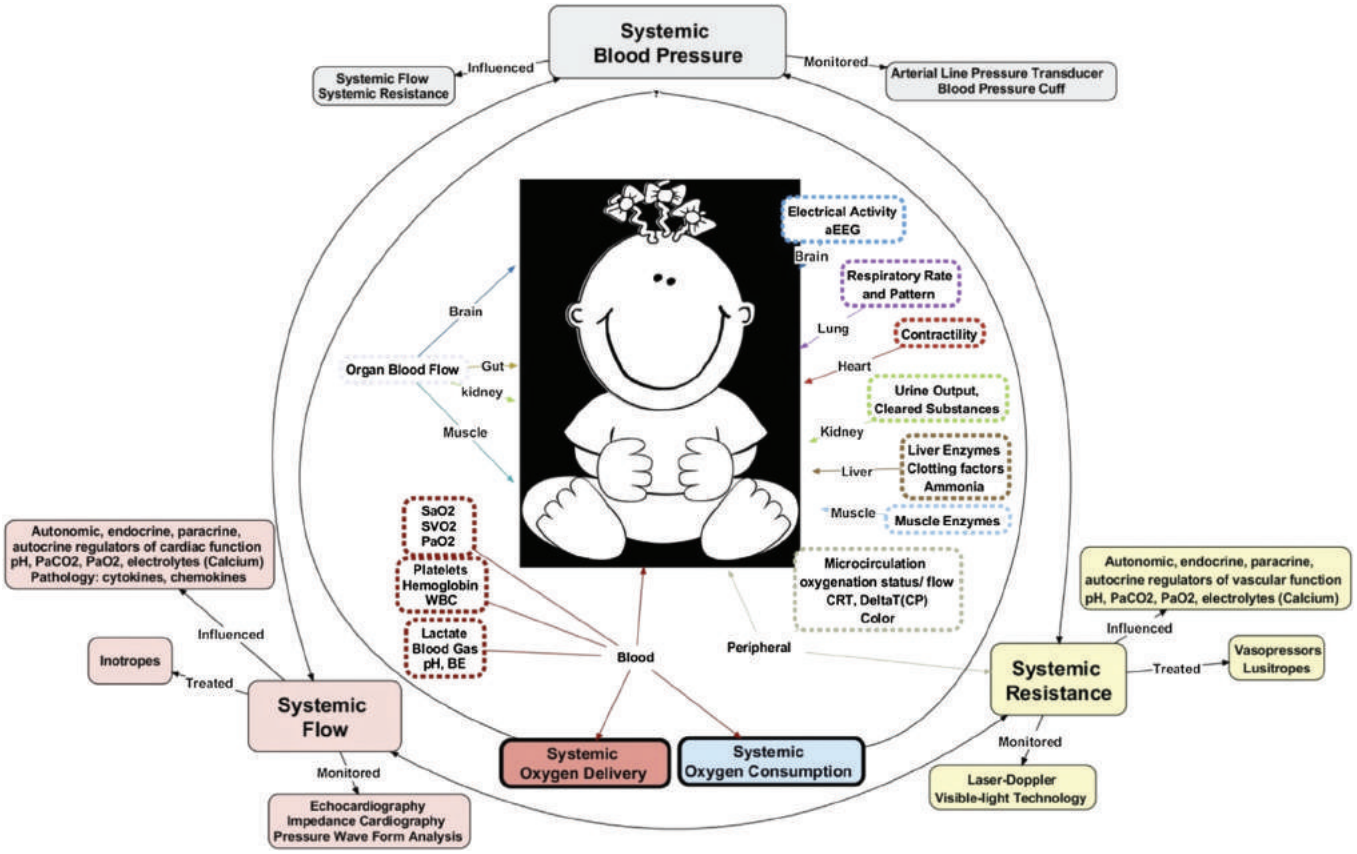
- It is difficult to diagnose neonatal shock in its *uncompensated phase* during the immediate transitional period, while it is even harder to diagnose neonatal shock in its *compensated phase* using standard clinical monitoring and clinical approach.
- It is unclear what gestational- and postnatal-age-dependent blood pressure and systemic and organ blood flow values represent hypotension and poor tissue perfusion (respectively), warranting timely intervention in the neonate.
- Although there is some recent evidence that management of neonatal hypotension/systemic blood flow improves clinically relevant outcomes, more data are needed.
- A thorough understanding of the principles of developmental cardiovascular physiology, the etiology, pathophysiology, and clinical presentation of neonatal shock, and the mechanisms of action, pharmacokinetics, and pharmacodynamics of medications used to treat cardiovascular compromise are essential for neonatal care providers.
- Recent advances in bedside monitoring technologies such as near-infrared spectroscopy and the application of point of care ultrasonography in assessing cardiovascular function hold promise for early diagnosis and better management of circulatory failure in neonates.

Although the prevalence of hypotension in neonates admitted for intensive care is unclear, up to 50% of very low birth weight (VLBW) neonates present with blood pressure values considered to be low in the immediate transitional period.[1] However, VLBW neonates account for only approximately 25% of all patients diagnosed with hypotension in neonatal intensive care units. The lack of clear data on the prevalence of neonatal hypotension is primarily due to the uncertainty about the lower limit of the gestational- and postnatal-age-dependent normal blood pressure range in neonates.[2] This is illustrated, among others, by the significant differences in the prevalence of the use of vasopressor/inotropes in preterm neonates during the transitional period among different intensive care units.[3–5] In addition, depending on the ability of the patient to compensate for the cardiovascular compromise, a given blood pressure value in a given patient might represent appropriate systemic and organ blood flow while at another point in time, it may signal compromised tissue perfusion. Therefore, considering only blood pressure and the fairly inaccurate indirect clinical signs of tissue perfusion makes it difficult if not impossible to accurately diagnose neonatal shock in time. Also, a timely diagnosis is one of the cornerstones of initiating effective treatment modalities.

As long as pulmonary gas exchange is adequate, shock is caused by hypovolemia, cardiac failure, vasoregulatory failure, or a combination of these etiologies. Shock has been defined as a "state of cellular energy failure resulting from an inability of tissue oxygen delivery to satisfy tissue oxygen demand."[6] According to this definition, when oxygen delivery is inadequate to meet oxygen demand, the organs start failing and, if corrective measures are not effective, will result in irreversible organ damage and ultimately death. Although oxygen delivery to the organs is dependent on several factors, it is fundamentally driven by both the oxygen content of the blood and the volume of blood flowing to those organs. Because oxygen content is primarily determined by the hemoglobin concentration and oxygen saturation, with less contribution from the dissolved oxygen (see later discussion), it is relatively easily evaluated and monitored in the neonatal intensive care unit. However, reliably assessing systemic and organ blood flow and tissue oxygen delivery and consumption at the bedside is challenging because, to provide adequate information on the rapidly changing hemodynamic status of the critically ill neonate, it requires continuous monitoring of key hemodynamic parameters in absolute numbers (Fig. 46.1).

Recent advances in our ability to monitor systemic and organ blood flow and tissue oxygenation as well as vital organ (brain) function at the bedside will likely lead to a better understanding of the complex hemodynamic changes associated with neonatal cardiovascular compromise.[7,8] These advances should lead to the development of treatment modalities more appropriately based on the etiology, pathophysiology, and phases of shock, thereby improving clinically relevant outcomes. The impact of treatment using some of these advances is currently under investigation.[9]

At present in clinical practice, tissue perfusion is routinely assessed by monitoring heart rate, blood pressure, capillary refilling time, acid-base status, serum lactate levels, and urine output. However, Doppler ultrasound and near-infrared spectroscopy



• **Fig. 46.1** Tools of Comprehensive Hemodynamic Monitoring. Tools used to provide a global *(outside circles)* and regional *(inside the circles)* assessment of developmental hemodynamics. Global monitoring of the relationship among systemic flow, blood pressure, and resistance and arterial/venous oxygen content provides information on systemic oxygen delivery and consumption. Regionally monitored parameters provide direct or indirect information on a specific organ blood flow, function, and vital or non-vital blood flow regulatory assignments. *aEEG*, Amplitude-integrated electroencephalography; *BE*, base excess; *CRT*, capillary refill time; *$PaCO_2$*, arterial partial pressure of carbon dioxide; *PaO_2*, arterial partial pressure of oxygen; *SaO_2*, arterial oxygen saturation; *SvO_2*, venous oxygen saturation; *WBC*, white blood cells. (Modified from Azhibekov T, Noori S, Soleymani S, et al. Transitional cardiovascular physiology and comprehensive hemodynamic monitoring in the neonate: relevance to research and clinical care. *Semin Fetal Neonatal Med*. 2014;19:45–53.)

(NIRS) data have highlighted that these parameters are relatively poor indicators of acute changes in organ blood flow and tissue oxygen delivery in critically ill neonates.[10–14] These observations and the very limited evidence that treatment of neonatal cardiovascular compromise and hypotension improves outcomes[3,15–17] call for a paradigm shift in our thinking about pathophysiology, diagnosis, and treatment of neonatal shock. This suggests that the assessment of the hemodynamic status in critically ill neonates should include the complex interactions between blood flow and blood pressure as well as tissue oxygen delivery and consumption.[18] A comprehensive, real-time hemodynamic monitoring and data acquisition system has been developed,[7,19,20] although its use is limited to clinical research at present. However, integration of point of care echocardiography and NIRS in clinical management of circulatory compromise has now been used at a number of centers and preliminary results are encouraging.[21] The reader is referred to the recent review on the applications and limitations of various advanced hemodynamic monitoring tools in neonates.[22]

Principles of Developmental Cardiovascular Physiology and Pathophysiology, Phases, and Etiology of Neonatal Shock

Principles of Oxygen Delivery

Oxygen is essential for mitochondrial respiration but is not stored in the body. Thus, interruption of oxygen supply to cells can result in irreversible damage (sometimes within minutes), particularly in vital organs such as the brain and myocardium.

The primary function of the cardiorespiratory system is to provide adequate oxygen delivery to tissues. Accordingly, shock is defined as inadequate systemic tissue oxygen delivery (see earlier). Oxygen delivery can be expressed as

$$DO_2 = \text{cardiac output (CO)} \times \text{arterial oxygen content } (CaO_2)$$

where

$$CO = \text{heart rate (HR)} \times \text{stroke volume (SV)}$$

and

$$CaO_2 = [1.34 \times \text{hemoglobin concentration (Hb)} \times \text{arterial oxygen saturation } (SaO_2)] + [0.003 \times \text{arterial partial pressure of oxygen } (PaO_2)]$$

Stroke volume is the result of a complex interplay among preload, afterload, and contractility (Fig. 46.2), all three of which are, at present, impossible to monitor reliably and continuously at the bedside. Although these parameters are more fully described below, in brief preload is the end-diastolic volume of the ventricle (a three-dimensional reflection of pre-contractile myocardial cell fiber length), and, up to a point, the greater the preload, the larger the stroke volume (the Frank-Starling relationship). Afterload is the force the ventricle must generate against the systemic or pulmonary vascular resistance. As long as appropriate perfusion pressure is ensured, the lower the afterload, the higher the cardiac output. Contractility (the intrinsic ability to generate force per unit time) may be assessed noninvasively but not continuously by echocardiogram. Since at present most of the measures of cardiac contractility are both preload and afterload dependent, contractility is not truly an independent variable. In addition, cardiac output in neonates is considered more dependent on heart rate than contractility because the ability of neonates to augment their stroke volume is limited when compared to children or adults.

From this simple model, it is easily appreciated that if there is an acute decline in CaO_2, by a decrease in either the hemoglobin concentration or SaO_2 or both, the cardiac output will increase in response to maintain DO_2. On the other hand, because neither hemoglobin concentration nor SaO_2 can be physiologically increased rapidly, other than increasing tissue oxygen extraction and selective blood flow distribution, there is no acute compensation for a low cardiac output due to decreases in myocardial contractility and/or preload.

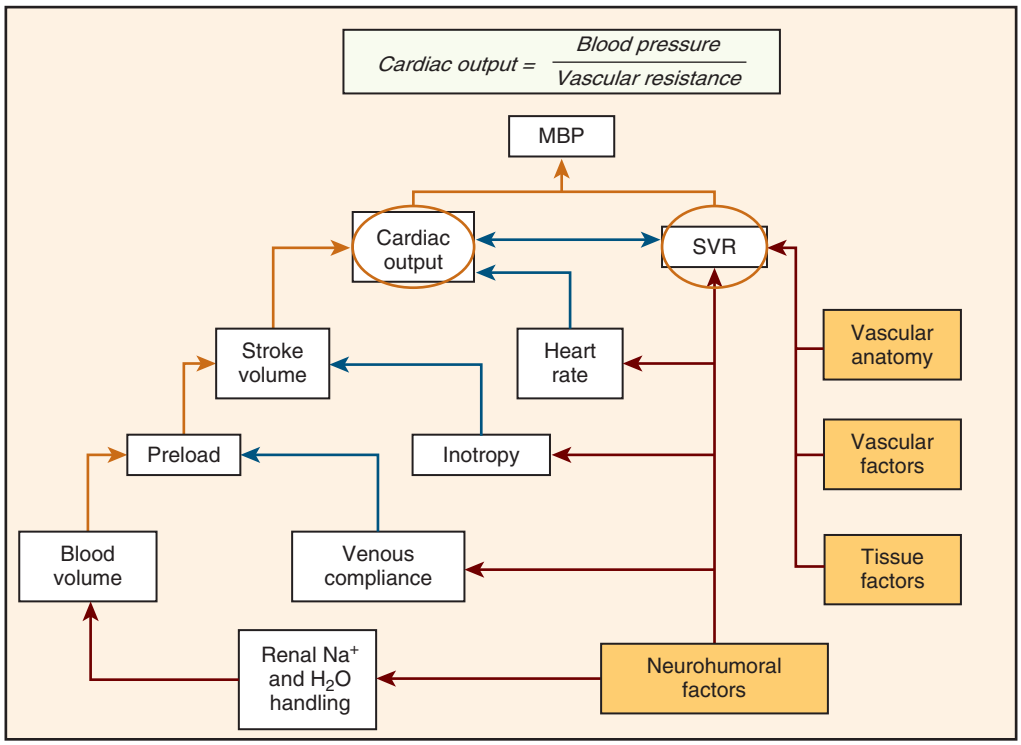
The purpose of oxygen delivery is to provide for oxygen consumption (VO_2), which can be expressed as

$$VO_2 = CO \times (CaO_2 - CvO_2)$$

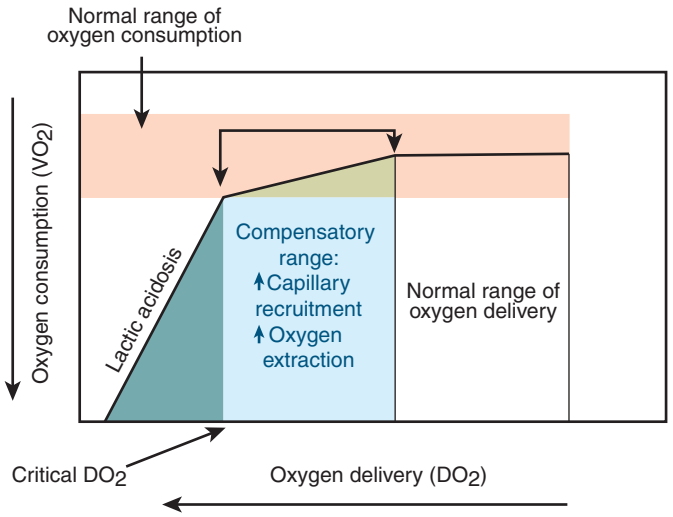
where CvO_2 is the mixed venous oxygen content.

This relationship is based on the Fick principle, from which, knowing the flow rate and arterial-venous content difference of a trace element (in this case, oxygen), one can calculate the uptake or removal rate of the tracer.

Normally DO_2 and VO_2 are well matched, with O_2 extraction being approximately 25%. Accordingly, if the SaO_2 is 100%, SvO_2 would be expected to be 75%. If cardiac output falls, VO_2 may be maintained constant by capillary bed vasodilation and recruitment and/or by increased O_2 extraction by the tissues. Increased O_2 extraction is manifested as a lower CvO_2 and therefore greater $CaO_2 - CvO_2$ difference. The relationship between DO_2 and VO_2 may be graphically displayed as in Fig. 46.3. Once oxygen extraction is maximal, at the critical DO_2 threshold, anaerobic metabolism ensues, resulting in lactic acidosis. If not reversed, the oxygen debt accumulates, and organ failure and death will ensue. In general, during aerobic metabolism 38 mol adenosine triphosphate (ATP) is produced per 1 mol glucose, whereas during anaerobic metabolic conditions, 2 mol ATP and 2 mol lactate are produced per 1 mol glucose.



• **Fig. 46.2** Factors Regulating Cardiac Output, Blood Pressure, and Systemic Vascular Resistance. From a physiologic standpoint, SVR and cardiac output are the regulated (independent) variables and MBP is the dependent variable. *MBP*, Mean blood pressure; *Na*+, sodium; *SVR*, systemic vascular resistance. (Modified from Klabunde RE, www.cvphysiology.com.)



• **Fig. 46.3** Relationship Between Oxygen Consumption and Delivery. In the normal range of oxygen delivery, oxygen consumption is unaffected by changes in the delivery rate of oxygen to the tissues. As oxygen delivery decreases below the normal range, tissue oxygen consumption remains in the normal range for a while because of activation of local compensatory mechanisms such as capillary recruitment and increased oxygen extraction. However, when oxygen delivery decreases to the "critical" point, compensatory mechanisms can no longer satisfy tissue oxygen demand, and anaerobic metabolism commences, resulting in significantly decreased adenosine triphosphate and increased lactate production.

Before reaching the stage of delivery-dependent VO_2, the SvO_2 can be used as a proxy for DO_2. Assuming VO_2, hemoglobin concentration, and SaO_2 are constant over a short period of time, a decline in SvO_2 represents a decrease in cardiac output. This relationship is clinically important, as SvO_2 can be measured intermittently via a venous catheter, ideally placed in the pulmonary artery in a patient without intracardiac shunts in order to obtain a true mixed venous sample. Central venous oxygen saturation ($ScvO_2$) may also be used as a proxy for DO_2, measured via a venous catheter with its tip at the superior vena cava—right atrial junction. It is of note that the location of the catheter tip is critical; if the tip is located lower in the right atrium, it will sample more desaturated blood streaming from the coronary sinus and/or hepatic veins. However, for several reasons, measurement of $ScvO_2$ is not done routinely in neonates in neonatal intensive care units except for some neonates with congenital heart disease in the postoperative period following surgical correction of the underlying cardiac condition.

In general, these principles are valuable guides to understanding and managing global DO_2 and VO_2, but they do not readily assist with the assessment of individual organ DO_2. Furthermore, the limitations of measuring VO_2, DO_2, and even just $ScvO_2$ are often daunting. However, advances in noninvasive regional tissue oxygen saturation (rSO_2) monitoring via NIRS have increasingly allowed for such assessments in different tissues including the brain, kidneys, intestine, and muscle (see later discussion).

Finally, there is a class of neonates where calculations using the Fick principle can be critical in directing therapy. Newborns with congenital heart disease and intracardiac shunts may have perturbations in the usual pulmonary to systemic blood flow ratio (Qp:Qs). Normally, of course, in patients with the two circulations in series and no shunts, Qp:Qs = 1. By comparing the oxygen utilized by the body with the oxygen taken up by the lung, Qp:Qs can be estimated.

$$Qs = VO_2/(CaO_2 - CvO_2)$$

and

$$Qp = O_2\text{uptake}/(CpvO_2 - CpaO_2)$$

where pv and pa represent pulmonary vein and pulmonary artery, respectively.

After substituting and eliminating common terms

$$Qp{:}Qs = (SaO_2 - SvO_2)/(SpvO_2 - SpaO_2)$$

This formula requires two assumptions unless saturation values are determined directly, as done in the cardiac catheterization laboratory: first, $SpvO_2$ is 95% to 100%, and second, SvO_2 measured through a central venous line reflects a mixed venous sample. A Qp:Qs ratio of < 1 would suggest the presence of a right-to-left shunt and, typically, decreased pulmonary circulation, both of which result in less oxygenated blood entering the systemic circulation with resultant cyanosis. A Qp:Qs ratio of>1, in turn, would suggest left-to-right shunting with resultant pulmonary overcirculation. Of note is that changes in Qp:Qs ratio in either direction will affect oxygen content and/or oxygen delivery to the organs.

The value of this calculation can be illustrated with the following example. A newborn infant with hypoplastic left heart syndrome (HLHS) is found to have an SaO_2 of 95% and an SvO_2 of 80%. Using the formula just given, assuming a $SpvO_2$ of 100% and recognizing that SaO_2 and $SpaO_2$ are the same in this patient, we arrive at a Qp:Qs ratio of 3:1. If systemic blood flow is to be preserved, the single right ventricle will need to sustain a fourfold increase in cardiac output while the significant pulmonary overcirculation will lead to the development of congestive heart failure. The inability of the right ventricle to sustain such an increase in cardiac output will then lead to inadequate systemic blood flow, resulting in shock. Importantly, both conditions can be present at the same time. However, by maintaining an SaO_2 within the target range of 75% to 85% in these patients, the Qp:Qs ratio will shift closer to 1:1, providing a simplistic rationale for using such an SaO_2 target range. However, in patients with HLHS, the effects of shunting on blood oxygen content and/or delivery are far more complex as systemic oxygen availability is determined, along with other factors, by the relationship among $SpvO_2$, SaO_2, SvO_2, and cardiac output.[23] Accordingly, in addition to a targeted SaO_2 range, management of these patients must be guided by the difference in systemic arterial and venous oxygen saturations and other indicators of tissue perfusion (see also Chapter 50).

Developmental Regulation of Cardiac Output and Its Determinants

Cardiac output is the product of stroke volume and heart rate and is determined by the amount of blood returning to the heart (preload), the strength of myocardial contractility, and the load against which the heart must pump (afterload). Unlike preload, afterload is in general more difficult to conceptualize and therefore often used interchangeably with the simpler but less accurate term, vascular resistance. However, although afterload is altered by changes in vascular resistance, other factors also determine its magnitude. Afterload is the load or force the heart faces during contraction and is affected by the impedance of the central vasculature, the

resistance of the peripheral vascular beds, the ventricular mass, blood pressure, and the inertia of the blood. In addition, it is affected by myocardial contractility and preload as well. If the myocardial function is intact, cardiac output depends solely on preload and afterload according to the relationships described by the Starling curve.

Therefore, low cardiac output and thus low systemic blood flow can result from various combinations of abnormalities of the three determinants of cardiac output: low cardiac preload, poor myocardial contractility, or high cardiac afterload. In addition, extremes of these variables in the opposite direction (i.e., high cardiac preload, increased myocardial contractility, or low cardiac afterload) can contribute to cardiovascular insufficiency, albeit not as commonly. This is due to the fact that these three variables as well as heart rate affect one another. For example, in an infant of a diabetic mother with hypertrophied cardiomyopathy, increased contractility and low afterload can further compromise systemic flow by reducing preload and worsening left ventricular outflow tract obstruction.

Preload

Decreases in preload lead to diminished stroke volume and cardiac output and are most often caused by low effective circulating blood volume. This can be due to loss of circulating blood volume following hemorrhage (absolute hypovolemia), or the circulating volume may be inadequate for the vascular space as in vasodilatory shock or as a side effect of administration of lusitropes (relative hypovolemia). Because approximately 75% of the circulating blood volume is on the venous side of the circulation at any given point in time, the increases in venous capacitance caused by venodilation significantly contribute to relative hypovolemia under these circumstances. The interaction between the respiratory and cardiovascular system is complex, and the provision of positive pressure respiratory support alters this interaction.[24] Excessive mean airway pressure, especially in compliant lungs, increases pulmonary vascular resistance and decreases pulmonary blood flow with a resultant decrease in systemic blood flow. Because preload is also augmented by the negative intrathoracic pressure generated at each spontaneous inspiration, the positive intrathoracic pressure associated with positive pressure mechanical ventilation reduces venous return and hence preload and cardiac output.[25,26] However, the impact in neonates with low lung compliance ventilated on appropriate positive pressure ventilatory settings appears to be modest.[27–29]

Contractility

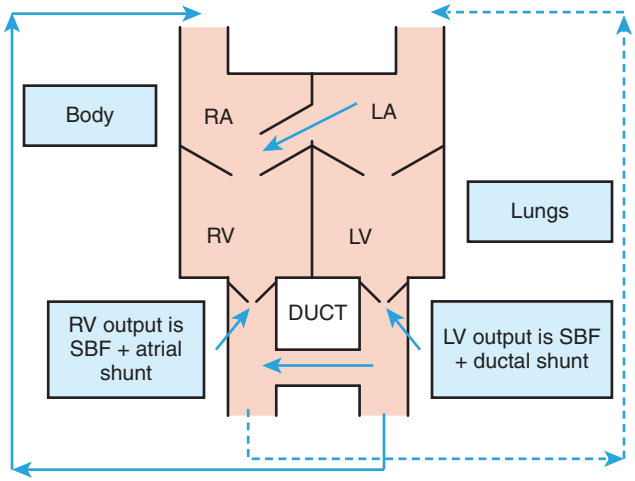
The strength of myocardial contractility depends on the filling volume and pressure, and the maturity and integrity of the myocardium.[30] Thus, decreases in preload (hypovolemia, cardiac arrhythmia), as well as prematurity (especially extreme immaturity), hypoxic insults, and infectious (viral or bacterial) agents,[31] all negatively affect the ability of the myocardium to contract with resultant decreases in cardiac output.

Afterload

If cardiac afterload is too high, the ability of the myocardium to contract and pump may become compromised, and cardiac output may fall.[32,33] Such increases in afterload are associated with enhanced endogenous catecholamine release during the period of immediate postnatal adaptation along with loss of the low-resistance placental circulation. Similar increases in afterload are seen in hypovolemia, hypothermia, or when inappropriately high doses of vasopressor-inotropes are being administered to a patient with intact cardiovascular adrenoreceptor responsiveness.[34] High afterload can affect either ventricle and if the output of one of the ventricles is reduced, this will affect the function of the other ventricle, especially when the fetal channels are closed. For instance, if the right ventricular output is low because of high pulmonary vascular resistance, the amount of blood traversing the lungs to the left ventricle will be reduced, leading to low systemic blood flow with blood pooling in the systemic venous system.

Changes in Preload, Contractility, and Afterload During Transition

With delivery and the separation of the placenta, the fetal circulation begins its transition to the mature (adult-type) circulation in which the systemic and pulmonary circuits are in series and, with the closure of the fetal shunts between the two circulations, the right and left cardiac outputs are equal. However, some component of the normal transition (e.g., removal of placental circulation) is rather abrupt while others (cessation of flow through the ductus arteriosus and foramen ovale) are more gradual.[35,36] With the initiation of breathing resulting in lung expansion and the separation of the placenta, the pulmonary vascular resistance drops precipitously and systemic vascular resistance increases, respectively. The resultant increase in the left ventricular (LV) afterload could lead to a decrease in myocardial contractility, which in selected populations of vulnerable preterm infants may result in decreased cardiac output. In addition, the above-mentioned changes in the pulmonary and systemic vascular resistance lead to an evolution in ductal flow pattern from a purely right-to-left to bidirectional and, eventually, purely left-to-right ductal flow. In healthy term infants, LV stroke volume and output increase in the first few minutes after birth. This change coincides with increasing net left-to-right ductal shunting.[37] The increase in LV preload from ductal shunting likely explains the increase in LV output, and the increase in LV output may offset the potential impact of the left-to-right ductal shunt on the systemic circulation. Therefore, there is usually no evidence of circulatory compromise in the healthy neonate. Subsequently, the ductus arteriosus constricts and then closes in the vast majority of term neonates within 24-to-48 hours. Therefore, in the healthy term neonate, the rapidly constricting ductus arteriosus prevents the development of hemodynamically significant left-to-right shunting across the ductus. However, the transition to adult-type circulation is prolonged in preterm, especially in extremely preterm infants and can result in circulatory compromise.[38] Indeed, by 2 months of age, less than 50% of preterm infants born at <26 weeks' gestation close their ductus arteriosus spontaneously[39] and, as the right-sided pressures fall, blood will shunt left-to-right from the systemic circulation back into the pulmonary circulation. In most VLBW neonates, pulmonary vascular resistance (PVR) initially decreases relatively rapidly for physiologic and non-physiologic reasons.[40] Physiologic mechanisms most important in the postnatal decrease of PVR include the mechanical effects of initiation of breathing on PVR and the increased postnatal oxygenation-associated direct, paracrine, and endocrine vasodilation.[41] Iatrogenic causes include surfactant administration or the inappropriate targeting of higher arterial oxygen saturations.[38,42] With the left-to-right ductal shunting, pulmonary overcirculation develops and left ventricular output, the gold standard of bedside assessment of systemic perfusion, cannot be used as a measure of systemic perfusion (Fig. 46.4).[43]



• **Fig. 46.4** Impact of Left-to-Right Shunting Across the PDA and PFO on LVO and RVO Measurements. Effect of left-to-right shunting across the PDA and foramen ovale on LVO and RVO. Under these circumstances, LVO represents the sum of total pulmonary venous return and ductal blood flow, whereas RVO measures the sum of systemic venous return and left-to-right shunting across the PFO. *LVO*, Left ventricular output; *PDA*, patent ductus arteriosus; *PFO*, patent foramen ovale; *RVO*, right ventricular output; *SBF*, systemic blood flow. (From Kluckow M, Seri I. Cardiovascular compromise in the preterm infant during the first postnatal day. In: Seri I, Kluckow M, eds. *Neonatology Questions and Controversies: Hemodynamics and Cardiology*. 3rd ed. Philadelphia: Elsevier; 2019:471–488.)

Indeed, under these circumstances, left ventricular output measures systemic perfusion and ductal blood flow. In earlier studies investigating the post-transitional changes in systemic perfusion and/or the effects of vasoactive agents on cardiovascular function, this fact has often not been acknowledged.[33,44] Therefore, the conclusions drawn from some of these studies[33] need to be reevaluated. More recent studies have acknowledged this hemodynamic paradigm and used right ventricular output to assess systemic perfusion in the VLBW neonate during the transitional period. However, right ventricular output only represents systemic perfusion as long as left-to-right shunting across the foramen ovale does not become significant. In many preterm neonates, however, as left-to-right shunting across a nonconstricting patent ductus arteriosus (PDA) increases during the first 12 to 36 hours, left atrial volume and pressure increase, often leading to the development of a significant left-to-right shunt across the foramen ovale.[45] The left-to-right shunt through the patent foramen ovale (PFO) will then render the use of right ventricular output as a measure of systemic blood flow inaccurate because right ventricular output now represents systemic inflow and PFO flow.[43] This hemodynamic scenario results in the lack of an acceptable conventional measure of systemic blood flow in these neonates. To circumvent this problem, superior vena cava (SVC) flow has been used as a measure of upper body blood flow in preterm neonates with the fetal channels open.[11,46] The use of SVC flow has provided novel insights into the mechanisms of transitional hemodynamics, such as the observation that intraventricular hemorrhage (IVH) develops in many VLBW neonates as systemic blood flow improves, resulting in reperfusion of the brain (see discussion below).[47] However, the vulnerability of SVC flow measurements to error and the technical difficulties associated with its use as a surrogate measure of systemic blood flow have forced these measurements to remain primarily a research rather than a clinical tool.[48–50]

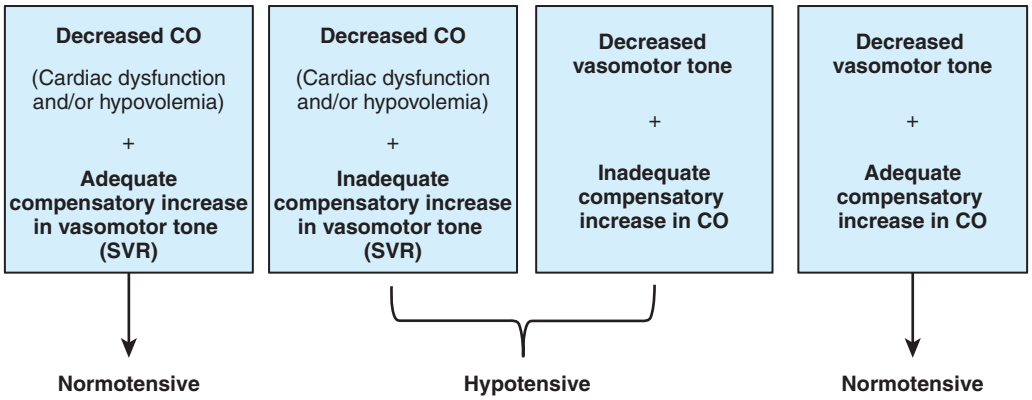
Developmental Regulation of Systemic Blood Pressure

Systemic blood pressure is the product of systemic blood flow and systemic vascular resistance. There is an association between low blood pressure and central nervous system injury in the preterm neonate.[3,51–55] Yet, blood pressure correlates only weakly with blood flow in this patient population during the period of immediate postnatal adaptation when the fetal channels are open.[11] Thus, in preterm infants during the first postnatal day, blood pressure may be low because resistance (vasomotor tone) is low even in the presence of normal or high blood flow (Fig. 46.5). Alternatively, blood pressure may be normal or high because resistance is high in the presence of normal or low blood flow.[10] The lack of clarity surrounding the nature of the relationship between blood pressure and systemic blood flow during the transitional period results, at least in part, from our inability to appropriately define the normal blood pressure range[2] and systemic blood flow (see earlier), and to characterize the developmental regulation of organ blood flow and vital organ assignment (see later discussion) in the preterm neonate. Indeed, the recent findings of a higher rate of survival without severe morbidity and a lower rate of severe IVH and cerebral injury among preterm infants with isolated (asymptomatic) hypotension who were treated compared to those who were not treated highlights the complexity of the blood pressure and blood flow interaction during the postnatal transitional period.[3] These findings also challenge the "permissive hypotension" strategy[56] stemming from the uncertainty surrounding the nature of the relationship between blood pressure and systemic blood flow during the transitional period as well as from the potential side effects of vasopressor-inotropes, especially when not appropriately titrated.[57]

Developmental Regulation of Organ Blood Flow and Its Autoregulation and Vital Organ Assignment

Cerebral Blood Flow Autoregulation

Even very immature preterm neonates autoregulate their cerebral blood flow (CBF).[14,58,59] However, the autoregulatory blood pressure range in this patient population is believed to be narrow, and thus "normal" blood pressure is very close to the lower elbow of the autoregulatory curve.[60,61] Some data suggest that CBF autoregulation is different during the cardiac cycle with CBF being pressure-passive mainly during diastole in preterm infants <34 weeks' gestation.[62] Organ blood flow autoregulation is impaired in preterm neonates who are sicker and/or more immature.[1,59,63,64] In these patients, changes in blood pressure are mirrored by changes in CBF with a high coherence, and these babies are at higher risk for cerebral injury.[59,64–66] Similarly, impairment of the autoregulatory system in dampening the impact of changes in blood pressure on CBF can increase the risk of brain injury in preterm infants.[67] Factors that impair cerebral and other organ blood flow autoregulation include birth asphyxia, acidosis, infection, hypoglycemia, tissue hypoxia and ischemia, and sudden alterations in arterial carbon dioxide tension ($PaCO_2$).[60] It is of clinical importance that the CO_2-CBF reactivity is more robust than the pressure-CBF



• **Fig. 46.5** Pathophysiology of Neonatal Cardiovascular Compromise in Primary Myocardial Dysfunction and Primary Abnormal Vascular Tone Regulation With or Without Compensation by the Unaffected Other Variable. This figure illustrates why blood pressure can remain in the "normal" range when there is appropriate compensatory increase in either vasomotor tone or cardiac output. In the hypotensive scenarios, the compensatory mechanisms have been exhausted. *CO*, Cardiac output. (From Wu TW, Noori S, Seri I. Neonatal hypotension. In: Polin RA, Yoder MC, eds. *Workbook in Practical Neonatology*. 5th ed. Philadelphia: Elsevier; 2014:230–243.)

reactivity, as 1 mm Hg change in $PaCO_2$ results in ~4% change in CBF, whereas 1 mm Hg change in blood pressure is associated with only a ~1% to 2% change in CBF.[68,69] The impairment of CBF autoregulation in the preterm neonate during the immediate postnatal period has been proposed to contribute to cerebral injury with loss of vascular reactivity to both blood pressure and CO_2.[65,66] However, the finding that impaired autoregulation may also be a consequence of a preceding ischemic insult[60] makes clarification of this question particularly difficult.

Vital Organ Assignment

The vessels of the vital organs respond to decreased perfusion pressure and/or oxygen delivery with vasodilation (i.e., high-priority vascular beds), whereas the vessels of the nonvital organs, with low-priority vascular beds, vasoconstrict. Several lines of evidence in human neonates and developing animals suggest that the assignment of the forebrain circulation to a high-priority vascular bed may not be complete at birth.[70–72] For instance, in response to hypoxic exposure, the forebrain vessels of newborn dogs vasoconstrict like those of a nonvital organ whereas the hindbrain vessels vasodilate.[71] The finding that CBF autoregulation also appears in the brainstem first and in the forebrain only later in gestation[70] supports the notion that there are developmentally regulated differences in the timing of the blood flow autoregulatory functions and vital organ assignment characteristics between the forebrain and the hindbrain. The cellular mechanisms responsible for the assignment of vital and nonvital organ status from a blood flow regulatory standpoint are poorly understood. Based on these findings, it is tempting to speculate that the diminished capacity of the forebrain vessels to vasodilate in the very preterm neonate during the complex process of cardiovascular transition after delivery may contribute to hypoperfusion of the forebrain. These neonates may present with blood pressure values in the perceived normal range while being in the compensated phase of shock. Because this early phase of shock is difficult to recognize immediately after delivery, forebrain hypoperfusion can go unnoticed. Similarly, the subsequent evolution to the reperfusion phase as a result of the eventual adaptation of the cardiovascular system to the extrauterine environment may not be detected clinically. This proposed vital organ assignment-associated hypoperfusion-reperfusion cycle might contribute to cerebral injury in the very preterm neonate (see below).[73]

Developmental Regulation of Cerebral Oxygen Demand-Delivery Coupling

Very little is known about the regulation of oxygen demand–oxygen delivery coupling in neonates, especially in the transitional period. Yet, several lines of evidence indicate that the very preterm neonate is unable to couple cerebral oxygen demand with blood flow, and instead increases oxygen extraction when oxygen demand is increased.[72,74] This phenomenon may be linked to the developmental delay in the vital organ assignment of the forebrain immediately after delivery (see earlier discussion).

Phases of Shock

From a pathophysiologic standpoint, three phases of shock depicting advancing severity have been identified.[18,75]

In the "compensated phase," complex neuroendocrine and autonomic compensatory mechanisms maintain perfusion and oxygen delivery in the normal range to the vital organs (brain, heart, and adrenal glands) at the expense of decreased perfusion to the remaining organs (nonvital organs). This is achieved by vasodilation and vasoconstriction of the vessels to vital and nonvital organs, respectively, in response to a fall in perfusion pressure and/or oxygen delivery.[76,77] Blood pressure is maintained within the normal range, and heart rate increases. As perfusion of nonvital organs is decreased because of the compensatory vasoconstriction of their vascular beds, there often are clinical signs of compromised nonvital organ function such as decreased urine output. In addition, signs of poor peripheral perfusion can often be detected, such as cold extremities and prolonged capillary refill time.

If adequate treatment is not commenced, compensatory neuroendocrine and autonomic mechanisms begin to be exhausted and hypotension develops as the shock enters its "uncompensated phase." Systemic perfusion (cardiac output) will decrease, perfusion of all organs including the vital organs becomes compromised, and lactic acidosis develops.[75]

If treatment is ineffective in the uncompensated phase of shock, multiorgan failure develops and shock may enter its "irreversible phase," where permanent damage to the various organ systems occurs and further interventions will be ineffective in reversing the patient's condition.

Pathogenesis of Neonatal Shock

Etiologic Factors

The etiologic factors leading to the development of neonatal shock include hypovolemia, myocardial dysfunction, abnormal peripheral vasoregulation, or a combination of two or all three of these factors.

Hypovolemia

Hypovolemia may be absolute (loss of intravascular volume), relative (increased venous capacitance), or combined, such as is often seen in septic shock (Fig. 46.6). Hypovolemia results in cardiovascular compromise primarily by the decrease in cardiac output (systemic blood flow) caused by the decrease in preload. In addition, if blood loss is the primary cause of hypovolemia, the associated decrease in oxygen carrying capacity contributes to the development of the circulatory compromise. Because of the weak relationship between blood pressure and blood volume in hypotensive preterm neonates, hypovolemia was traditionally thought to be a relatively uncommon primary cause of circulatory compromise, especially during the first postnatal day.[78,79] However, given the difficulty in assessing intravascular volume, especially during the transition, hypovolemia can be difficult to detect clinically. Therefore, the true contribution of hypovolemia to circulatory failure is uncertain. Interestingly, recent studies comparing the effects of delayed umbilical cord clamping or cord milking with immediate cord clamping found increased blood pressure and decreased use of vasopressor-inotropes suggestive of improved hemodynamic status in the delayed cord clamping and cord milking groups.[80–82] In addition, patients with delayed cord clamping have higher blood volume and fewer receive transfusions.[82–84] These findings imply that hypovolemia might be a more common presentation in preterm neonates who receive standard care with immediate cord clamping than previously thought.

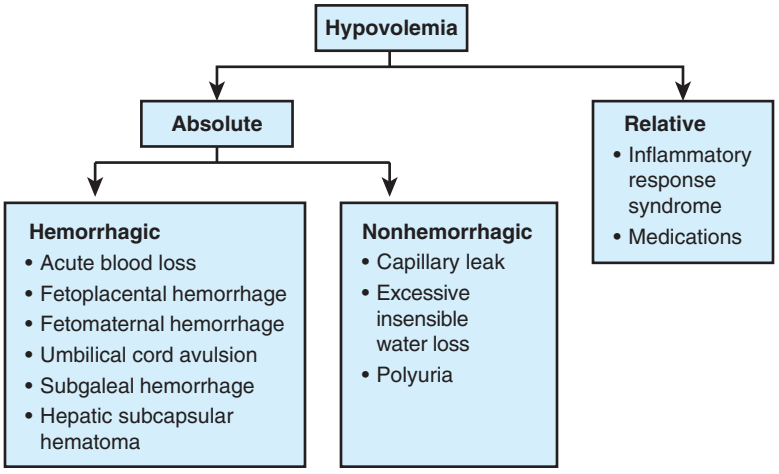
Absolute hypovolemia in the newborn can be due to several conditions. Intrapartum fetal blood loss is usually caused by open bleeding from the fetal side of the placenta, and therefore it is likely to be detected. More difficult to diagnose is the closed bleeding of an acute fetomaternal hemorrhage or an acute feto-placental hemorrhage. The latter can occur during delivery when the umbilical cord comes under some pressure (breech presentation or nuchal cord). Because the umbilical vein is more compressible, it is occluded before the artery and blood continues to be pumped into the placenta. If the cord is clamped early, the excess blood remains trapped in the placenta. This probably happens to some degree in all babies with tight nuchal cords who, as a group, have lower hemoglobin levels.[85] However, in some neonates, a tight nuchal cord may also cause severe circulatory compromise.[86] Postnatally, acute blood loss may occur from any site and is frequently associated with perinatal infections or severe asphyxia-induced endothelial damage and the ensuing disseminated intravascular coagulation. Finally, acute abdominal surgical problems and conditions associated with the nonspecific inflammatory response syndrome and subsequent increased capillary leak with loss of fluid into the interstitium can lead to significant decreases in the circulating blood volume. Iatrogenic causes of absolute hypovolemia include inadequate fluid replacement in conditions of increased insensible losses in the very preterm neonate and gastroschisis before closure of the defect, or the inappropriate use of diuretics.

Relative hypovolemia, that is, a decrease in the effective circulating blood volume, may occur in pathologic conditions leading to vasodilation, such as those associated with the nonspecific inflammatory response syndrome (sepsis, necrotizing enterocolitis [NEC], asphyxia, major surgical procedures, use of extracorporeal membrane oxygenation [ECMO]). In addition, the use of afterload-reducing agents (e.g., milrinone, PGE_2) may cause significant vasodilation (especially venodilation), thereby decreasing the effective circulating blood volume.

Finally, absolute and relative hypovolemia most frequently occurs in conditions associated with the nonspecific inflammatory response syndrome such as sepsis, asphyxia, and major surgical procedures.

Myocardial Dysfunction

Both systolic and diastolic cardiac dysfunction can cause circulatory failure. As echocardiographic assessment of diastolic function is complex and not well established, except in extreme



• **Fig. 46.6** Etiology of Hypovolemia in Neonates. Etiological factors of absolute (hemorrhagic and non-hemorrhagic) and relative hypovolemia are shown.

cases, diastolic dysfunction often goes undetected. Nevertheless, diastolic dysfunction is recognized to be the primary cause of circulatory failure associated with hypertrophic cardiomyopathy in infants of diabetic mothers. In addition, extrinsic factors such as pericardial effusion evolving to tamponade and tension pneumothorax can lead to diastolic dysfunction. Systolic dysfunction, on the other hand, is easier to diagnose using echocardiography.

Acquired heart disease presenting as circulatory compromise includes cardiomyopathies, postasphyxial myocardial dysfunction due to hypoxic-ischemic injury, viral myocarditis, and myocardial dysfunction in the late stages of septic shock. For more detail on structural heart disease and cardiomyopathies, see Chapter 50.

Among the different types of congenital heart disease, structural heart defects that produce a ductus arteriosus–dependent systemic circulation such as the hypoplastic left heart syndrome, critical coarctation, and critical aortic stenosis, if not diagnosed prenatally or immediately after delivery, classically present as acute circulatory compromise with pallor, tachypnea, impalpable pulses, and hepatomegaly as the ductus starts closing. The presentation may be initially misdiagnosed as sepsis.

Abnormal Peripheral Vasoregulation

Peripheral vasodilation causes circulatory compromise by resultant decreases in perfusion pressure. However, patients with intact myocardial function usually present with normal or high cardiac output as they attempt to compensate for the decrease in organ blood flow. Pathologic peripheral vasodilation in neonates occurs primarily in conditions associated with the nonspecific inflammatory response syndrome such as sepsis, NEC, severe asphyxia, major surgical procedures, use of ECMO, or respiratory distress syndrome of prematurity. It is of clinical importance that preterm neonates born to mothers with chorioamnionitis, especially if they have evidence of funisitis (fetal vessel inflammation), frequently present with hypotension and hyperdynamic, vasodilatory cardiovascular compromise at birth or shortly afterward.[87–89]

Clinical Presentations of Shock in Neonates Associated With Multiple Etiologic Factors

Transitional Circulatory Compromise of the Very Preterm Neonate

The transitional circulatory changes at birth and in the first 12 to 24 hours after birth denote a period of unique circulatory vulnerability, especially for the extremely preterm infant. As mentioned earlier, the timing of umbilical cord clamping has a significant effect on volume status and systemic hemodynamics. During normal postnatal adaptation, pulmonary vascular resistance falls, systemic vascular resistance rises with removal of the placenta from the circulation, the ductus arteriosus closes, and the foramen ovale is closed by the reversal of the atrial pressure gradient. During this time frame, the left ventricle must double its output. Given that the very preterm infant's cardiovascular system is adapted to the low-resistance intrauterine environment and its myocardium is immature, it is not surprising that these patients have difficulties during this critical period. In addition, as discussed earlier, developmentally regulated factors such as the state of vital organ assignment of the forebrain and cerebral oxygen demand-flow coupling make cardiovascular adaptation of the very preterm neonate an even more complex process. It is important to note that there is much more to understand about the complex interactions between immediate postnatal cardiovascular adaptation and immaturity, organ development, myocardial and vasoregulatory function, and vital organ assignment.

Low Preload and Immediate Umbilical Cord Clamping

Following delivery of a preterm infant, immediate clamping of the umbilical cord had been the standard of clinical care since the 1960s. However, as this practice may be associated with inadequate transfer of blood from the placenta to the newborn, the approach to the timing of cord clamping has recently changed. Both animal and human studies have demonstrated that delayed umbilical cord clamping increases blood volume (preload), confers a more gradual rise in left ventricular afterload, and allows for a smoother cardiorespiratory transition in the first few minutes after birth. These beneficial effects are likely responsible for the observation that preterm infants delivered with delayed cord clamping have higher brain tissue oxygen saturation and indices of CBF compared to those with immediate cord clamping.[90,91] Interestingly, initiation of breathing seems to be a key factor in aiding the effective placental transfer of blood to the infant.[92,93] However, as cord milking, compared to immediate cord clamping, also improves hemodynamics and indices of CBF,[81,94] it appears that the most important factor in improving systemic blood flow and reducing the incidence of circulatory compromise during the transitional period is placental transfusion itself rather than the process of maintaining a placental connection to preterm infants for a period of time immediately after birth. But a recent RCT reported a higher rate of severe IVH with cord milking as compared to delayed cord clamping among preterm infants <32 weeks' gestation, which is concerning.[95] The reason for this increased risk of IVH is unclear although a rapid rise in CBF has been postulated. In a subset of the population studied in that RCT, cerebral oxygen saturation was monitored in the delivery room.[96] Arterial oxygen saturation was higher in the cord milking group without a difference in cerebral tissue oxygen saturation. Therefore, as a group, those who underwent cord milking did not seem to have excessive CBF. However, it is possible that in selected vulnerable individuals, a rapid increase in the preload and CBF played a role. Therefore, while cord milking could reduce the risk of cerebral ischemia by rapidly increasing circulating blood volume (preload) and improving systemic blood flow, it might increase the risk of IVH through yet unidentified mechanism(s), and its use is currently not recommended.

The improvement in systemic hemodynamics with placental transfusion, including CBF, explains, at least in part, the finding of a lower incidence of overall peri/intraventricular (P/IVH) in preterm infants with delayed cord clamping compared to those with immediate cord clamping.[97] This finding along with a host of other benefits has initiated support for the use of delayed cord clamping by the American College of Obstetricians and Gynecologists[98] as well as by the Neonatal Resuscitation Program.[99] However, little is known about the optimal timing of cord clamping after delivery, the populations who benefit most from it, the position of the baby relative to the placenta, and the preferred mode of respiratory support before clamping the cord.[97,100] Finally, although cord milking appears to exert a better hemodynamic response compared to delayed cord clamping in preterm infants delivered via c-section,[81] the higher rate of IVH[95,101] associated with cord milking renders its use potentially harmful. It is clear that more needs to be understood about the most appropriate way or ways to promote placental transfusion in the preterm neonate.

Myocardial Dysfunction and High Afterload

As mentioned earlier, the abrupt increase in left ventricular afterload following the sudden removal of the low-resistance placenta can be detrimental to the immature myocardium. There is a normal inverse linear relationship between heart rate-corrected velocity of circumferential shortening (VCF_C, an index of contractility) and wall stress (WS, an index of afterload). Even in term infants, the myocardium is particularly sensitive to a rise in afterload as demonstrated by a steeper inverse relationship between VCF_C and WS compared to the mature heart of older children.[102] As preterm infants have anatomically and functionally immature myocardium, they are especially prone to systolic dysfunction. Indeed, the ensuing systolic dysfunction is thought to be one of the key factors in the development of low cardiac output in a subset of high-risk preterm infants during the transitional period.[103,104]

Patent Ductus Arteriosus

As mentioned earlier, a significant proportion of preterm infants fail to constrict and close their ductus arteriosus. The impact of prolonged left-to-right shunting on the pulmonary circulation and the resultant systemic hypoperfusion are discussed in Chapter 48. However, even a short exposure to significant left-to-right PDA shunting in susceptible preterm infants can result in pulmonary hemorrhage and systemic hypoperfusion and hypotension during the transitional period. Furthermore, it is important to recognize that ductal shunting can have significant hemodynamic effects before it can be clinically detected. In healthy term neonates, PDA shunting changes to predominantly left-to-right within the first few minutes after birth.[37,105] Although no data are available on PDA shunting at the time of delivery in preterm infants, a small study showed exclusively left-to-right shunting in most of the patients studied by 20 minutes after birth.[106] In a larger study population of preterm infants, by 5 hours after birth the vast majority of patients had a completely or at least predominantly left-to-right shunt across the ductus arteriosus.[38] The heart significantly increases its output in an attempt to compensate for the left-to-right ductal shunt. There is some evidence that, at least in a subset of preterm neonates, this compensation may be inadequate in the early postnatal period.[37,38] Indeed, preterm infants with low SVC flow have a larger PDA compared to those with normal SVC flow but only during the first 6 to 12 postnatal hours.[47] This suggests that inadequate adaptation to ductal shunting early after birth may contribute to cerebral hypoperfusion immediately after delivery. Therefore, with regard to the negative effect of PDA shunting on CBF, the most vulnerable period might be the first 6 to 12 hours of postnatal life.[103]

Respiratory Support and Hemodynamics

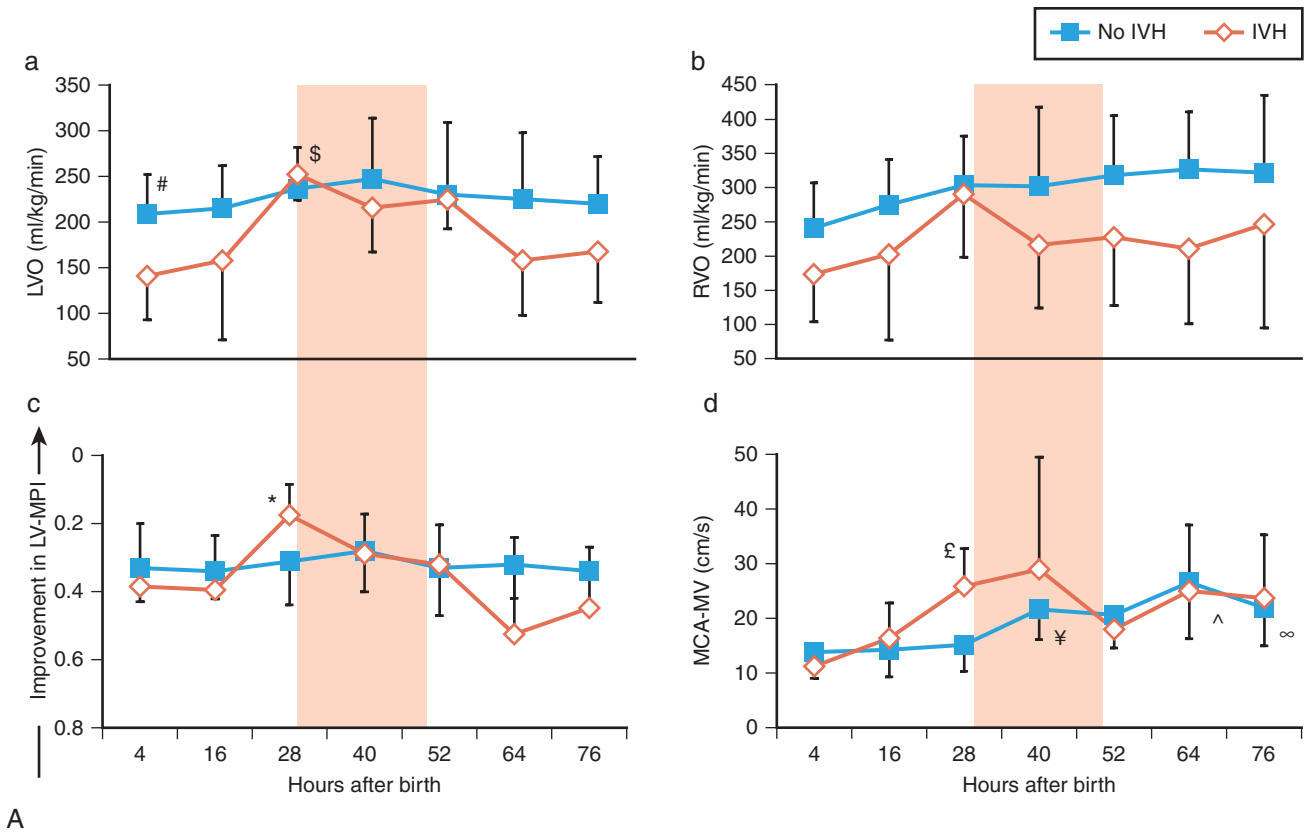
Extremely preterm infants commonly require respiratory support during the transitional period. However, positive intrathoracic pressure associated with the provision of respiratory support could potentially adversely affect venous return and result in decreased preload and cardiac output.[24] In poorly compliant lungs, for example in patients with respiratory distress syndrome prior to administration of surfactant, the effect on venous return is minimal.[27] However, in compliant lungs, venous return can be severely impaired.[26] These findings highlight the importance of understanding the possible adverse effects of respiratory support on pulmonary and systemic hemodynamics so that the most appropriate settings and weaning strategies can be employed.

Permissive hypercapnia is a commonly used strategy aimed at reducing both the need for ventilator support and ventilator-induced lung injury in preterm infants.[107,108] However, little is known about the safety and the optimal carbon dioxide (CO_2) range of permissive hypercapnia.[109–114] In addition to its direct effects on cerebral hemodynamics (see below) and due to the immature renal compensatory mechanisms of the preterm neonate, permissive hypercapnia invariably leads to more severe respiratory acidosis during the transitional period. The effects of acidosis on the cardiovascular system in the neonate in general and the preterm infant, in particular, are largely unknown.[115] In adults, respiratory acidosis decreases myocardial contractility and systemic vascular resistance but, presumably due to the resultant lower afterload, also increases cardiac output.[116] In hemodynamically stable preterm infants, an observational study showed no association between acidic pH and indices of myocardial function and contractility, and systemic vascular resistance (SVR) during the first three postnatal days.[115] While this finding might be considered reassuring, it is important to note that the cardiovascular effects of an acidic pH in hemodynamically unstable neonates are not known.

Ischemia-Reperfusion

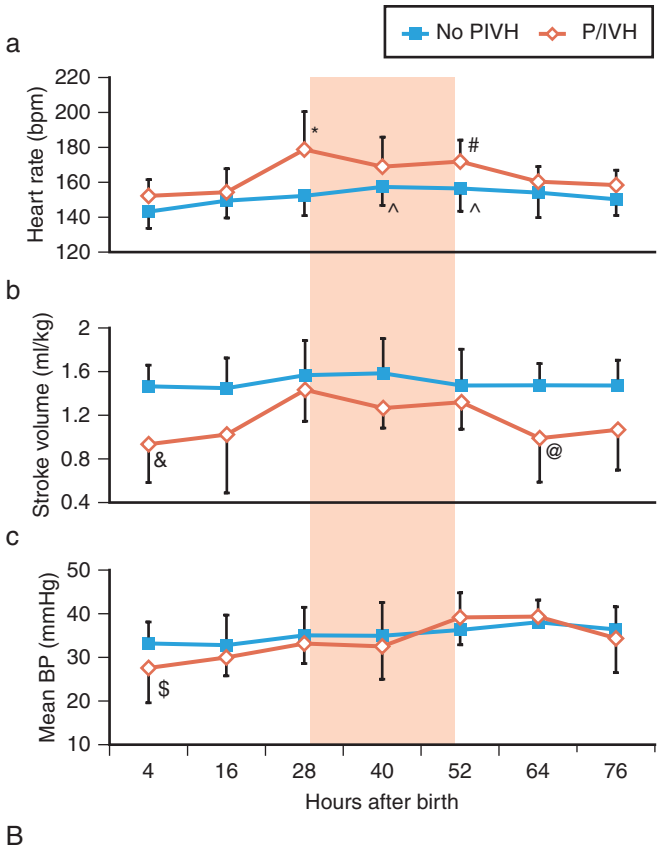
It has long been recognized that preterm infants presenting with low CBF during the immediate postnatal period are at greater risk for developing P/IVH,[117] The decrease in CBF in these patients has been documented using different techniques.[47,118] In addition, in some patients with low SVC flow, it was observed that P/IVH occurred after improvements in SVC flow.[47] Similarly, higher cerebral regional oxygen saturation ($CrSO_2$) and lower oxygen extraction were recently reported in preterm infants during the 24 hours before detection of P/IVH.[119] These findings might suggest that cerebral reperfusion leads to the development of P/IVH. However, these observations are not universal as others have only documented persistently low CBF for over a week after birth.[120] Advances in NIRS technology have made continuous monitoring of indices of CBF feasible (see section on diagnosis).[121] When, during the first three postnatal days, extremely preterm infants were comprehensively evaluated by, among others, continuous monitoring of $CrSO_2$ using NIRS and periodic cardiac function, systemic and cerebral perfusion assessments using ultrasonography, a phase of initial cerebral ischemia, and lower systemic perfusion (Fig. 46.7A and 46.7B) followed by a reperfusion phase preceding the detection of P/IVH were documented (Fig. 46.8).[122] The cerebral hypoperfusion and reperfusion coincided with low cardiac output and improvement in cardiac output, respectively.

Although, as mentioned earlier, myocardial dysfunction may contribute to the occurrence of low cardiac output, this finding has not been consistently documented.[104,122] There is some evidence that altered cerebral hemodynamics predisposing to P/IVH could be present already in the delivery room. Indeed, a case-control study found lower $CrSO_2$ among preterm infants who later developed P/IVH.[123] The lower $CrSO_2$ could be the result of inadequate placental transfusion as lower hematocrit is associated with an increased risk of developing P/IVH.[124] Furthermore, higher mean airway pressure in the delivery room may also result in lower $CrSO_2$ possibly by negatively impacting cardiac output or CBF.[125] Therefore, the initial low cardiac output predisposing to cerebral hypoperfusion[47,122] is likely multifactorial. Such factors include a decreased preload, especially with immediate cord clamping

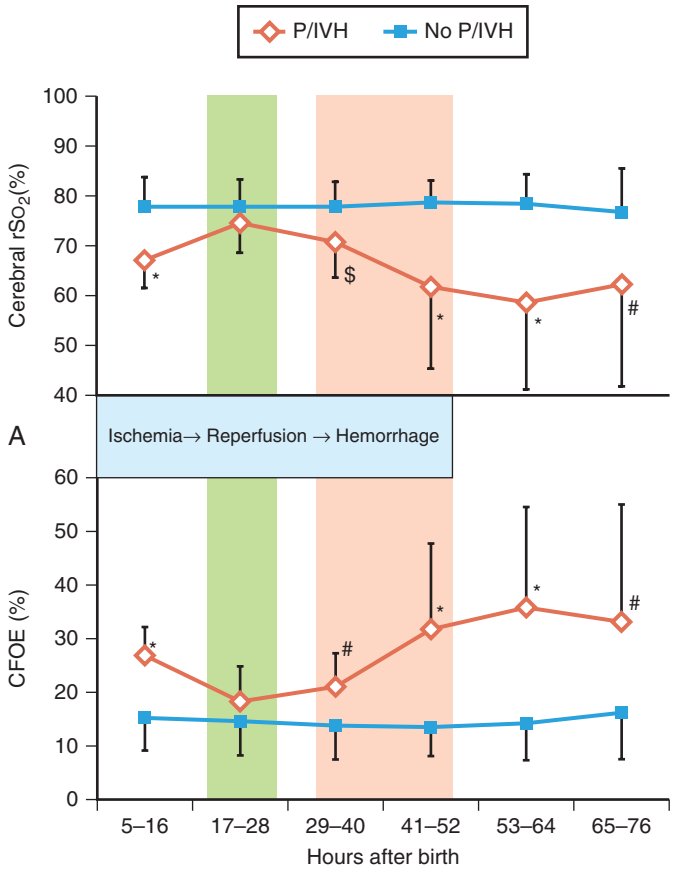


• **Fig. 46.7A** Changes in Selected Hemodynamic Parameters (Left and Right Ventricular Output, Left Ventricular Myocardial Performance Index and Middle Cerebral Artery Mean Velocity) During the First 76 Hours in Very Preterm Neonates With and Without Periventricular/Intraventricular Hemorrhage. Changes in LVO (*a*), RVO (*b*), left ventricular myocardial performance index (LV-MPI) (*c*) and MCA-MV (*d*) in the two groups during the study are shown. There was a trend for a lower LVO in the P/IVH group at baseline with a trend for improvement before the occurrence of P/IVH (highlighted in pink). Lower MPI (i.e., better function) and higher MC-MV in the P/IVH group preceded occurrence of P/IVH. The pattern of changes in LVO between the two groups tended to be statistically significant (P = 0.068) while in RVO, MPI and MCA-MV did not reach statistical significance between the two groups. Statistically significant differences between groups: $^{*}p = 0.04$ and $^{£}p = 0.016$. No-P/IVH group; compared to baseline: $^{¥}p = 0.02$, $^{\wedge}p < 0.0001$, $^{\infty}p = 0.044$.Differences approaching statistical significance and suggesting a difference between the groups: $^{\#}p < 0.055$, and within the P/IVH group compared to baseline: $ p = 0.07. The values represent the mean ± SD of the data obtained upon entry into the study and every 12 hours thereafter. *LVO*, Left ventricular output; *MCA-MV*, middle cerebral artery mean velocity; *MPI*, myocardial performance index; *P/IVH*, periventricular/intraventricular hemorrhage; *RVO*, right ventricular output. (From Noori S, McCoy M, Anderson MP, et al. Changes in cardiac function and cerebral blood flow in relation to peri/intraventricular hemorrhage in extremely preterm infants. *J Pediatr*. 2014;164: 264–270.e1–e3.)

and/or with the use of inappropriately high mean airway pressure, and a poor myocardial contractility in the setting of myocardial immaturity, high afterload, and uncompensated ductal shunting.[103] On the other hand, the reperfusion phase could also be a consequence of the recovery of cardiac function potentiated by hypercapnia and/or the inappropriate titration of medications used in the management of neonatal shock. As increases in CO_2 result in increases in CBF, permissive hypercapnia may also exaggerate the reperfusion phase. Although a recent RCT with two different threshold targets of permissive hypercapnia with the goal to decrease the rate of bronchopulmonary dysplasia or death did not show a higher rate of P/IVH within the higher hypercapnia target population,[107] epidemiologic data reveal an association between hypercapnia and P/IVH.[126–128] Furthermore, $PaCO_2$ values above the low-to-mid 50 mm Hg strongly correlate with indices of increased CBF, suggesting a significant rise in CBF with the use of more extreme levels of permissive hypercapnia.[111] Hypercapnia also attenuates CBF autoregulation, likely putting preterm infants at higher risk for uncontrolled increases in CBF.[111,129] As hypotensive preterm infants also have impaired CBF autoregulation,[130] excessive and abrupt increases in blood pressure with the inappropriate use of vasopressors/inotropes can result in sudden, excessive increases in CBF, which in turn might potentiate the reperfusion injury.[103] As mentioned earlier, the safe threshold for permissive hypercapnia is unknown, and therefore the risk and benefits of permissive hypercapnia above mild values, especially in the most vulnerable population during the first 3 postnatal days, should be carefully considered.



• **Fig. 46.7B** Changes in Selected Hemodynamic Parameters (Heart Rate, Left Ventricular Stroke Volume, and Mean Blood Pressure) During the First 76 Hours in Very Preterm Neonates With and Without Periventricular/Intraventricular Hemorrhage. Changes in heart rate (*a*), left ventricular stroke volume (*b*), and mean blood pressure (*c*) in the two groups during the study are shown. Heart rate significantly increased in the No-P/IVH group (ANOVA p = 0.004), while there was only a trend for an increase in the P/IVH group (ANOVA p = 0.051) during the study. Compared to the No-P/IVH group, left ventricular stroke volume in patients of the P/IVH group was lower at the baseline but similar before the occurrence of the P/IVH. Mean blood pressure (c) tended to increase in the P/IVH group (ANOVA *P* = .052) while it remained unchanged and relatively stable in the No-P/IVH group (ANOVA *P* = .2). In addition, mean blood pressure at baseline also tended to be lower in patients in the P/IVH group. The pattern of changes in heart rate and stroke volume, but not in mean blood pressure, was different between the two groups. See text for details. No-P/IVH group; compared to baseline: ^p = 0.007. Between groups: *p = 0.004, # p = 0.03, & p = 0.007, @ p = 0.048, $ p = 0.085. The values represent the mean ± SD of the data obtained upon entry into the study and every 12 hours thereafter. Area highlighted in pink represents the period when P/IVH occurred. *bpm*, Beats per minute; *P/IVH*, periventricular/intraventricular hemorrhage. (From Noori S, McCoy M, Anderson MP, et al. Changes in cardiac function and cerebral blood flow in relation to peri/intraventricular hemorrhage in extremely preterm infants. *J Pediatr*. 2014;164:264–270.e1–e3.)



• **Fig. 46.8** Changes in Cerebral Regional Oxygen Saturation (rSO_2) and Cerebral Fractional Oxygen Extraction (CFOE) in Two Groups With (Red) and Without (Blue) Peri/Intraventricular Hemorrhage (PIVH) During First 3 Postnatal Days. The No-P/IVH group exhibited stable cerebral rSO_2 (A) and CFOE (B) values while the P/IVH group presented with a characteristic pattern of changes. The P/IVH group had lower cerebral rSO_2 and higher CFOE during the first 12 hours of the study followed by normalization of these parameters *(highlighted in green)* just before the two study periods when P/IVH was detected *(highlighted in pink)*. These findings suggest initial cerebral hypoperfusion followed by a period of reperfusion before the occurrence of the bleeding. After the second study period, cerebral rSO_2 decreased and CFOE increased suggesting a decrease in CBF during and after the development of P/IVH. Statistically significant differences between the two groups: *p < 0.005, # p < 0.04 and $ p < 0.05. The values represent the mean ± SD of the data obtained in each 12-hour data collection period. (From Noori S, McCoy M, Anderson MP, et al. Changes in cardiac function and cerebral blood flow in relation to peri/intraventricular hemorrhage in extremely preterm infants. *J Pediatr*. 2014;164:264–270.e1–e3.)

Vital Organ Assignment

As discussed earlier, compared to most other organs, blood supply to the brain is relatively protected during hypoxia by its vascular property; hence, the brain is considered a vital organ. However, this vascular capacity undergoes a maturation process with the vessels of the forebrain (including the cerebral cortex and basal ganglia) reacting like those of a non-vital organ in the very preterm infants during the early postnatal period.[70–73] This developmentally regulated phenomenon has significant implications as most extremely preterm infants are in compensated or uncompensated shock, with lower systemic flow, immediately following delivery. In these infants, instead of vasodilating, the vessels of the forebrain vasoconstrict just like those of the non-vital organs (e.g., kidneys or intestine). Accordingly, immaturity of vital organ assignment in the forebrain likely contributes to the observed cerebral ischemia even in non-hypotensive extremely preterm neonates during the immediate postnatal period (see above).

Vasopressor-Resistant Hypotension

Although the pathologies of transitional hemodynamics described earlier may or may not present with low blood pressure (compensated versus uncompensated phase of shock), there is a group of preterm neonates in whom persistent hypotension that is resistant to conventional vasopressor-inotropic support develops.[131,132] The underlying systemic hemodynamic changes of this condition have also been characterized.[133] These babies are more likely to be extremely preterm (≤27 weeks) and/or have been critically ill or suffered a degree of perinatal asphyxia. Generally, corticosteroid-responsive refractory hypotension manifests during the first 2 weeks of postnatal life, although it may also occur at later times.[131] The problem may be apparent as early as the first postnatal day and may persist beyond and represent a state of vasodilatory shock with normal to high systemic blood flow and possibly supranormal cardiac output. There are striking analogous features of this presentation in preterm neonates to those of vasodilatory shock described in adults, particularly the lack of responsiveness to vasopressor-inotropes. Potential mechanisms for the uncontrolled vasodilation include dysregulated cytokine release, excess nitric oxide synthesis, overactivation of the K_{ATP} channels in the vascular smooth muscle cell membrane in response to tissue hypoxia, and downregulation of the cardiovascular adrenergic receptors. In the preterm infant, the foregoing mechanisms are exacerbated by immaturity-associated relative adrenal insufficiency[134] or by preceding asphyxia or may be secondary to transitional circulatory failure. Recently, polymorphism in the glucocorticoid receptor gene was shown to be associated with refractory hypotension in premature infants.[135] In the term neonate, congenital diaphragmatic hernia has been associated with relative adrenal insufficiency and refractory hypotension.[136]

Sepsis

Although clinical evidence of circulatory compromise is a leading feature of many infectious processes in the newborn, only limited data are available on the hemodynamics in neonatal septic shock.[137] In older subjects, two distinct hemodynamic patterns occur: warm shock, characterized by loss of vascular tone, increased systemic blood flow, and low blood pressure; and cold shock, characterized by increased vascular tone, low systemic blood flow, and eventually falling blood pressure. Cold shock has been well described in the newborn,[138] whereas the warm shock pattern is more difficult to recognize clinically unless the blood pressure and the cardiovascular status, in general, are being closely monitored. A study in preterm infants demonstrated high cardiac output and low systemic vascular resistance consistent with the presence of warm shock.[139] Another study also found higher left ventricular output in preterm infants with septic shock compared to a control group, suggestive of vasodilation as the dominant pathophysiologic factor.[137] However, because a significantly higher proportion of neonates in the septic group had a PDA, the increase in left ventricular output in this study could have been due, at least in part, to the presence of the left-to-right shunt rather than high systemic flow per se. We don't know how common myocardial dysfunction is in neonates with septic shock. In older children, about half of them have either systolic or diastolic dysfunction,[140] and the prevalence of myocardial dysfunction in fluid- and catecholamine-refractory septic shock is even higher.[141] The mediators of neonatal warm septic shock remain unclear, but in adult sepsis, dysregulated cytokine release and upregulated nitric oxide productions as well as deficiency of vasopressin production, play important roles. The significance of this to newborn sepsis remains unclear, but it may have relevance to the vasopressor-resistant hypotension seen in preterm babies.[142]

Diagnosis of Circulatory Compromise

There is no agreement regarding what constitutes the gold standard in diagnosing circulatory compromise in the neonate. However, blood pressure remains the most commonly used criterion for diagnosis and initiation of treatment in this patient population.[3,143,144] As mentioned earlier, available data suggest that sole reliance on blood pressure can lead to inaccurate or, in other cases, significantly delayed diagnosis of circulatory compromise, especially in the very preterm infant during the immediate postnatal period. On the other hand, it is clear from the observations about vasodilatory shock that continuous blood pressure monitoring is imperative. The other commonly used clinical signs of circulatory compromise, such as increased heart rate, slow skin capillary refill time (CRT), increased core-peripheral temperature difference, low urine output, and acidosis due to increased lactate production, aid in establishing the diagnosis of circulatory compromise in the preterm or term infant but also have significant limitations.

With recent advancements in biomedical technology, the armamentarium of devices for bedside cardiorespiratory monitoring has broadened enabling the clinician to perform intermittent and/or continuous assessment of systemic and regional perfusion in neonates (Table 46.1). While being used mostly for research applications, these devices have also started to be increasingly utilized for routine clinical care.[7,21]

Heart Rate and Blood Pressure

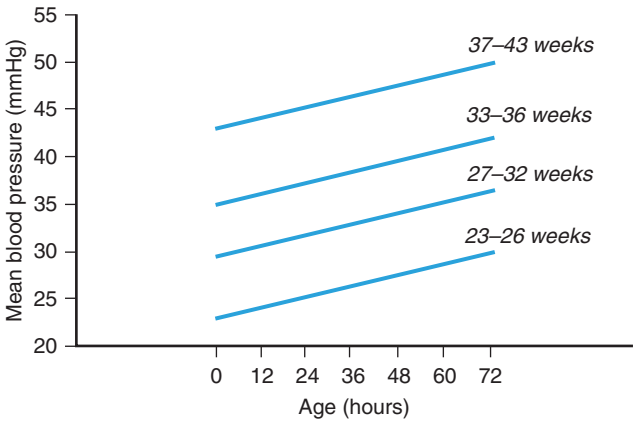
Heart rate is continuously, accurately, and routinely monitored in neonates requiring admission to neonatal intensive care units (NICUs). Because many factors other than those regulating the cardiovascular system affect heart rate, it has a limited yet widely utilized role in the diagnosis of circulatory compromise. As for the blood pressure, in babies with invasive arterial access, continuous and accurate measurement of this parameter is routinely done. The accuracy of the noninvasive oscillometric method is less certain, especially when severe hypotension develops. Normal ranges for blood pressure in babies of different gestations have been defined in the literature (epidemiologic definition of hypotension), and it is clear that gestation and postnatal age are the dominant influences on blood pressure.[2] The nomogram from the data of Nuntnarumit et al. in Fig. 46.9 shows the 10th percentile for mean blood pressure of babies from different gestations at different postnatal ages.[145]

The normal gestational- and postnatal-age-dependent blood pressure range is not known, primarily because it is affected by additional factors such as disease severity, history of perinatal insult, presence of infection or ductal shunting, and interindividual variations.[1,2,146] In addition to the epidemiologic definition of hypotension, increasing severity of hypotension can be defined from a pathophysiologic standpoint as the blood pressure value at which CBF becomes pressure-passive (autoregulatory blood pressure threshold; the definition used for the pediatric and adult patient population), brain function is affected (functional blood pressure threshold), and tissue ischemia develops (ischemic

TABLE 46.1 Systemic and Regional Hemodynamic Parameters Most Commonly Monitored in Neonates

	Parameter	Technology/Method	Purpose and Acquisition [C, I or C/I]
Systemic perfusion (BP and CO)	Heart rate	ECG (electrodes)	In conjunction with stroke volume gives flow status [C]
	BP	Arterial line/cuff (oscillometry; Doppler-US)	Perfusion pressure [C/I]
	Stroke volume/CO	Echocardiography	Systemic, pulmonary (CO) and organ blood flow, cardiac function [I]
		IEC	Systemic blood flow (CO) [C]
Systemic oxygenation	SPO_2	Pulse oximetry	Oxygenation on the arterial side [C]
CO_2 status	TCOM	CO_2 diffusion through skin	Effect on cerebral vasculature (changes in CBF) [C]
Regional perfusion	Regional O_2 saturation	NIRS	Tissue oxygenation and (indirectly) organ perfusion [C]
Peripheral perfusion	Microcirculation (oxygenation; blood flow velocity; capillary recruitment)	Visible light technology	Peripheral perfusion [C]
		Laser Doppler flowmetry	Peripheral perfusion [C]
		OPS and SDF	Peripheral perfusion [C]
Indirect assessment of perfusion	Capillary refill time	Visual	Systemic perfusion (indirectly) [I]
	Delta T (C-P)	Temperature	Systemic perfusion (indirectly) [I]
	Color	Visual	Peripheral perfusion [I]
Organ function	Brain electrical activity	aEEG	Assessment of brain activity [C]
	Urine output	Urinary catheter	Assessment of renal function [I]

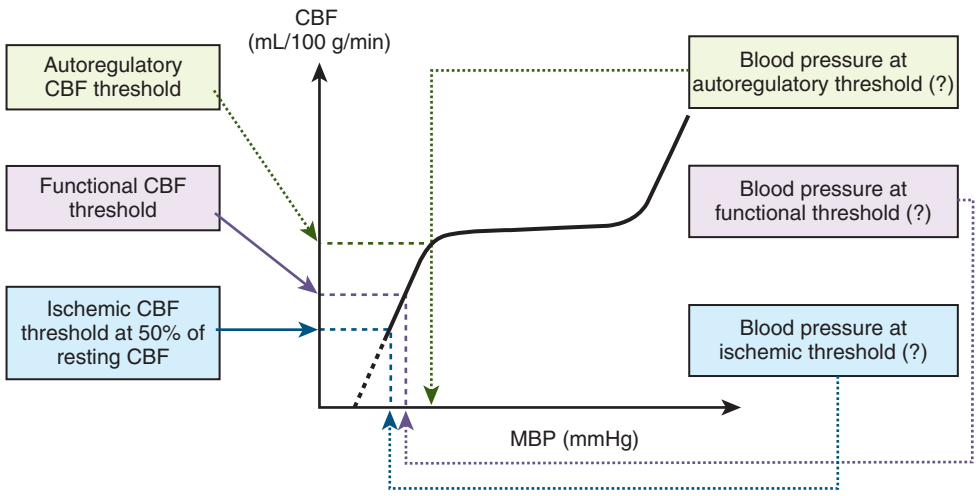
Various components of systemic perfusion (BP and CO) and oxygenation, carbon dioxide production and elimination, regional (organ) and peripheral (microcirculation) perfusion, and organ function (aEEG) that can be monitored at the bedside along with the indirect methods used in the clinical practice to assess perfusion and organ function. Acquisition can be continuous [C], intermittent [I], or both [C/I]. *aEEG*, Amplitude-integrated electroencephalography; *Delta T (C-P)*, difference core and peripheral temperature; *OPS*, orthogonal polarization spectral (imaging); *SDF*, side-stream dark-field imaging; *TCOM*, transcutaneous CO_2 monitoring; *US*, ultrasonography. (Modified from Azhibekov T, Noori S, Soleymani S, et al. Transitional cardiovascular physiology and comprehensive hemodynamic monitoring in the neonate: Relevance to research and clinical Care. *Semin Fetal Neonatal Med* 2014;19:45–53.)



• **Fig. 46.9** Gestational and Postnatal Age-Dependent Nomogram for Mean Blood Pressure Values in Neonates During the First 3 Days of Postnatal Life. The nomogram is derived from continuous arterial blood pressure measurements obtained from 103 neonates with gestational ages between 23 and 43 weeks. Each line represents the lower limit of 80% confidence interval of mean blood pressure for each gestational age group. Thus, 90% of infants for each gestational age group are expected to have a mean blood pressure equal to or greater than the value indicated by the corresponding line (the lower limit of confidence interval). (From Nuntnarumit P, Yang W, Bada-Ellzey HS. Blood pressure measurements in the newborn. *Clin Perinatol*. 1999;26:981–996.)

blood pressure threshold, Fig. 46.10).[1] Some data are available for the autoregulatory and functional blood pressure threshold in very preterm neonates during the first few postnatal days.[72,130] However, there is no information on the ischemic threshold in very preterm neonates, and very little is known about these pathophysiologic measures of hypotension in the term neonate or in any neonate beyond the transitional period. Perhaps the best method to define hypotension would be the demonstration of causation between gestational- and postnatal-age-dependent blood pressure values and clinically relevant outcome measures such as mortality or long-term neurodevelopmental disability. However, because there are no data available on this interaction, there is uncertainty about the normative values. Besides, treatment may have its own side effects; hence there is controversy about when and how to intervene in the critically ill neonate with suspected hypotension and circulatory compromise.[15,16,147]

In clinical practice, hypotension during the first postnatal day is usually defined in one of three ways: as a mean blood pressure less than 30 mm Hg, a mean blood pressure below the patient's gestational age in weeks at the time of birth, or, more recently, a blood pressure value that is accompanied by clinically detectable evidence of circulatory compromise (decreased urine output, poor peripheral perfusion, and/or lactic acidosis). A threshold of 3 or 5 mm Hg below the gestational age in weeks for mean blood pressure also has been used for initiating vasopressors-inotropes.[148]



• **Fig. 46.10** Definition of Hypotension by Three Pathophysiologic Phenomena of Increasing Severity: Autoregulatory, Functional, and Ischemic Thresholds of Hypotension. CBF, cerebral blood flow; MBP, mean blood pressure. (From Noori S, McLean CW, Wu T-W, et al. Cerebral circulation and hypotension in the premature infant: diagnosis and treatment. In: Perlman JM, Cilio MR, eds. *Neurology: Neonatology Questions and Controversies*. 3rd ed. Philadelphia: Elsevier; 2019:1–26.)

However, in preterm neonates with clinically asymptomatic hypotension (blood pressure value in mm Hg below the gestational age in weeks), antihypotensive treatment is associated with improved outcomes (see below).[3] Thus, at present the clinician must rely on his/her understanding of developmental hemodynamics, pharmacodynamics, and the pathophysiology of the transitional period as well as the findings in the literature to make the most appropriate decision at the bedside of the individual patient with hemodynamic compromise.

As mentioned earlier, there is only a weak relationship between mean blood pressure and SVC flow, a surrogate measure of systemic blood flow in preterm neonates with the fetal channels open in the immediate postnatal period.[11] Thus, if blood pressure alone is used to guide treatment, patients in the unrecognized compensated phase of shock may not be treated appropriately. For instance, in the extremely preterm neonate with mean blood pressure between 20 and 40 mm Hg in the immediate postnatal period, the state of systemic blood flow and CBF is unclear based on the blood pressure values alone. Recent advances in our ability to apply complex, continuous cardiovascular monitoring providing data in absolute numbers using impedance-based electrical cardiometry, NIRS, and amplitude-integrated EEG combined with the intermittent use of Doppler flow measures hold the promise of gaining a better understanding of the hemodynamic changes occurring during transition and beyond in the neonatal patient population.[7]

Capillary Refill Time (CRT)

Several studies have attempted to validate this widely used clinical tool for accuracy. However, there is no validated standard for assessing decreased peripheral perfusion and its relationship to systemic flow in the newborn. Further, in the different types of shock (warm vs. cold shock), the state of peripheral perfusion is different (vasodilation vs. vasoconstriction). Hence it is impossible to objectively assess the practical utility of this clinical tool in neonates. Accordingly, in VLBW neonates, a poor correlation was found between the capillary refill time, assessed on the forehead, sternum, and toe, and mean blood pressure, urine output, and SVC flow.[149] An earlier study documented a similar lack of tight relationship in VLBW neonates; only when CRT was greater than 5 seconds did it have any clinically relevant degree of specificity.[150] Although a CRT of ≤3 seconds is traditionally accepted as normal, a CRT of 4.23 ± 1.47 seconds with a range of 1.63 to 8.78 seconds was reported in a large population of healthy newborns during the first 72 postnatal hours. CRT does not appear to change during the first 72 postnatal hours in healthy term newborns.[151] Environmental, axillary, hand, and foot temperatures have been reported to indirectly relate to CRT.[151] Interestingly, the duration of the pressure applied when eliciting CRT affects the measurement in term neonates less than 4 hours after delivery.[152] This study also found an unanticipated moderate, direct correlation between blood pressure and CRT with a prolongation of CRT at higher blood pressures in this patient population.[152] In general, interobserver variability has been reported to be fair, but a variation in the measurement among the different sites (sternum, forehead, hand, and feet) may be significant, although this issue needs to be more systematically studied.

Core-Peripheral Temperature Difference

There are few data to support the accuracy of this test in older infants.[153] As for preterm neonates less than 30 weeks' gestational age, there was also no relationship between this measure and SVC flow in the immediate postnatal period.[150] However, because SVC flow measurements have their limitations, these studies need to be repeated when a more accurate and continuous measure of systemic blood flow assessment becomes available for neonates with fetal channels open.

Low Urine Output and Hyperkalemia

Urine output is a frequently utilized clinical measure to assess renal perfusion and function. However, because urine output is low during the first postnatal day as a result of delivery-associated increases in stress hormones (catecholamines, vasopressin, renin-angiotensin) causing renal vasoconstriction and increased tubular reabsorption of sodium and water, its value in assessing

compensated shock during the transitional period is limited. After the first 2 to 3 days, however, a decrease in urine output may be the earliest clinical sign of compensated shock in neonates of all gestational ages. In addition, a relationship has been documented in very preterm infants between low SVC flow and subsequent low urine output and hyperkalemia.[154] However, because hyperkalemia may occur in the very preterm neonate without oliguria (i.e., nonoliguric hyperkalemia of the extremely preterm neonate),[155] and because there could be other causes of hyperkalemia (acidosis), hyperkalemia alone should not be used as a measure of poor systemic perfusion.

Lactic Acid, pH, and Base Excess

In a large series, pH and base excess were found to have little relationship with low SVC flow in preterm babies in the immediate postnatal period.[154] This finding is likely explained by the lack of a strong relationship between pH, base excess, and lactic acid levels in neonates.[156] However, because serum lactate levels can be sequentially followed routinely on blood gases, and because changes in serum lactate are informative of changes in the cardiovascular status, this indirect measure of cellular oxygen delivery and consumption has been used in clinical practice. Interestingly, combining a CRT of >4 seconds with an elevated serum lactate concentration of >4 mmol/L has a specificity of 97% for detecting a low SVC flow state in VLBW neonates during the first postnatal day.[149] One needs to keep in mind, though, that a given serum lactate level primarily represents past hemodynamic events and not necessarily the present state of cardiovascular function. Thus, as with urine output, by the time lactic acidosis develops and is detected, the initiating event may or may not be present anymore. It is not a surprise, therefore, that rising lactate levels are more predictive of adverse outcomes than a high value early on followed by a subsequent decline.[156]

Organ Blood Flow

With the use of Doppler ultrasonography and, more recently NIRS, blood flow to various organs can be assessed at the bedside. As for CBF, several methodologies have been studied in both preterm and term neonates including Doppler ultrasonography, xenon clearance, and NIRS.[14,58,59,157–160] Because peripheral arteries tend to be too small for size measurement, Doppler studies in such vessels are limited to parameters of velocity from which it is not possible to derive blood flow. Consequently, peripheral artery Doppler tends to be more useful for assessing changes over a time frame in which it is unlikely that the vessel size will have changed.[58] Xenon clearance is not practical outside a research setting. However, advances in NIRS technology have allowed for continuous assessment of brain, renal, mesenteric, and muscle oxygenation (i.e., blood flow) at the bedside in the critically ill neonate (see next section).[161–163]

Near-Infrared Spectroscopy (NIRS)

NIRS uses changes in tissue oxygenation over time or the difference between oxygenated and deoxygenated hemoglobin to indirectly assess flow. The technology has been very useful as a research tool. In addition, with a better understanding of biophysical principles that govern light behavior in tissues, and the associated advances in software algorithms and sophisticated NIRS probe development, continuous assessment of regional tissue oxygenation (rSO_2) has become available and gained evidence-based application in neonatal intensive care.[164,165]

Indeed, commercially available monitors with up to six channels are now being applied to infants, typically on the forehead to gauge cerebral rSO_2 and over the flank, to measure renal rSO_2. Although more validation is needed using invasive measurements,[166–168] it is evident that when cardiac output and/or mean arterial blood pressure fall, the renal rSO_2 declines. First, cerebral rSO_2 may be preserved, but interference with global cerebral blood flow can also be detected with this monitor, provided that certain conditions are met. These monitors are useful during cardiac surgical procedures on infants,[169–171] where precipitous drops in cerebral rSO_2 may be the only warning of a malpositioned venous or arterial cannula while on cardiopulmonary bypass. In addition, emerging findings suggest that monitoring rSO_2 in critically ill term and preterm neonates without congenital heart disease during transition to extrauterine life[163] and treatment for various conditions such as preterm neonates with a PDA,[162,172] asphyxiated infants with or without cooling,[159,173–176] or term neonates receiving ECMO[177–179] provides clinically useful information.[163–165,180] Whether titration of therapy based on rSO_2 measurements in neonates with critical conditions other than congenital heart disease requiring surgery[169] improves patients' outcomes remains unknown.[181] In our experience, however, renal rSO_2 reliably declines well before signs of uncompensated shock such as hypotension or acidosis develop (see later discussion), enabling earlier intervention.[19]

Echocardiographic Systemic Blood Flow Measures

In the mature circulation, systemic blood flow is the cardiac output. Although the output of both ventricles will be the same, cardiac output is traditionally measured from the left ventricular output. In clinical practice, Doppler ultrasound offers a noninvasive but noncontinuous method to measure cardiac output. Measuring blood flow directly in the pulmonary artery and ascending aorta enables us to evaluate outputs from the right and left ventricles, respectively. However, as discussed earlier, in transitional circulation of the newborn infant, neither ventricular output will consistently reflect systemic blood flow because of the shunts across fetal channels. The use of SVC flow as a surrogate for systemic blood flow has been a valuable research tool offering insights into hemodynamic events unfolding during the immediate postnatal period when shunting across fetal channels precludes the use of traditional measures of systemic blood flow assessment (see earlier discussion).[182]

Doppler measures of left ventricular output have been validated against more invasive measures in neonates and older subjects.[183] Right ventricular output and SVC flow are less well validated but correlate well with left ventricular output in neonates with no confounding shunts.[11,45,184] Despite this, the validity of these measures has been questioned based on a comparison to cardiac output thermodilution measures in critical care patients.[185] However, thermodilution itself has an intrinsic error in that the volume of the required cold saline injection influences cardiac output.[186]

Recently, functional magnetic resonance imaging has become the "gold standard" for cardiac output measurements. However, this method is utilized primarily for research applications, and its use for bedside assessment in neonates remains limited due to a number of technical and logistical difficulties.[187] Thus, finding an ideal gold standard method capable of continuous bedside cardiac output measurements in absolute numbers remains elusive.

There are significant intrinsic errors in Doppler flow measures as well. Intraobserver variability rates of around 10% and interobserver variability rates up to 20% are common.[38,188] Most of the error relates to the vessel size measurement that is derived from its diameter. Thus, small differences are magnified in the conversion to a cross-sectional area. The other major problem of Doppler flow measurements is that there is reliance on ultrasound technology and echocardiographic skill that may not often be available 24 hours a day in many neonatal intensive care units.

In neonates, most studies quote a normal range for left and right ventricular output between 150 and 300 mL/kg/min.[189–192] It is important to note that, in the transitional neonatal circulation, left ventricular output can be affected by ductal shunting to a greater extent than the right ventricular output is affected by atrial shunting. Therefore, right ventricular output is a better measure of low flow than left ventricular output during the first 1 to 2 postnatal days. Accordingly, a relationship has been documented between indirect measurements of systemic (and thus cerebral) perfusion such as right ventricular output and blood pressure (and cerebral function) in preterm infants during the first 2 postnatal days. This is a period when ductal shunting significantly decreases the accuracy of left ventricular output measurements reflecting systemic blood flow.[192] SVC flow may also be used to estimate systemic flow in the early postnatal period. The normal range in well preterm babies is between 40 and 120 mL/kg/min, with the median rising from 70 mL/kg/min at 5 hours of age to 90 mL/kg/min at 48 hours.[11]

In summary, the mainstay of diagnosing neonatal circulatory compromise has been a combination of blood pressure measurement and evaluation of specific clinical parameters. However, none of these parameters has a sufficient degree of accuracy to allow it to be relied on as the sole evaluator of systemic blood flow and tissue perfusion. Therefore, the addition of echocardiographic and NIRS hemodynamic assessment to blood pressure monitoring and thorough continuous clinical evaluation of the patient are necessary to better understand changes in organ blood flow and tissue perfusion, especially in preterm neonates during the vulnerable period of immediate transition to extrauterine life. The goal should be to maintain normal systemic blood flow and thus oxygen delivery in the presence of an acceptable blood pressure using the normal range for blood pressure that controls for gestation and postnatal age and following the indirect clinical and laboratory signs of tissue perfusion (urine output, serum lactate levels, CRT). If there is no immediate access to echocardiography or NIRS technology, the clinician will have to rely on blood pressure monitoring while recognizing the limitations of this approach. Over the past decade, training in functional echocardiography for neonatologists has become available in a structured format in several programs in the United States and abroad,[193–195] although the medicolegal implications of such training remain largely unknown.[196]

Measurement of Systemic Blood Flow Using Electrical Impedance Velocimetry

Electrical velocimetry (EV) measures left cardiac output by continuous, noninvasive measurement of thoracic electrical bioimpedance.[197] It has also been validated against invasive methods of cardiac output measurements with very good correlations in animals,[198] and children[199] and against echocardiography in healthy term neonates during the first two postnatal days.[200] However, its evidence-based value in pediatric clinical practice in general[201] and neonatology, in particular, remains to be established.[202,203]

Treatment of Neonatal Shock

Selection of the most appropriate treatment strategy for neonatal shock requires identification of its pathogenesis. As described earlier, the most frequent etiologic factors responsible for neonatal cardiovascular compromise are inappropriate peripheral vasoregulation, resulting in vasodilation or vasoconstriction, and dysfunction of the immature myocardium. Although absolute hypovolemia was thought to be a less frequent primary cause of neonatal hypotension, especially in preterm infants in the immediate postnatal period,[79] recent findings suggest that this assumption is incorrect.[81]

Association Between Systemic Hypotension, Hypoperfusion, and Their Treatment and Mortality or Neurodevelopmental Impairment

The impact of hypotension and/or its treatment on mortality, brain injury, or neurodevelopmental impairment is unclear, primarily because it is assumed that treatment will improve outcomes, and therefore the common practice has been to treat hypotension.[73] Consequently, there have been no prospective studies evaluating the impact of untreated hypotension on clinically relevant short- and long-term outcomes. There have been two attempts at conducting prospective randomized controlled trials (RCT), each with a no-treatment arm, and with neither achieving its objectives.[148,204] The first RCT was a pilot study and found that such a trial is not feasible, at least in the US, primarily due to difficulty in obtaining consents but also because of the clinicians' bias favoring treatment.[204] The second RCT also had difficulty with recruitment and had to be terminated early at 7.7% of the target sample size.[148] Accordingly, the study was significantly underpowered to draw any meaningful conclusion on the impact of untreated hypotension. In addition to the paucity of data from RCTs on the effect of treatment on the outcome, the gestational- and postnatal-age-dependent definition of hypotension based on physiology, pathophysiology, and clinically relevant outcome measures is lacking.[2,146]

One prospective RCT found that hypotensive VLBW neonates overall had a higher rate of severe P/IVH than their non-hypotensive counterparts.[205] Interestingly, hypotensive infants responding with an increase in blood pressure to targeted titration of vasopressors/inotropes had similar rates of P/IVH when compared to non-hypotensive controls. The authors also did not find an association between abnormal ultrasound findings and the use of dopamine or epinephrine. At follow-up at 2 to 3 years of age, no difference was found in the rate of abnormal neurologic status and/or developmental delay between the formerly hypotensive but successfully treated and the control patients. Indeed, a lack of response to dopamine was found to be associated with a greater risk of IVH, whereas a strong response was associated with a decreased risk among preterm infants <28 weeks' gestation.[206] Although the results of the above studies suggest that carefully titrated use of dopamine or epinephrine in hypotensive preterm infants might be safe and even have potential benefits, the small sample size and the lack of untreated hypotensive controls in the RCT do not allow for generalization of the findings. Of note is that, although dopamine was found to be neuroprotective in an asphyxiated lamb model,[207] evidence in the human neonate is needed to establish the clinical relevance of this finding. On the other hand, there is an association between dopamine use and impaired cerebral autoregulation

in preterm infants.[208,209] However, it is unknown whether dopamine plays a role in the observed impairment in cerebral autoregulation or the impairment is simply reflective of illness severity necessitating vasopressor-inotrope use in the first place.

In the last decade, there has been a move toward less aggressive treatment of hypotension with some completely disregarding blood pressure values. This approach has provided a glimpse into the effect of isolated hypotension. A recent analysis from the French national prospective population-based cohort study found worse outcomes in preterm neonates with untreated isolated (clinically asymptomatic) hypotension.[3] They matched 119 extremely preterm infants with untreated isolated hypotension to 119 who were treated during the first 3 days of postnatal life. Hypotension was defined as the mean blood pressure value in mm Hg less than gestational age in weeks and the matched patients had no clinical signs of inadequacy of cardiovascular function. The treated group had a higher rate of survival without severe morbidity and a lower rate of severe IVH and cerebral injury. Of note, the association between treatment and better outcome was even stronger when hypotension was defined as mean blood pressure 5 mm Hg below the gestational age in weeks. This dose-effect relationship strengthens the possibility of causality.

Volume Administration

Volume administration is the most commonly used intervention for the treatment of hypotension in neonates.[144] However, observations that low blood pressure is frequently associated with normal or even high ventricular output and low index of resistance[13] and that dopamine is more effective in increasing blood pressure than volume administration[44] question the effectiveness of volume administration, primarily when isotonic saline is being used. Therefore, particularly in the preterm infant during the immediate postnatal period, only cautious, limited fluid resuscitation has been recommended.[16] In addition, expansion of blood volume using delayed umbilical cord clamping or cord milking has been shown to improve systemic hemodynamics in preterm infants.[81,90,91,94]

As myocardial dysfunction frequently contributes to the development of neonatal hypotension[210] and aggressive volume administration in this patient population increases pulmonary, cardiovascular, gastrointestinal, and central nervous system morbidity and mortality,[44,211,212] judicious use of fluid administration is warranted.[213] On the other hand, combined relative and absolute hypovolemia is clearly a major contributing etiologic factor to neonatal shock in neonates with sepsis and/or in the postoperative period in patients undergoing major surgery. Indeed, early and aggressive fluid resuscitation of neonates and children presenting with sepsis has been documented to decrease mortality[214] and has been recommended by the international "surviving sepsis campaign"—which recently published guidelines for the management of septic shock and sepsis-associated organ dysfunction in children.[215]

Further controversy has emerged concerning the type of fluid administered to preterm neonates with cardiovascular compromise. Most studies have shown that isotonic saline is as effective as 5% albumin in increasing blood pressure.[216,217] In addition, albumin may impair gas exchange and induce a fluid shift from the intracellular compartment[218] and is associated with increased mortality.[219] Therefore, given the documented comparable efficacy of isotonic saline and albumin in the face of differences in cost and the suggested increased mortality and morbidity associated with albumin administration,[44,211,212] isotonic saline has been the initial choice of treatment in the neonatal patient population. A recent multicenter study found isotonic saline to be the most commonly used solution for fluid bolus in the neonates.[220] The most frequent indications for fluid bolus were hypotension followed by poor perfusion and metabolic acidosis. Interestingly, in 40% of cases, there was minimal or no response to the saline bolus. Indeed, the blood pressure response to colloids, at least in the shorter run, might be better than normal saline. An RCT found that in hypotensive, mostly preterm neonates, albumin administration resulted in a greater likelihood of achieving normotension and decreased the subsequent use of vasopressors when compared to isotonic saline.[221] However, the findings of this study need to be replicated before the routine use of albumin can be considered as the initial treatment for neonatal cardiovascular compromise. Therefore, unless evidence of serum or blood loss or hypoalbuminemia is present, volume support in hypotensive preterm and term infants at present is still provided in the form of 10 to 20 mL/kg of isotonic saline.[17,18,213,222] It is also important to note that, because of the unbalanced nature of isotonic saline, caution should be exercised with the administration of large amounts over a short period of time, as this may worsen the metabolic acidosis.[223,224] Besides isotonic saline and albumin-containing solutions, other fluid replacement options are also available (Table 46.2).[17,213] Lactated Ringer's solution has lower chloride content than isotonic saline and 5% albumin and therefore carries a lower risk of the development of hyperchloremic acidosis. However, it has yet to be adequately studied in the neonatal population.[17] In adults, the use of lactated Ringer's solution is associated with better outcomes in patients with septic shock. A recent secondary analysis of a cluster-randomized trial of balanced crystalloids (either lactated Ringer or Plasma-Lyte A) versus isotonic saline found a lower 30-day in-hospital mortality in the balanced crystalloid group in critically ill adults with sepsis.[225] Another fluid choice is Plasma-Lyte A, the composition of which is very similar to plasma, making it an attractive option as a fluid bolus (see Table 46.2). However, there is no published data on the use of Plasma-Lyte A as a fluid bolus in the neonate. However, when used as a maintenance or replacement fluid, a recent RCT of Plasma-Lyte A versus moderately hypotonic saline solution in acutely ill children at or beyond 6 months of age found a greater risk of developing electrolyte disorders in the Plasma-Lyte A group.[226] Interestingly, there are no studies yet on the safety and efficacy of the use of whole blood transfusion for treatment of hypotension and shock in neonates in the transitional period or beyond.

Should careful volume administration be ineffective, pharmacologic cardiovascular support with a vasopressor-inotrope or an inotrope is recommended.[18] However, based on the recent findings of the aforementioned studies on using delayed cord clamping or cord milking, it is likely that once clinical practice changes as per the recommendations of expert panels,[98,99,227] the need for volume administration in the immediate transitional period will decline.

If there is an identifiable volume loss, the type of fluid lost should be replaced. In cases of blood loss, transfusion with packed red blood cells after the initial crystalloid or colloid bolus or packed red blood cells suspended in fresh frozen plasma with a hematocrit around 55% may be used. In cases of increased transepidermal water losses, higher free water administration without an increase in sodium supplementation is indicated. When polyuria is present, the composition and volume of the replacement fluid may be adjusted to the urinary sodium and free water losses. However,

TABLE 46.2 Electrolyte Content, pH, and Osmolarity of Various Fluid Replacement Options

Fluid Type	Na (mmol/L)	K (mmol/L)	Cl (mmol/L)	Mg (mmol/L)	Ca (mmol/L)	Acetate (mmol/L)	Gluconate (mmol/L)	Lactate (mmol/L)	pH	Osmolarity (mOsmol/L)
Plasma-Lyte A	140	5	98	1.5		27	23		7.4	294
0.9% NaCl	154		154						5.5	308
Lactated Ringer's	130	4	110		1.5				6.2	275
5% albumin*	145±15	<2							6.4–7.4	

Plasma-Lyte A (Baxter), 0.9% NaCl (Baxter), Lactated Ringer's (B. Braun Medical Inc.), 5% albumin (Flexbumin 5%, Takeda Pharmaceutical Company).
*No data available on osmolarity or electrolyte content other than sodium and potassium.
From Wu T-W, Noori S. Recognition and management of neonatal hemodynamic compromise. *Pediatr Neonatol.* 2021;62(Suppl 1):S22–S29.

the replacement of half of the excessive urinary losses with 0.45% saline will usually suffice.

Dopamine and Dobutamine

Dopamine and dobutamine treatments were introduced in the management of neonatal hypotension in the early and mid-1980s, respectively, without appropriately designed randomized and blinded clinical trials. Thus, as mentioned earlier, we have no clear evidence that the use of these sympathomimetic amines (or any other sympathomimetic amine) improves neonatal mortality or morbidity. By examining the drugs' effect on neonatal myocardial contractility, and systemic and organ blood flow, a number of studies have extended their focus beyond the dopamine- and dobutamine-induced heart rate and blood pressure changes.[32,33,44,58,133,228–232] Some of the earlier studies, however, used left ventricular output to assess the impact of these medications on systemic blood flow even when shunting across fetal channels occurred.[33,44,232] Therefore, the conclusions drawn in these studies need to be carefully reevaluated.[43]

Hemodynamic Effects of Dopamine

Dopamine, an endogenous catecholamine, is the sympathomimetic amine most frequently used in the treatment of hypotension in preterm infants.[4,146,233] It exerts its cardiovascular actions via the dose-dependent stimulation of the cardiovascular dopaminergic, α- and β-adrenergic, and serotoninergic receptors. In addition, by stimulating epithelial and peripheral neuronal dopaminergic and adrenergic receptors, the drug exerts significant renal and endocrine effects independent of its cardiovascular actions.[233] Although dopamine affects all three major determinants of cardiovascular function (preload, myocardial contractility, and afterload), the drug-induced increases in myocardial contractility[44,232] and peripheral vascular resistance (afterload)[33,44,232] are the most important factors in increasing systemic blood pressure and improving the cardiovascular status.

The original dose-range recommendation of 2 to 20 μg/kg/min of dopamine was based on pharmacodynamic data obtained in adults without cardiovascular compromise. However, changes in cardiovascular adrenergic receptor expression caused by critical illness[234] and relative or absolute adrenal insufficiency and immaturity,[235,236] as well as the dysregulated production of local vasodilators during severe illness, decrease the sensitivity of the cardiovascular system to dopamine, resulting in the emergence of hypotension resistant to conventional doses of the drug.[133,237–239] Thus, with the advancement of the disease process, higher doses of dopamine and other sympathomimetic amines may be needed to exert the same magnitude of cardiovascular response. Therefore, dopamine administration should be tailored to the drug's pharmacodynamic effects in a given patient at the bedside rather than driven by the conventional dose recommendations based on data obtained in healthy adults. Indeed, although many neonatologists do not advance the dose of dopamine beyond 20 μg/kg/min, there is no evidence that, when required to normalize blood pressure, high-dose dopamine treatment with or without additional epinephrine administration has detrimental vasoconstrictive effects.[222,240] However, there are no data available on changes in cardiac output and organ blood flow in response to high-dose catecholamine treatment in vasopressor-resistant neonatal shock, and close attention should be paid to signs of inappropriate vasoconstriction when this therapy is applied. Several earlier studies have shown that administration of low-dose hydrocortisone ameliorates the need for high-dose vasopressor administration in most patients.[34,133,134,238,239]

As hypotension and circulatory failure are most common in the first postnatal days, a period in which significant shunting at the PDA level is often present in VLBW infants, assessment of the cardiovascular effects of vasopressors/inotropes using echocardiography is challenging. This is especially true with regard to estimating the magnitude of the PDA shunt. Therefore, one needs to be cautious in interpreting these studies. With that caveat in mind, several studies suggest that dopamine does not worsen left-to-right PDA shunt. Rather, in hypotensive preterm neonates with a significant left-to-right shunt across the PDA, dopamine administration increased systemic blood pressure, pulmonary pressure, and SVC flow, used as a surrogate of systemic blood flow in these patients.[228] Similarly, dopamine administration has not been associated with evidence of increased pulmonary vascular resistance and decreased right ventricular output in neonates without a significant left-to-right shunting across the PDA.[241,242] This finding suggests that when pulmonary blood flow is increased, vasoconstrictive mechanisms may be upregulated in the pulmonary circulation, resulting in more effective α-receptor-mediated, dopamine-induced pulmonary vasoconstriction. The findings of an earlier study demonstrating a variable pulmonary resistance response to dopamine in preterm neonates with a hemodynamically significant PDA support this notion.[243]

The vasodilatory dopamine receptors are primarily expressed in renal, mesenteric, and coronary circulations.[233] Dopamine has been shown to selectively decrease renal vascular resistance[231,233] and increase glomerular filtration rate[244] in preterm infants as early as the 23rd week of gestation. However, dopamine appears to decrease mesenteric vascular resistance in preterm infants only beyond the first postnatal day,[58,231,245,246] and the effect may be variable.[232] Similarly, there are some differences in the reported magnitude of the drug-induced increases in ventricular function, cardiac output, and systemic vascular resistance.[33,44,232,241] These findings may be best explained by differences in intravascular volume status, gestational and postnatal age, developmentally regulated expression of cardiovascular adrenergic and dopaminergic receptors, and severity of adrenergic receptor downregulation among different populations of critically ill infants studied. It is important to note that none of the studies found evidence for a direct effect of dopamine on cerebral blood flow as long as blood pressure was in the autoregulatory range.[44,58,231,232] Thus, dopamine administration appears to be devoid of potentially harmful selective hemodynamic effects in the brain. As expected, in hypotensive neonates, dopamine (or epinephrine) increases both blood pressure and CBF.[230] As discussed earlier, there is an association between dopamine exposure and impaired cerebral autoregulation in preterm infants,[208,209] a finding most likely reflective of illness severity.[1,59,63,64]

Hemodynamic Effects of Dobutamine

Unlike dopamine, dobutamine is a relatively cardioselective sympathomimetic amine with significant α- and β-adrenoreceptor–mediated direct inotropic effects and limited chronotropic actions.[247] Dobutamine administration is usually also associated with a variable decrease in total peripheral vascular resistance and, at least in adults, with improved coronary blood flow and myocardial oxygen delivery.[247] Dobutamine's cardiovascular effect is dose-dependent with limited data suggesting an improvement in myocardial function and cardiac output at moderate to high doses.[246,248,249] However, and as seen with the other cardiovascular medications, there is a significant individual variability in the hemodynamic response to and the clearance of dobutamine.[248,249] Therefore dose-titration should always be individualized and the hemodynamic response followed by close monitoring of cardiac output and function, if possible.[7] Furthermore, unlike dopamine, dobutamine increases myocardial contractility exclusively through the direct stimulation of myocardial adrenergic receptors. Because myocardial norepinephrine stores are immature and rapidly depleted in the newborn, and because dobutamine may decrease afterload, newborns with primary myocardial dysfunction and elevated peripheral vascular resistance are most likely to benefit from dobutamine treatment.[32,250] However, dobutamine may offer few hemodynamic benefits if the primary underlying pathophysiology is not poor myocardial contractility. Indeed, a recent placebo-controlled pilot trial showed dobutamine to have no effect on SVC flow.[229] Interestingly, although the addition of dobutamine to dopamine in preterm infants with RDS was effective in increasing blood pressure, it was associated with supranormal cardiac output states and low systemic vascular resistance.[12] Whether the benefits of supranormal cardiac output by providing adequate tissue oxygen delivery throughout the body outweigh the risks of sustained hypercontractility, potentially resulting in myocardial injury, remains to be investigated.

There are few data available on direct renal, cerebral, or pulmonary hemodynamic effects of dobutamine in the newborn.[251] A nonrandomized study comparing the effects of dopamine and dobutamine on blood pressure and mesenteric blood flow in preterm infants found that both drugs increased blood pressure and were equally effective in decreasing mesenteric vascular resistance.[245] Because dobutamine does not stimulate the dopaminergic receptors, β-adrenoreceptor–induced selective vasodilation may be responsible for the observed mesenteric vasodilation in dobutamine-treated patients.

Dopamine Versus Dobutamine

Randomized studies have uniformly demonstrated that dopamine is more effective than dobutamine in increasing blood pressure in the preterm infant, and a meta-analysis of these studies confirmed that dopamine was more successful than dobutamine in treating hypotension, with fewer infants in the dopamine group facing treatment failure.[252] However, there was no difference in short-term adverse neurologic outcomes between the two groups. In the absence of long-term outcome data, no firm recommendations can be made regarding the choice of drug in treating hypotension of preterm infants in the immediate postnatal period. In addition, no information was forthcoming on changes in systemic blood flow. However, when carefully titrated to a predetermined "optimum" hemodynamic effect, dopamine (and epinephrine) increased blood pressure and CBF in hypotensive VLBW neonates during the first postnatal day.[230] Neurodevelopmental follow-up of patients enrolled in this study at 2 to 3 years of age did not reveal evidence of an independent vasopressor-inotrope–associated increase in morbidity (see earlier).[205] Although the findings of this study provide some reassurance against the possibility of a dopamine- (or epinephrine-) associated increase in neurodevelopmental morbidity in VLBW neonates during the first postnatal day, the original study[230] was not sufficiently powered to put these concerns to rest.

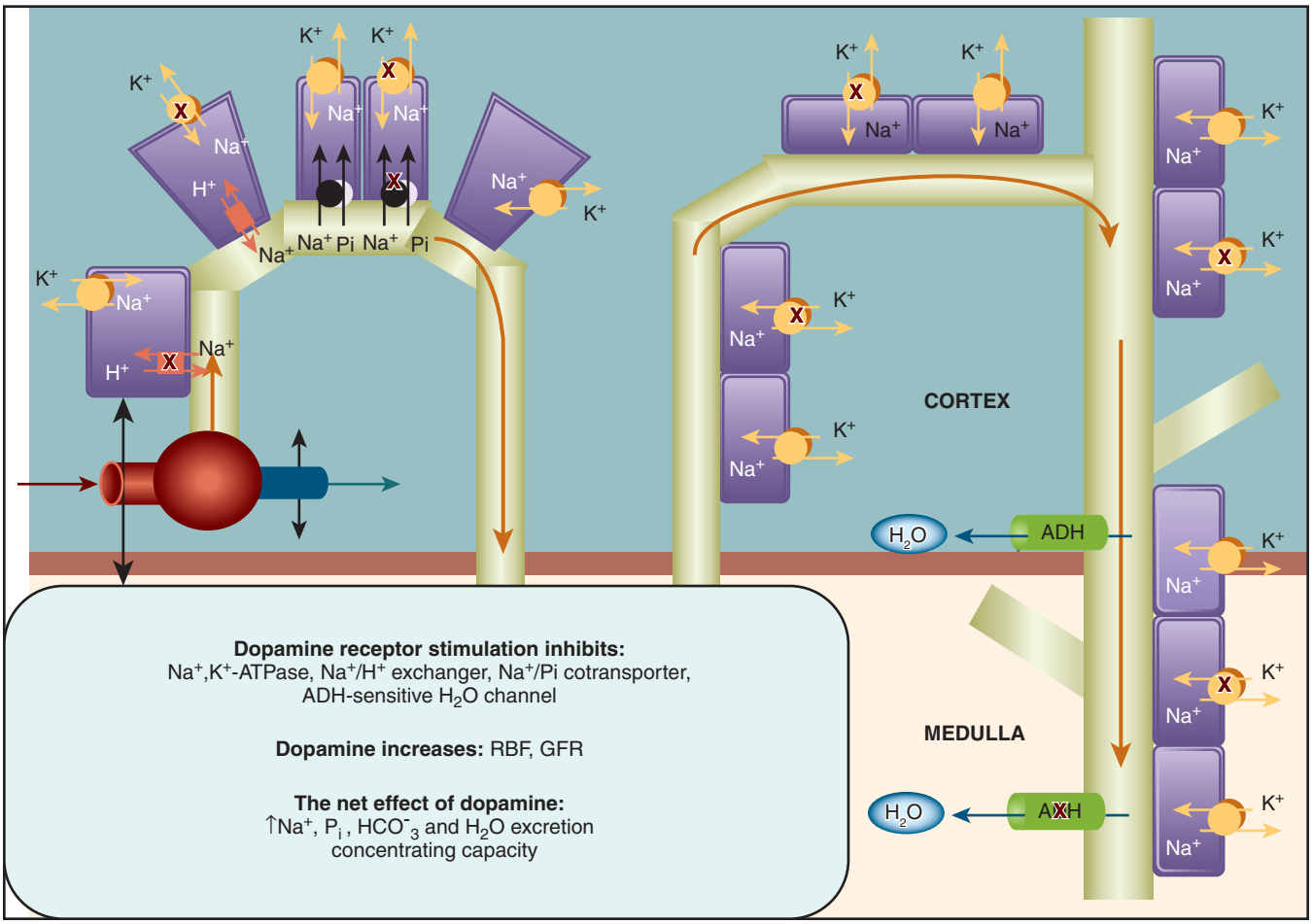
As discussed earlier, because of the weak relationship between blood pressure and systemic blood flow in very preterm neonates during the immediate postnatal period, an increase in blood pressure does not necessarily guarantee that tissue perfusion has improved along with the blood pressure.[10–13,43] Although one study using laser Doppler showed no evidence of peripheral vasoconstriction even with dopamine doses greater than 10 μg/kg/min,[253] an earlier study demonstrated impairment in systemic blood flow with higher doses of dopamine in a subset of patients, despite improvements in blood pressure.[32] Therefore, if there is evidence of peripheral vasoconstriction, especially in VLBW neonates during the first postnatal day, high-dose dopamine treatment should only be attempted if systemic blood flow can be monitored by functional echocardiography, NIRS, and/or other advanced perfusion monitoring techniques. However, in present clinical practice these measures of blood flow monitoring, with the possible exception of NIRS, are not routinely available, and the neonatologist must rely on monitoring blood pressure and the indirect measures of cardiovascular function. If evidence of vasoconstriction is present with higher doses of dopamine (or epinephrine), the neonatologist should consider accepting blood pressure values in the lower range for gestational and postnatal age and decrease the dose of vasopressor-inotrope to levels where significant α-adrenoreceptor stimulation is less likely.[43,233] In these cases and depending on the pathophysiology of the cardiovascular compromise, the addition of dobutamine or milrinone should be considered and the blood pressure maintained in the low-normal range. As systemic hypotension has been linked to poor long-term neurodevelopmental outcomes in the VLBW neonatal patient population,[51,53] careful

consideration of the lowest acceptable perfusion pressure in the given patient is warranted. A combination of dobutamine or milrinone and low-dose dopamine may achieve the most important goals of treatment by maintaining blood pressure and systemic blood flow in acceptable ranges if monitoring of both cardiovascular parameters is possible. In most of these patients, physiologic glucocorticoid and mineralocorticoid replacement with hydrocortisone is likely to be effective, although the potential side effects of early hydrocortisone exposure in preterm neonates should be kept in mind (see later discussion).[43,136] In summary, as maintenance of appropriate perfusion pressure is necessary to ensure appropriate tissue oxygen delivery (systemic and organ blood flow) and because both hypotension and low systemic blood flow have been associated with impaired neurodevelopmental outcomes, the primary goal of management of the hypotensive very preterm neonate should be the correction of both measures of cardiovascular function.

In hypotensive term and preterm neonates beyond the immediate postnatal period, where vasodilatory shock is the more likely presentation, dopamine (or epinephrine) administration in doses tailored to the cardiovascular response is warranted and appears to be beneficial[230,250,254,255] unless evidence of primary myocardial dysfunction is present.[222,233]

Epithelial and Neuroendocrine Effects

Independent of the just-described cardiovascular effects, dopamine exerts direct renal[233] and endocrine[233] actions in the newborn. Via its actions on renal blood flow and glomerular filtration rate as well as through its direct effects on sodium, phosphorous and water transport processes, and Na^+K^+-ATPase activity in renal tubules, dopamine increases sodium, phosphorus, and free water excretion (Fig. 46.11) and may increase the hypoxic threshold of renal tubular cells during episodes of hypoperfusion and hypoxemia.[233] Via its renal vascular and epithelial actions,



• **Fig. 46.11** Effects of Dopamine Along the Nephron. Dopamine increases renal blood flow (RBF) by selective renal vasodilation and by increasing mesangial cell surface area and glomerular hydrostatic pressure via the preferential dilation of the afferent arteriole *(black arrows)*. These changes result in an increased single-nephron glomerular filtration rate (GFR). Indeed, administration of dopamine to normotensive preterm neonates results in increased sodium, phosphorous, and free water excretion (Seri, 1993 and 1995). These effects are primarily caused by the drug-induced inhibition of Na^+-K^+-ATPase, Na^+/H^+ exchanger, and the Na^+/Pi cotransporter in the proximal tubule as well as inhibition of antidiuretic hormone (ADH)-induced phosphorylation of water channels in the collecting duct (Seri, 1995). The proposed resultant increase in bicarbonate excretion has not been documented in preterm infants. **X** *(in red)* = inhibition of enzyme or transporter function. (From Kelly L, Seri I. Renal developmental physiology: relevance to clinical care. *NeoReviews*. 2008;9:e150–e161.)

dopamine also potentiates the diuretic effects of furosemide[256] and theophylline.[257] Although dopamine has the theoretical potential to attenuate renal side effects of indomethacin, the data in the literature are contradictory.[233] Differences in the level of maturity, disease severity, ductal shunting, intravascular volume status, and indomethacin dose may be responsible for the conflicting results. Among its endocrine actions, the dopamine-induced decreases in plasma prolactin, thyrotropin, and growth hormone levels[233] may be of clinical importance. The decrease in plasma prolactin may attenuate the preterm infant's propensity to edema formation but may also have a modulating effect on immune function.[233] The inhibition of thyrotropin release necessitates the postponement of routine neonatal thyroid screening until after dopamine administration has been discontinued.[233,258] Dopamine, compared to dobutamine, decreases serum TSH and T4 levels by approximately 70% to 80% and 50%, respectively.[258] Indeed, as dobutamine does not stimulate dopaminergic receptors, its administration is devoid of neuroendocrine effects. The potential impact on long-term neurodevelopmental outcome and immunologic function of the drug-induced alterations in neuroendocrine function is not known in the preterm and term neonates.

Epinephrine, Norepinephrine, and Other Cardiovascular Agents and Hormones

Epinephrine

In an RCT, low to medium doses of epinephrine, when titrated to optimal hemodynamic response, have been shown to increase blood pressure and CBF in hypotensive VLBW neonates.[230] Because of epinephrine's significant effect on glycogenolysis, its administration is associated with an increase in serum lactate levels independent of the drug's cardiovascular actions.[57] This effect should be kept in mind when following serum lactate levels to assess the changes in cardiovascular status due to epinephrine administration. Therefore, the epinephrine-induced improvement in perfusion cannot be ascertained by following serum lactate levels alone, because these levels will likely increase despite drug-induced improvement in the cardiovascular status. A recent double-blinded cross-over RCT of epinephrine versus dopamine for neonatal septic shock showed no difference in the reversal of shock by 45 minutes and mortality in 28 days.[259] It is of note that on analysis stratified for gestational age groups, epinephrine seemed to be favored in neonates $\leq 30^{6/7}$ weeks' gestation.[259] Finally, in a recent meta-analysis on the use of epinephrine versus dopamine in patients with pediatric and neonatal septic shock, the drugs showed similar efficacy in the three available RCTs.[260]

It is not known whether there is a difference in the cardiovascular response and/or side effects of sympathomimetic amines with the combined use of epinephrine and dopamine compared to the use of increasing doses of dopamine beyond 20 μg/kg/min with or without dobutamine. In preliminary publications, there have been no detrimental vasoconstrictive effects reported using either high doses of epinephrine with or without dopamine, or norepinephrine in sick neonates and children.[261–263] These findings are best explained by the down-regulation of cardiovascular adrenergic receptors and thus the decreased cardiovascular sensitivity of critically ill preterm infants to catecholamines, necessitating escalating sympathomimetic support. However, in a retrospective study of VLBW infants with dopamine-resistant hypotension, epinephrine administration was associated with an increase in blood pressure and heart, no effect on urine output, and worsening metabolic acidosis.[264] Therefore, one must be extremely careful when escalating treatment with these potent vasopressor-inotropes in order to decrease the risk of unwarranted severe vasoconstriction and compromised tissue perfusion. As referred to earlier, with the appropriate use of low-dose hydrocortisone in patients with evidence of relative adrenal insufficiency, high-dose vasopressor support is rarely required.

Norepinephrine

Norepinephrine is the drug of choice for the treatment of septic (vasodilatory) shock in adult patients and is being increasingly used in children with septic shock.[215,265] However, due to a number of developmentally regulated factors, the pharmacokinetics and pharmacodynamics of norepinephrine are different in neonates. Unfortunately, limited data are available on the effectiveness and safety of norepinephrine in the neonatal population.[266] An observational study in late preterm and term neonates with persistent pulmonary hypertension of the newborn (PPHN) treated with inhaled nitric oxide (iNO) who also presented with signs of circulatory failure, found that medium doses of norepinephrine improved systemic and pulmonary cardiovascular function by decreasing the pulmonary artery–to–aortic pressure gradient and improving cardiac performance.[267] Another observational study also found improvement in blood pressure, urine output, and lactic acidosis in term infants with refractory hypotension unresponsive to conventional treatment.[268] A recent retrospective study of preterm infants with septic shock and refractory hypotension to epinephrine or dopamine reported improvement in blood pressure and urine output but no change in pH or base deficit 8 hours after initiating norepinephrine therapy.[269] Forty-three percent of patients were also receiving hydrocortisone, which likely had an impact on the finding. Another retrospective study of preterm infants with cardiovascular compromise (mostly due to sepsis or pulmonary hypertension) also found improvement in blood pressure and oxygenation but no change in pH, lactate, or urine output following initiation of norepinephrine infusion.[270] Ninety-four percent of the patient were on other vasopressor-inotropes, and more than half also received hydrocortisone. Despite normalization of blood pressure in all except one of the patients, 1 hour after starting norepinephrine, 45% of the patients died. Although some of the above findings are encouraging, the routine use of norepinephrine in the treatment of neonates with PPHN and cardiovascular compromise requires further investigation.

Milrinone

There are limited data available on the pharmacokinetics, pharmacodynamics, and cardiovascular effects of milrinone, a phosphodiesterase-III (PDE-III) inhibitor in the neonatal patient population.[271–273] It has been widely used to reduce afterload in neonates with congenital heart disease with low cardiac output syndrome after surgery.[274] As for its use in term infants without congenital heart disease, milrinone administration has been shown in a small case series to improve the oxygenation index without compromising systemic blood pressure when given to term neonates with PPHN treated with iNO during the first 2 postnatal days.[275] Another small retrospective study found improvement in indices of cardiac function about 24 hours after initiation of milrinone in late preterm and term infant with pulmonary hypertension treated with iNO.[276] The preferential effect of milrinone on the pulmonary circulation in neonates with PPHN treated with iNO may be due to the upregulation of PDE-III in pulmonary vessels in response to iNO administration.[277] It is unclear

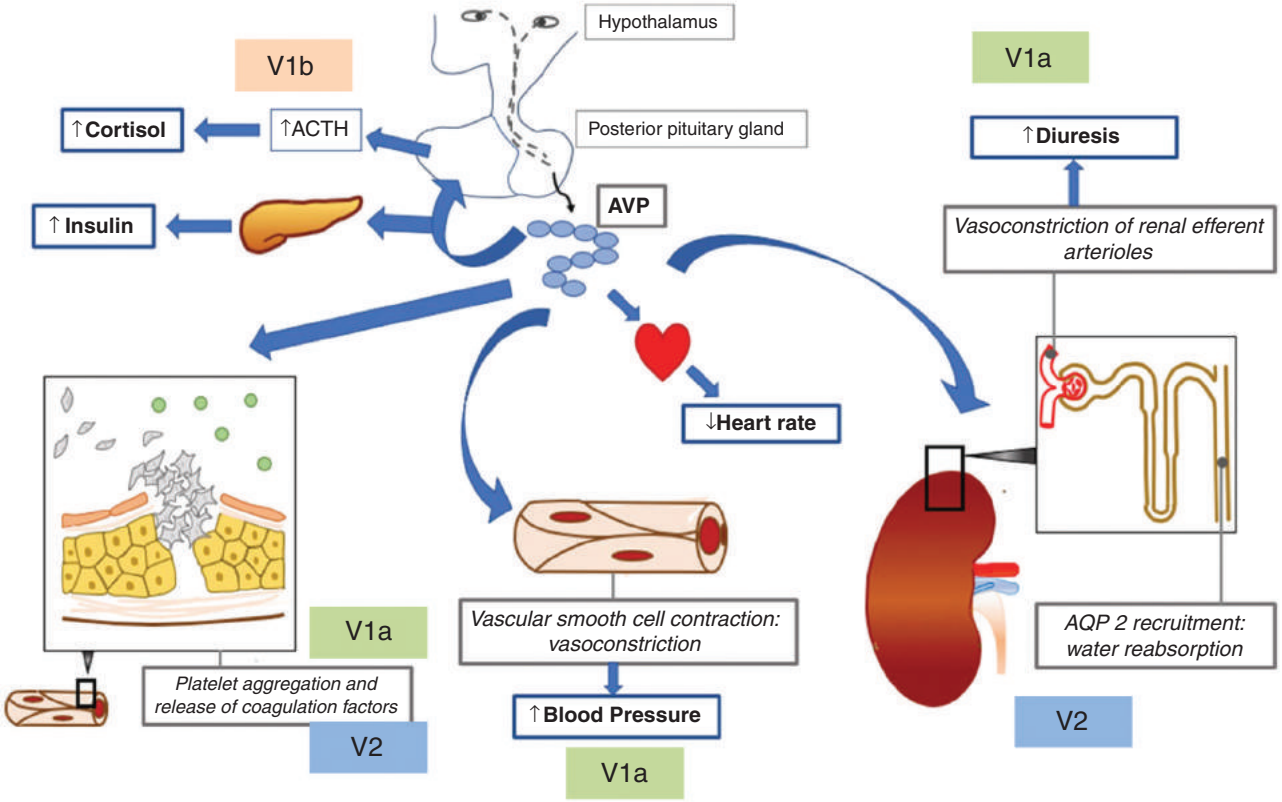
if milrinone has a positive inotropic effect in neonates.[278] A retrospective study of 1446 neonates exposed to milrinone found that 42% of the infants had adverse effects attributed to the drug administration.[273] Arterial hypotension prompting vasopressor-inotrope administration and thrombocytopenia were the most frequently noted adverse effects. A recent case-control study found hypotension to be particularly severe after initiating milrinone in patients with hypoxic-ischemic encephalopathy receiving hypothermia and inhaled nitric oxide therapies.[279]

A randomized placebo-controlled blinded clinical trial also investigated whether the use of milrinone would minimize or prevent the suspected increase in systemic vascular resistance and the associated systemic hypoperfusion in VLBW infants during the first postnatal day.[280] However, there was no improvement in the incidence of low SVC flow used as a surrogate of systemic blood flow in patients treated with milrinone compared to the placebo group. These findings may be explained, at least in part, by the little-appreciated complexity of the pathophysiology of cardiovascular compromise in the very preterm neonate after delivery.[281] They further suggest that systemic and cerebral hypoperfusion and reperfusion in this patient population may not solely occur because of the inability of the immature myocardium to pump against the sudden postnatal increase in systemic vascular resistance.[73,122]

Vasopressin

Vasopressin acts on various organs and vascular beds via three receptor subtypes.[282] The stimulation of V_{1a} receptor is responsible for its vasoconstrictive effects in the systemic vascular bed and renal efferent arterioles. Stimulation of this receptor also leads to platelet aggregation (Fig. 46.12). Yet, the hemodynamic effects of this receptor subtype are more complex and, in animal models, stimulation of the V_{1a} receptor is associated with both an increase and decrease in coronary blood flow and contractility.[283] The activation of the V_{1b} receptor results in ACTH and insulin release from the anterior pituitary gland and the pancreas, respectively. V_2 receptors are involved in the recruitment of aquaporin in the collecting ducts and promote renal absorption of free water. V_2 receptor stimulation also leads to the release of clotting factors.

Despite the multiple physiologic roles of vasopressin, the primary cardiovascular effect of exogenous vasopressin in the systemic circulation is vasoconstriction. However, in the pulmonary circulation, low doses of vasopressin have been shown to induce



• **Fig. 46.12** Effects of Vasopressin on Various Organs and Vascular Beds. Vasopressin (AVP, arginine vasopressin) is synthesized in the hypothalamus and carried via the nerve tracts to the posterior pituitary gland. Once secreted from the posterior pituitary gland into the bloodstream, it acts on different vasopressin receptor subtypes. The stimulation of V_{1a} receptor is responsible for its vasoconstrictive effects in the systemic vascular bed and renal efferent arterioles. Stimulation of this receptor also leads to platelet aggregation. The activation of the V_{1b} receptor results in adrenocorticotropic hormone (ACTH) and insulin release from the anterior pituitary gland and the pancreas, respectively. V_2 receptors are involved in the recruitment of aquaporin (AQP) in the collecting ducts and promote renal absorption of free water. V_2 receptor stimulation also leads to the release of clotting factors. (Modified from Demiselle J, Fage N, Radermacher P, et al. Vasopressin and its analogues in shock states: a review. *Ann Intensive Care*. 2020;10:9.)

vasodilation in adults. In neonates with pulmonary hypertension including patients with a congenital diaphragmatic hernia, several case series have also reported improvement in oxygenation and a decrease in pulmonary pressure following low-dose vasopressin administration.[284–287] However, as expression of vasopressin receptors is developmentally regulated, it is unclear if vasopressin truly induces selective pulmonary vasodilation in the neonatal population.[288]

Its systemic pharmacodynamic profile makes vasopressin a good candidate for the treatment of circulatory failure when the underlying pathophysiology is primarily that of vasodilation such as in septic shock without myocardial dysfunction or systemic inflammatory response following cardiac surgery. However, there are only case series reporting improvement in systemic hemodynamics in preterm and term infants with refractory hypotension or septic shock.[289–293] In adults, some RCTs have demonstrated a reduction in mortality with the use of vasopressin in septic shock.[294,295] A randomized pilot study compared vasopressin to dopamine as the first line of treatment in hypotensive preterm infants.[296] This study found that vasopressin resulted in similar improvement in blood pressure and urine output compared to dopamine. In addition, the vasopressin group had less tachycardia. Interestingly, a recent meta-analysis of the available three RCTs and five prospective clinical trials in the pediatric age groups found no clinical benefit of vasopressin or terlipressin use on mortality and length of stay and the results suggested a potential increase in the risk of ischemic events compared to conventional vasopressor-inotrope use.[297]

Clinical scenarios in which the use of a vasopressor without significant inotropic effect may be advantageous include hypotensive neonates with hypertrophic cardiomyopathy. In such cases, increasing inotropy will worsen the dynamic obstruction of left ventricular outflow tract and lead to worsening systemic hypoperfusion. Apart from volume administration and the use of beta-blockers, a pure vasopressor without significant inotropic effects such as phenylephrine and vasopressin can be helpful. Indeed, a recent case series reported improvement in blood pressure and oxygenation after starting vasopressin in neonates with hypertrophic cardiomyopathy.[298] Although, as discussed earlier, vasopressin acts on a number of organ systems, RCTs in adults have not shown an increase in adverse cardiac effects or coagulation problems in the vasopressin arm.[294,299] However, a metanalysis of RCTs in adults has shown an increased risk of digital ischemia.[295] Vasopressin-induced hyponatremia has been reported in adults, and the limited experience in neonates indicates that it is a common side effect of vasopressin administration.[291,298] Therefore, more data on the safety and efficacy of vasopressin are needed before it can be introduced in the routine clinical care of critically ill neonates with vasodilatory shock.[300]

Steroid Administration

Steroid Administration as Primary or Rescue Treatment

Over two decades ago, a small RCT showed that low-dose hydrocortisone improved blood pressure but was less effective than dopamine in hypotensive preterm infants.[301] However, given the concerns over adverse long-term effects of steroids (primarily dexamethasone) and inadequate data on their efficacy, steroids are not recommended for first-line treatment of hypotensive preterm infants.[236,302]

As for rescue treatment, there is now overwhelming evidence provided by descriptive studies and confirmed by randomized blinded prospective trials that brief steroid treatment stabilizes the cardiovascular status and decreases the need for vasopressor-inotropic support in the critically ill preterm and term newborn with vasopressor-resistant hypotension. Because there is overwhelming evidence that early and/or medium-to-high cumulative doses of dexamethasone have detrimental effects on the developing brain, and because in addition to glucocorticoids, mineralocorticoids also have significant effects on the cardiovascular system, even low-dose dexamethasone administration to treat vasopressor-resistant hypotension has fallen by the wayside in recent years.[238] This section, therefore, addresses only the actions, side effects, recommended doses, and remaining clinically relevant concerns of hydrocortisone administration to treat vasopressor-resistant hypotension in the critically ill neonate. It is of note that the available evidence for the effectiveness of low-dose hydrocortisone to increase blood pressure and decrease vasopressor-inotrope requirement in the preterm neonate is so strong that it would take 74 and 188 future studies demonstrating no effect of hydrocortisone on blood pressure increase and on the decrease in vasopressor requirement, respectively, to eliminate the statistical power of the present findings.[303]

Rationale for Hydrocortisone Treatment

Because in most patients and after some time, cardiovascular stability will be achieved by the use of vasopressors-inotropes and/or inotropes alone, it is important to understand the rationale for hydrocortisone administration, especially because the drug has its own side effects.

First, it is widely accepted although not proven that the sooner one normalizes blood pressure and systemic perfusion, especially in the VLBW neonate during the immediate postnatal period, the better the outcome. However, because normal blood pressure in the first 24 hours may not guarantee normal cerebral perfusion (see earlier discussion), and because an association but not causation has been documented between hypotension and adverse outcomes, stabilization of the cardiovascular status with little blood pressure fluctuation remains an intuitively desirable goal but one without much direct evidence to support it.

Second, and more importantly, hydrocortisone specifically addresses the underlying etiology of the cardiovascular instability in most critically ill neonates and thus is the logical choice of treatment. Indeed, findings of developmental endocrinology and cardiovascular physiology as well as recent clinical data support a role for hydrocortisone use in critically ill hypotensive neonates, especially in VLBW neonates.[236] In critical illness, desensitization of the cardiovascular system to catecholamines takes place through the downregulation of cardiovascular adrenergic receptors and second messenger systems.[234] This process is attenuated or prevented by the regulatory actions of glucocorticoids on the expression of cardiovascular adrenergic receptors and second messenger systems. In addition, the direct increase in myocardial and vascular smooth muscle cell contractility induced by mineralocorticoids also plays an effective role.[304,305] Furthermore, corticosteroids contribute to the maintenance of capillary integrity, inhibit catecholamine metabolism and reuptake of norepinephrine into sympathetic nerve endings, increase the expression of angiotensin type 2 receptors in the myocardium, and inhibit prostacyclin production and the induction of inducible nitric oxide synthase. Each of these actions of corticosteroids aids in maintaining the sensitivity of the

cardiovascular system to catecholamines in response to acute stress or critical illness.

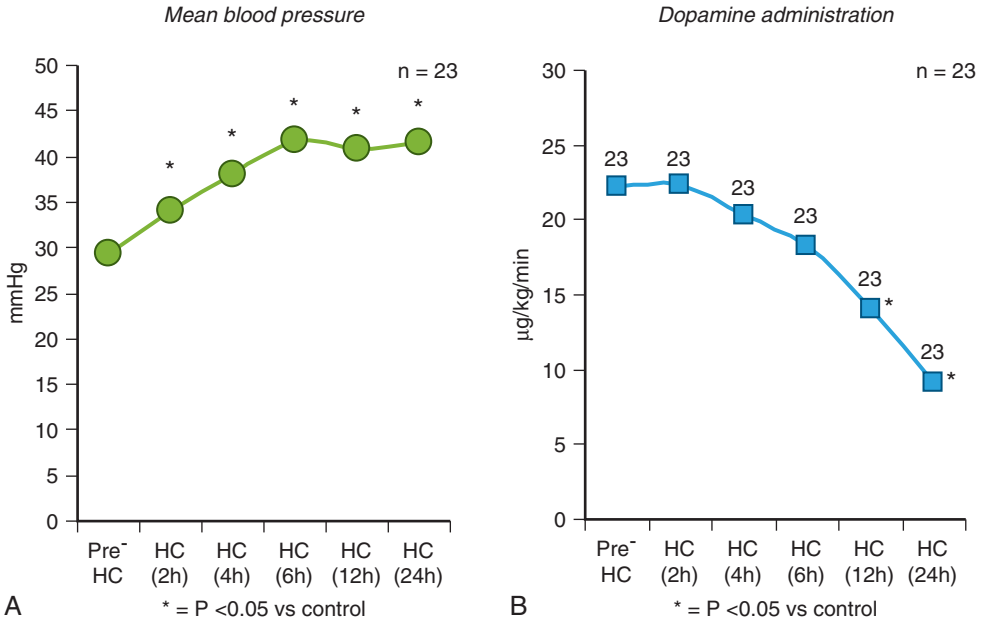
Third, because of the role of glucocorticoids in the physiologic regulation of adrenergic receptor expression, the emergence of vasopressor resistance in itself may indicate a state of relative adrenal insufficiency. Indeed, the findings of several studies indicate that critically ill preterm and term infants are likely to develop relative adrenal insufficiency because of their immature adrenal function[235,306,307] and hypothalamopituitary axis,[308,309] respectively. It is important to emphasize that, based on these findings, it appears that in the VLBW neonate, adrenal unresponsiveness to endogenous or exogenous ACTH[235,306] and in the late preterm and term neonate, unresponsiveness of the hypothalamopituitary axis to stress[308,309] are the primary causes for the development of relative adrenal insufficiency. Thus, these patients have limited capacity to mount sufficient adaptive increases in endogenous steroid production to prevent the development of cardiovascular adrenergic receptor downregulation and desensitization of the cardiovascular system to catecholamines during their illness. Therefore, in critically ill preterm and term infants with vasopressor-resistant hypotension, steroid administration also serves as hormone substitution therapy.

Clinical Applications of Hydrocortisone

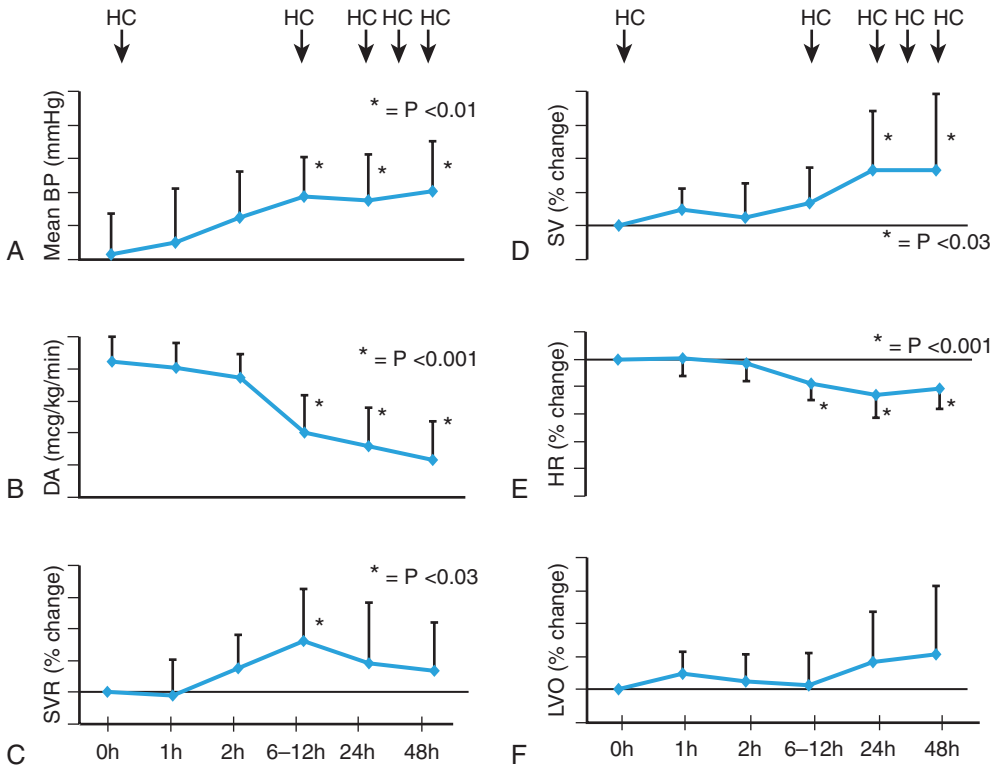
As mentioned earlier, the use of hydrocortisone as primary treatment in hypotensive preterm infants is not recommended. On the other hand, although long-term effects need to be further studied (see below), there is justification for the use of hydrocortisone as a secondary rescue treatment. Available evidence indicates that hydrocortisone in preterm and term infants with vasopressor-resistant hypotension increases blood pressure within 2 hours of the initiation of treatment (non-genomic effects) and decreases vasopressor requirement within 8 to 12 hours of the first dose of the drug (genomic effects) (Fig. 46.13).[133,237,238] A study in preterm infants without a PDA also documented that the hydrocortisone-induced improvement in blood pressure is associated with improvements in all aspects of cardiovascular function including stroke volume and tissue perfusion (Fig. 46.14).[133] It is important to note that due to the decreased clearance of hydrocortisone, lower and less frequent dosing is sufficient to induce the desired hemodynamic effects in preterm and term infants.[133,236,310,311]

Short-term Side Effects

As for the side effects of early or late low-dose hydrocortisone administration, the potential occurrence of short- and long-term sequelae is of great interest. As for the short-term side effects, it has been repeatedly documented that coexposure of preterm neonates to indomethacin and hydrocortisone during the first postnatal week significantly increases the risk of spontaneous gastrointestinal (mostly ileal) perforations.[312,313] This serious untoward effect curtails the use of hydrocortisone in the VLBW neonate during the first postnatal week—the very patient population in whom vasopressor-resistant hypotension and relative adrenal insufficiency are most prevalent during the immediate postnatal period. It is interesting to note that preliminary findings of one of these studies might suggest that VLBW neonates



• **Fig. 46.13** Effect of Hydrocortisone on Mean Blood Pressure and the Dose of Dopamine During the First 24 Hours of Hydrocortisone Treatment in 23 Preterm Neonates With Vasopressor-Resistant Shock. (A) Mean blood pressure (mean ± SD) and (B) dopamine requirement (mean ± SD) during the 12 hours before and the first 24 hours after the first dose of hydrocortisone. Before hydrocortisone administration, blood pressure remained low (A), despite significantly increased dopamine doses (B, ∗ = $P < .05$ vs. baseline [0 h]). However, mean blood pressure increased significantly by 2 hours after the first dose of hydrocortisone (A, ∗ = $P < .05$ vs. baseline [0 h]) and continued to rise until 6 hours of hydrocortisone therapy, remaining stable thereafter (A, ∗ = $P < .05$ vs. baseline [0 h]; ∗ = $P < .05$ vs. HC [2 h]). In addition, the dose of dopamine significantly decreased by 12 and 24 hours of hydrocortisone therapy (B, ∗ = $P < .05$ vs. baseline [0 h]). (From Seri I, Tan R, Evans J. Cardiovascular effects of hydrocortisone in preterm neonates with pressor resistant hypotension. *Pediatrics*. 2001;107:1070–1074.)



• **Fig. 46.14** Effect of Hydrocortisone on Systemic Hemodynamics and Dopamine Requirement During the First 48 hours of Hydrocortisone Treatment in 15 Preterm Neonates With Vasopressor-Resistant Shock. Changes in mean BP (A) and dopamine dosage *(DA)* (B) and percentage changes relative to baseline (0 hour) in SVR (C), stroke volume *(SV)* (D), heart rate *(HR)* (E), and left ventricular output *(LVO)* (F) are shown at 1, 2, 6 to 12, 24, and 48 hours after the first dose of hydrocortisone. *HC* with arrows indicate the approximate timing of hydrocortisone doses. Significant P values for pairwise comparisons versus the baseline (0 hour) with adjustment for multiple comparisons (Bonferroni) are shown. See text for details. *BP*, Blood pressure; *SVR*, systemic vascular resistance. (From Noori S, Friedlich P, Wong P, et al. Hemodynamic changes following low-dose hydrocortisone administration in vasopressor-treated neonates. *Pediatrics*. 2006:118:1456–1466.)

with low baseline serum cortisol levels may be at lower risk for developing ileal perforations when treated with low-dose hydrocortisone and a cyclooxygenase inhibitor during the first postnatal days.[312] However, this observation needs to be confirmed before it can be used to predict the likelihood of spontaneous intestinal perforation based on the basal serum cortisol level alone. As for the other short-term side effects of hydrocortisone, one earlier study found an increase in the incidence of systemic fungal infections.[314] However, none of the other studies have documented a significant increase in bacterial or fungal infections in neonates. Finally, hydrocortisone administration–associated transient hyperglycemia and hypertension have been reported in a few cases in the literature.

Long-term Side Effects

As for the potential long-term side effects, the most pressing question is whether low to moderate doses of hydrocortisone interfere with neurodevelopment, especially in preterm neonates.

Two placebo-controlled trials showed that early, low-dose hydrocortisone treatment for the prevention of BPD used in the first 10 postnatal days was not associated with an increased incidence of cerebral palsy[313,315] and neurodevelopmental impairment.[315] Findings of one of the trials even suggested improvement in some measures of neurodevelopmental outcome at 18 to 22 months, corrected age when compared to controls.[316] On the other hand, the 5- to 7-year follow-up of a small RCT of early hydrocortisone treatment for prevention of BPD showed a lower performance intelligence quotient and a higher need for physiotherapy in the hydrocortisone group.[317]

As for the use of higher cumulative doses of hydrocortisone for the treatment of evolving bronchopulmonary dysplasia outside the immediate transitional period, the results of a study examining structural and functional brain development at an age of 8 years suggest that hydrocortisone, used for the treatment of bronchopulmonary dysplasia in ventilator-dependent preterm neonates at a median age of 18 days and at cumulative doses over 50 mg, does not interfere with brain development.[318] In addition, case-control studies have shown no difference in cerebral and cerebellar tissue volumes on MRI at term equivalent in preterm infants treated with hydrocortisone for BPD compared to the controls.[319,320] A small RCT on hydrocortisone with a cumulative dose of 17 mg/kg starting moderately early (at 10 to 21 days) also showed no difference in regional brain volume at term equivalent compared to the controls.[321]

Although overall, hydrocortisone appears to be safer than dexamethasone, the lack of power of the RCTs and the retrospective nature of the observational or retrospective studies warrant caution before the use of early or late and low- to medium-dose hydrocortisone could be declared safe in preterm neonates, at least from a neurodevelopmental standpoint.

Extracorporeal Membrane Oxygenation (ECMO) for Circulatory Support

ECMO or extracorporeal life support (ECLS) has been used for decades to provide cardiorespiratory support for neonatal cardiorespiratory failure refractory to conventional management.[322] There are two types of ECMO: veno-venous (VV) and veno-arterial (VA). In VV ECMO, blood is drained from the venous system via one of the ports of the cannula placed in the right atrium via the internal jugular vein. The blood first flows through the circuit and the oxygenator, and the oxygenated blood is then returned to the right atrium via a different port in the venous cannula. Therefore, VV ECMO does not provide direct cardiac support, although improved blood oxygen content in the heart and coronary arteries may improve cardiac function. Furthermore, as the oxygenated blood first circulates through the pulmonary vasculature, VV ECMO can improve cardiac function by reducing pulmonary vascular resistance in conditions such as persistent pulmonary hypertension (PPHN) with circulatory failure, wherein elevated pulmonary vascular resistance contributes to the circulatory collapse. In VA ECMO, blood is also drained from the venous system via cannula with a single port placed in the right atrium through the internal jugular vein. Blood is then returned to the arterial system via an arterial cannula typically placed in the right common carotid artery in the neonate and advanced to the transverse aortic arch. By bypassing the heart, VA ECMO also provides direct circulatory support and is the preferred modality when significant circulatory support is needed. Such conditions include refractory septic shock,[323] acute myocarditis associated with severe myocardial dysfunction, low cardiac output following repair of congenital heart defects, or severe hypertrophic cardiomyopathy seen in infants born to diabetic mothers. The recent surviving sepsis campaign international guidelines suggest using VV ECMO in children with sepsis-induced pediatric acute respiratory distress syndrome and using VA ECMO as a rescue therapy in children with refractory septic shock.[215]

In the past two decades, the number of neonates placed on ECMO for cardiac indications has increased. The survival of neonates receiving ECMO support for cardiac indications varies significantly and depends on the primary diagnosis. However, the overall survival is approximately 50%, which is lower than the survival of infants treated with ECMO for respiratory indications.[324] Of note is that neonates tend to have a better outcome than older children.[325,326] For example, survival among 191 neonates with group B streptococcal sepsis treated with ECMO was 71%.[327] However, little is known about the impact of various ECMO treatment strategies on outcomes. A recent study of children (older than 30 days) with refractory septic shock demonstrated a higher survival using a high (>150 mL/kg/min) ECMO blood flow strategy during the early phase of ECMO support.[328]

In summary, neonatal ECMO provided for circulatory failure is a relatively effective last resort for the sickest and most complex neonatal patients cared for in experienced neonatal centers.

General Supportive Measures

Maintenance of a normal intravascular volume, arterial pH, and serum ionized calcium concentration is necessary for the optimum cardiovascular response to catecholamines. A previous study found that metabolic acidosis of less than 7.25 may compromise myocardial function in the preterm infant,[329] and so it has been recommended that the arterial pH be maintained at or above this range in cases of acidosis with a significant metabolic component. On the other hand, respiratory acidosis has been shown to have no to minimal effect on cardiac function, contractility, and systemic vascular resistance in hemodynamically stable preterm infants during the transitional period.[115] However, and as discussed earlier, respiratory acidosis has significant effects on cerebral hemodynamics, and its cardiovascular effects in neonates receiving cardiovascular support are not known (see section on respiratory support and hemodynamics). Therefore, more data are needed to define the optimal pH and acid-base status in preterm and term infants receiving respiratory support. Administration of sodium bicarbonate[329] or, in cases with severe combined respiratory and metabolic acidosis, the administration of tromethamine, rapidly improves arterial pH. However, the efficacy and potential short- and long-term adverse effects of such supportive treatment measures have not been studied in the neonatal patient population, and there is indeed very little evidence that bicarbonate administration for metabolic acidosis caused by tissue hypoperfusion is beneficial.[330] Finally, although positive pressure ventilation may raise pleural pressure and has the potential to reduce venous return, it may also reduce left ventricular afterload by reducing transmural pressure and decreasing or eliminating the work of breathing. Decreasing or eliminating the work of breathing will also decrease or eliminate the concomitant cardiac output diverted to respiratory muscles. Therefore, the net hemodynamic effect of positive pressure ventilation is improved systemic oxygen delivery independent of any potential improvement in pulmonary gas exchange. On the other hand, excessive positive pressure and lung hyper-expansion are associated with decreases in pulmonary and systemic blood flow.

In summary, sustained stabilization of the cardiovascular status with the provision of appropriate blood pressure, cardiac output, and tissue perfusion and oxygenation remains a difficult task in most critically ill hypotensive neonates. Treatment of these patients requires the ability to continuously monitor the most important measures of cardiovascular function (blood pressure, systemic blood flow, and tissue oxygenation); a thorough understanding of the pathogenesis and pathophysiology of neonatal shock; and the mechanisms of action, pharmacodynamics, and potential side effects of sympathomimetic amines and other medications used in the management of neonatal shock.

Suggested Readings

de Boode WP. Advanced hemodynamic monitoring in the neonatal intensive care unit. *Clinics in Perinatology*. 2020;47(3):423–434.

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47

Persistent Pulmonary Hypertension

ERICA MANDELL, ROBIN H. STEINHORN, AND STEVEN H. ABMAN

KEY POINTS

- Persistent pulmonary hypertension of the newborn (PPHN) can occur with parenchymal lung disease, with pulmonary hypoplasia, or without associated lung disease (*idiopathic*) and has an incidence of 0.2% in term infants and up to 2% in preterm infants.
- Maternal factors such as diabetes, high body mass index, smoking, use of selective serotonin receptor inhibitors or nonsteroidal anti-inflammatory drugs, and cesarean delivery increase the risk of PPHN. Postnatal factors include perinatal asphyxia, hyperoxia, hypoxia, infection, and lung inflammation.
- Medical management of PPHN requires careful optimization of right and left ventricular function. Lung recruitment strategies should be optimized in patients with parenchymal lung disease.
- Inhaled nitric oxide improves oxygenation and reduces the need for extracorporeal membrane oxygenation support in term and near-term infants with PPHN.
- Extracorporeal membrane oxygenation support is indicated for term and near-term neonates with severe pulmonary hypertension and/or hypoxemia that is refractory to inhaled nitric oxide therapy and optimization of respiratory and cardiac function.
- Chronic pulmonary hypertension occurs most often in a subset of infants with congenital diaphragmatic hernia and/or bronchopulmonary dysplasia and substantially increases morbidity and mortality.
- Surviving infants of moderate to severe PPHN are at high risk of neurodevelopmental impairment and should undergo neuroimaging and neurodevelopmental follow-up.

Introduction

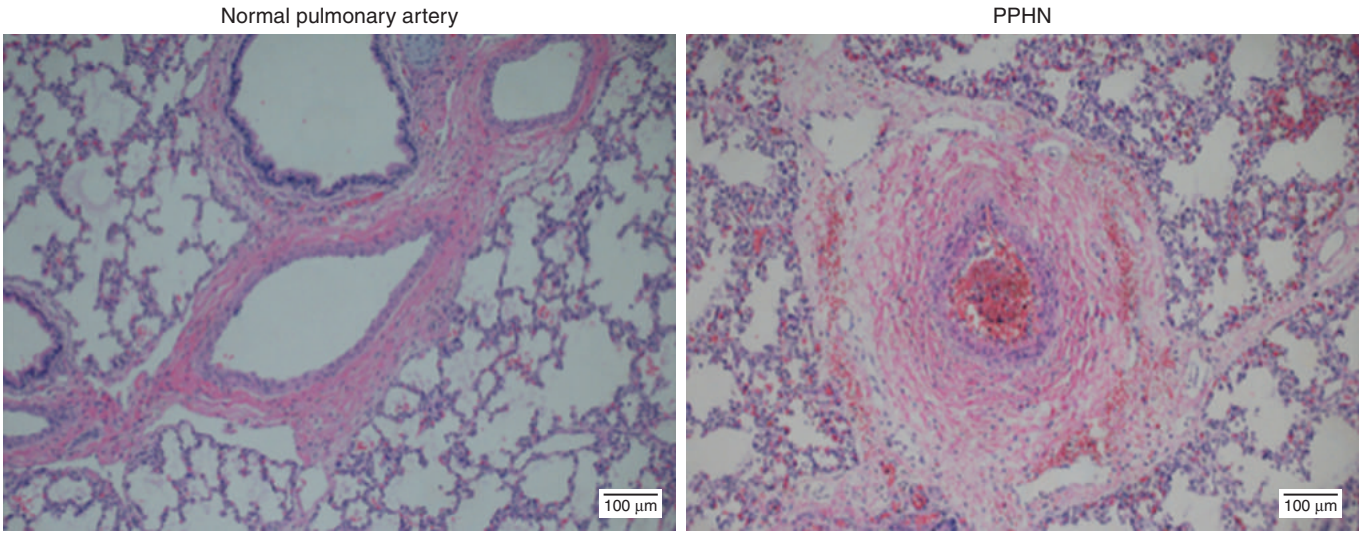
Persistent pulmonary hypertension of the newborn (PPHN) can be seen with many cardiopulmonary disorders with an incidence ranging from 0.4 to 6.8 per 1000 live births and 5.4 per 1000 live births in late preterm infants.[1,2] The etiology of PPHN in preterm infants is usually secondary to hypoxemic respiratory failure related to significant lung pathology soon after birth such as respiratory distress syndrome (RDS), preterm premature rupture of membranes (PPROM), or oligohydramnios causing pulmonary hypoplasia.[3,4] In the term infant, PPHN is most commonly seen with conditions such as congenital diaphragmatic hernia (CDH), meconium aspiration syndrome (MAS), and transient tachypnea of the newborn (TTN). Mortality of *all* newborns with PPHN has been reported at 7.6% and 10.7% infants with severe PPHN.[2] Surviving infants with PPHN have increased risk of long-term morbidities, including ~25% neurodevelopmental impairment at 2 years.[5] PPHN is defined as the failure to achieve or sustain the normal decrease in pulmonary vascular resistance (PVR) at birth. PPHN can produce severe respiratory distress and hypoxemia secondary to severe pulmonary vascular disease remodeling (Fig. 47.1) in term and near-term infants. Chronic pulmonary hypertension is associated with lung diseases such as bronchopulmonary dysplasia (BPD) and CDH and is a common complication of congenital heart disease. This chapter will review the pathophysiology of PPHN, diagnosis and clinical treatment of newborns with severe PPHN disease, and outcome data.

Normal Fetal Pulmonary Vascular Development and Transition at Birth

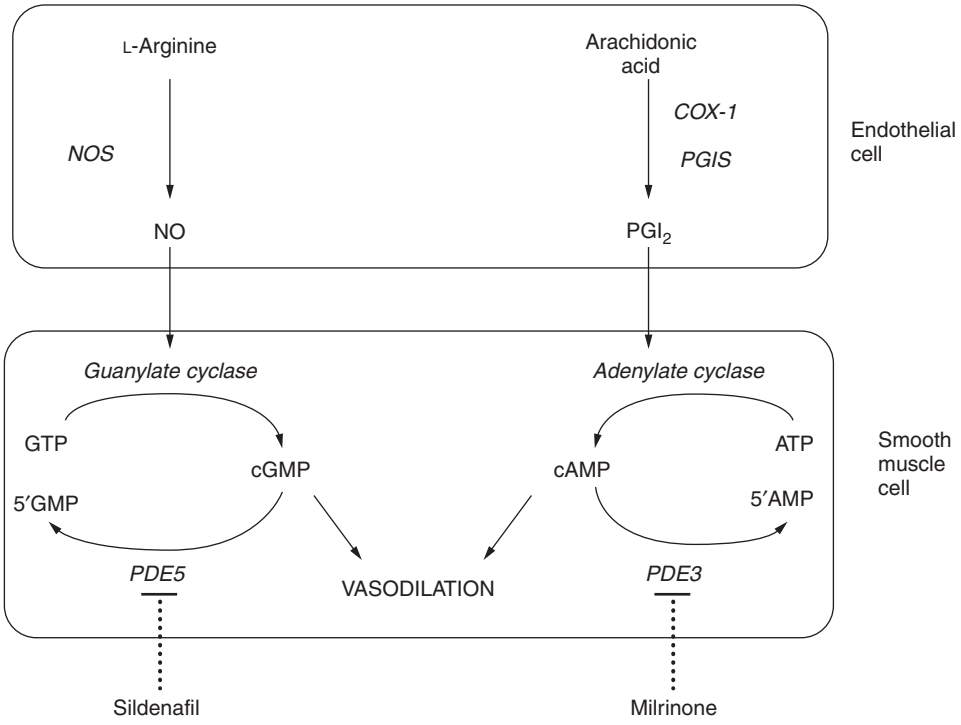
During fetal life, placental vascular resistance is low and PVR is high. This state of physiologic pulmonary hypertension is needed to maintain the patterns of blood flow that support gas exchange by the placenta. In the human fetus, only 10% to 20% of the combined ventricular output is directed to the pulmonary vascular bed with most of the right ventricular output crossing the ductus arteriosus to the descending aorta. Throughout gestation both pulmonary artery pressure (PAP) and pulmonary blood flow progressively increase with the developing growth of the lung vasculature.[6] Despite the marked increase in cross-sectional area of the pulmonary vascular bed, high PVR is maintained and even increases during gestation when corrected for gestational age.[6] This persistent elevation in PVR throughout gestation is mediated by changes in fetal pulmonary vessel vasoregulation. Initially, the fetal pulmonary circulation is poorly responsive to diverse stimuli, but reactivity to vasoconstrictor and vasodilator agonists increases during late gestation.[7] This normal maturational change in vasoregulation leads to increased vascular tone and sustained elevations in PVR in late gestation.[9,10]

The elevated PVR throughout fetal life is maintained by many factors, such as mechanical compression of pulmonary blood vessels from fluid-filled alveoli, hypoxic pulmonary vasoconstriction, increased production of circulating *vasoconstrictors* (endothelin-1, thromboxane, and leukotrienes), low levels of *vasodilator* products (nitric oxide [NO] and prostacyclin [PgI_2]), and abnormal smooth muscle cell reactivity leading to enhanced myogenic tone.[10,11] The vasodilatory response to oxygen and acetylcholine emerges in late gestation.[12–14]

At birth, a rapid and dramatic decrease in PVR redirects half of the combined ventricular output to the lung and increases pulmonary blood flow by 8- to 10-fold. Increased pulmonary blood flow increases pulmonary venous return and left atrial pressure, promoting functional closure of the one-way valve of the



• **Fig. 47.1** Histologic appearance of a pulmonary vessel from an infant with fatal persistent pulmonary hypertension of the newborn *(PPHN)* illustrating the dramatic remodeling that can be associated with severe PPHN.



• **Fig. 47.2** Nitric oxide *(NO)* and prostacyclin *(PGI_2)* signaling pathways that regulate pulmonary vascular tone in the developing lung. *ATP*, Adenosine triphosphate; *cAMP*, cyclic AMP; *cGMP*, cyclic GMP; *COX-1*, cyclooxygenase 1; *GTP*, guanosine triphosphate; *NOS*, nitric oxide synthase; *PDE3*, phosphodiesterase 3; *PDE5*, phosphodiesterase 5; *PGIS*, prostacyclin synthase.

foramen ovale. Clamping of the umbilical cord removes the low-resistance placental circulation, thus increasing systemic vascular resistance (SVR). The largest drop in PVR and pulmonary arterial pressure (PAP) occurs shortly after birth, although both will continue to fall during the first few months of life until levels are similar to that of typical adult circulation pressure levels. As PVR falls below systemic levels, blood flow through the patent ductus arteriosus reverses. During the first several hours of life the ductus arteriosus functionally closes, largely in response to the increased oxygen tension of the newborn. This effectively separates the pulmonary and systemic circulations and establishes the normal postnatal circulatory pattern.

These normal transitional changes in the pulmonary vasculature are initiated by ventilation of the lung and an increase in oxygen tension at birth and are mediated by alterations in several vasoactive compounds prior to birth and immediately following delivery. The fetus prepares for the extra-uterine transition late in gestation by increasing pulmonary vascular expression of NO synthases and soluble guanylate cyclase (sGC) (Fig. 47.2). At the time of birth, pulmonary endothelial NO production increases

markedly, partly as a response to increased oxygen tension and shear stress. NO exerts its actions through sGC[15] to increase the levels of cyclic guanosine monophosphate (cGMP), a central mediator responsible for vascular relaxation. Phosphodiesterase 5 (PDE5) catalyzes the breakdown of cGMP and, similarly to sGC, exhibits peak expression and activity in the immediate newborn period.[16,17] The arachidonic acid–prostacyclin pathway also plays a significant role in the pulmonary vascular transition at birth (see Fig. 47.2). The enzyme cyclooxygenase acts on arachidonic acid to produce prostaglandin endoperoxides. Prostaglandins activate adenylate cyclase to increase cyclic adenosine monophosphate (cAMP) concentrations in vascular smooth muscle cells, which, similarly to increases in cGMP concentrations, leads to vasorelaxation. Phosphodiesterase 3A catalyzes the breakdown of cAMP.[12]

Pathophysiology

Infants who develop PPHN after birth display failure of the normal cardiopulmonary transition. The first reports of PPHN described term newborns with profound hypoxemia who lacked radiographic evidence of parenchymal lung disease and echocardiographic evidence of structural cardiac disease.[18,19] In these patients, refractory hypoxemia was caused by sustained elevations of PVR and low pulmonary blood flow leading to right-to-left extrapulmonary shunting of deoxygenated blood across the patent ductus arteriosus or patent foramen ovale. Because of the persistently elevated PVR and blood flow through these "fetal shunts," the term *persistent fetal circulation* was originally used to describe these findings.

PPHN physiology can complicate the clinical course of term or preterm neonates with many different causes of hypoxemic cardiopulmonary failure, such as meconium aspiration, sepsis, pneumonia, asphyxia, CDH, and respiratory distress syndrome. As a result, the term *persistent pulmonary hypertension of the newborn* now denotes a syndrome characterized by sustained elevation of PVR and hypoxemia due to right-to-left extrapulmonary shunting of blood flow across the ductus arteriosus or foramen ovale. Hypoxemic respiratory failure in term infants is often presumed to be secondary to PPHN physiology, however, many hypoxemic newborns lack echocardiographic findings of extrapulmonary shunting across the PDA or PFO. Thus, PPHN describes hypoxemic newborns with evidence of extrapulmonary shunting.

The clinical presentation of infants with PPHN includes labile hypoxemia and often includes the findings of a gradient in oxygen saturations between pre-ductal (right upper extremity) and post-ductal values greater than 10%.[20] The presence of a pre- and post-ductal oxygen saturation gradient over 10% suggests the presence of extrapulmonary right-to-left shunting at the ductus arteriosus. Infants with PPHN can have wide swings in arterial oxygen saturation levels, which is due to rapid changes in pulmonary blood flow and right-to-left shunting associated with acute changes in PVR in response to minimal stimulation. Physical exam findings are often subtle but may include a loud second heart sound and a systolic murmur of tricuspid regurgitation. A chest radiograph is often helpful to differentiate primary parenchymal lung disease (meconium aspiration syndrome [MAS] or respiratory distress syndrome [RDS]) from other non-pulmonary etiologies of PPHN. Typical radiographic findings in *idiopathic* PPHN include pulmonary vascular oligemia, normal or slight hyperinflation, and a lack parenchymal infiltrates. In primary PPHN, the degree of hypoxemia is disproportionate to the severity of radiographic findings of lung disease.

PPHN is associated with many diverse cardiopulmonary disorders (Table 47.1) with an incidence ranging from 0.4 to 6.8 per 1000 live births and 5.4 per 1000 live births in late preterm infants.[1,2] One year mortality of *all* newborns with PPHN has

TABLE 47.1 Etiology of Persistent Pulmonary Hypertension of the Newborn by System

Pulmonary	Genetic/Rare Lethal Lung Developmental Disorders
• Meconium aspiration syndrome • Respiratory distress syndrome • Pulmonary hypoplasia (oligohydramnios) • Congenital diaphragmatic hernia • Pneumonia/sepsis • Idiopathic • Pulmonary interstitial glycogenosis • Congenital pulmonary lymphangiectasia	• Congenital surfactant deficiencies (SP-B/C, ABCA3) • TTF-1/Nkx 2.1 • FOXF1 mutation (ACD) • Mutation of CRH receptor-1 • TBX-4 mutation • Inborn error of metabolism • Trisomy 21
Cardiovascular	**Other**

• Myocardial dysfunction
• Structural cardiac disease
  • Mitral stenosis
  • Pompe disease
  • Aortic atresia
  • Coarctation of the aorta
  • Interrupted aortic arch
  • Transposition of great vessels
  • Ebstein anomaly
• Hepatic arteriovenous malformations (AVM)
• Cerebral AVMs
• Total anomalous pulmonary venous return
	• Neuromuscular disease
	• Polycythemia
	• Thrombocytopenia
	• Maternal drug use or smoking

PPHN, Persistent pulmonary hypertension of the newborn.

been reported at 7.6%, rising to 10.7% for infants with severe PPHN.[2] Surviving infants with PPHN are at increased risk of long-term morbidities, including ~25% neurodevelopmental impairment at 2 years.[5]

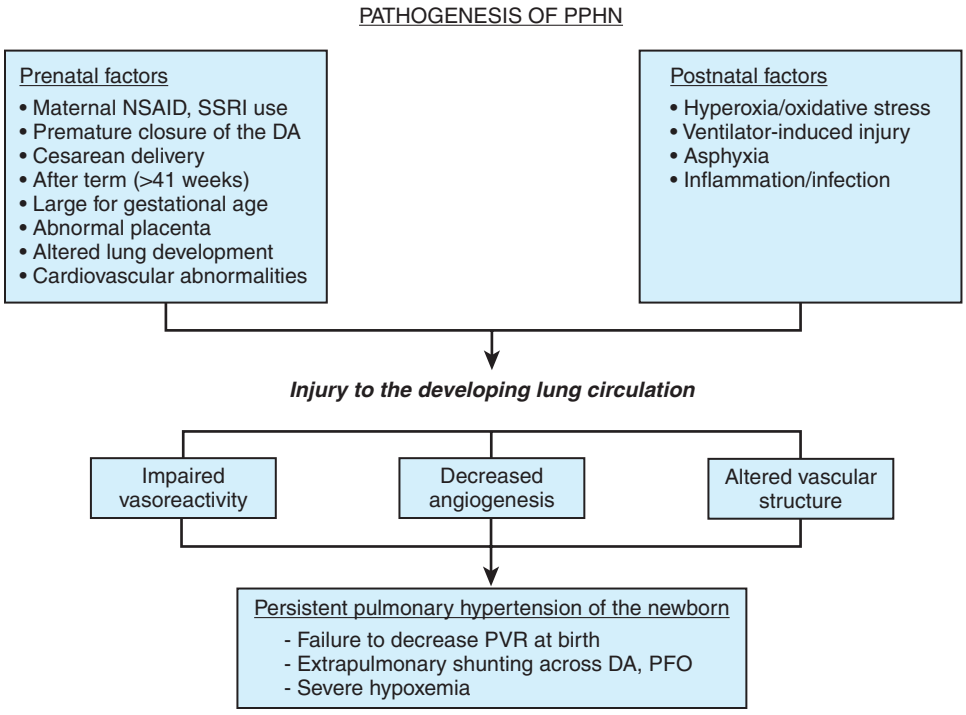
In 2019 the Pediatric Task Force of the World Symposium on Pulmonary Hypertension (WSPH) updated the WSPH Classification of pulmonary hypertension to differentiate PPHN as an important cause of pediatric PH.[21] Because of its specific anatomic and physiologic characteristics, PPHN was assigned a separate subcategory within WSPH Group 1, or pulmonary arterial hypertension, as Group 1.7. In Group 3 pulmonary hypertension (pulmonary hypertension due to lung disease or hypoxia), developmental lung diseases are specifically listed because of the important role that abnormal lung vascular growth plays in the pathogenesis of impaired lung development and pulmonary hypertension. CDH, BPD, and several other developmental lung disorders, such as surfactant protein deficiencies and alveolar capillary dysplasia (ACD), are now understood to be important causes of pulmonary hypertension in infants.

The etiology of PPHN is broad, but can generally be classified into one of three categories: *(1)* maladaptation: pulmonary vessels have normal structure and number but have abnormal vasoreactivity (respiratory distress syndrome [RDS], meconium aspiration syndrome [MAS], sepsis, or pneumonia); *(2)* excessive muscularization: increased smooth muscle cell thickness and abnormal distal extension of muscle into vessels that are usually not muscularized (chronic fetal hypoxia, idiopathic PPHN); and *(3)* underdevelopment: lung tissue hypoplasia associated with decreased pulmonary artery number (congenital diaphragmatic hernia [CDH], oligohydramnios) (see Table 47.1). There are many disorders associated with PPHN in the newborn period (see Table 47.1). Idiopathic PPHN is the least common, occurring in about 10% of cases. More commonly, PPHN is usually associated with an acute respiratory condition, such as meconium aspiration syndrome, respiratory distress syndrome, or pneumonia, and is referred to as *secondary PPHN*. This designation is imprecise, however, as many patients often have elevated PVR that involves overlapping categories. For example, infants with CDH-related PPHN may be due not only to underdevelopment of the pulmonary vasculature, but also to abnormal maladaptation and vasoreactivity, events that often culminate in chronic pulmonary vascular disease. Similarly, in infants with PPHN secondary to MAS the pulmonary vasculature can exhibit excessive muscularization of vessels in addition to abnormal vasoreactivity. In many cases it can be more difficult to separate chronic intrauterine remodeling from acute pulmonary vasoconstriction due to parenchymal lung disease.

Risk Factors

Based on the significant remodeling found in lethal cases of PPHN, intrauterine events have been presumed to affect pulmonary vascular growth, reactivity, and structure (see Fig. 47.1).[22] Pulmonary vascular development *in utero* can be disrupted by environmental, placental, toxic, or other influences (Fig. 47.3). Case-control surveillance studies indicate that maternal risk factors of black or Asian maternal race, elevated body mass index (>27 kg/m^2), diabetes, and asthma predict a higher risk of PPHN. Neonatal risk factors include male sex, large for gestational age infants, birth by cesarean delivery, and delivery before 37 weeks' gestation or after 41 weeks' gestation.[23]

Recent animal and epidemiologic studies also suggest that maternal exposures can alter fetal pulmonary vascular development and function. There are strong associations between PPHN and maternal smoking, and two classes of maternal medications, nonsteroidal anti-inflammatory drugs and selective serotonin receptor inhibitors, have also been implicated.[24] Exposure to nonsteroidal anti-inflammatory drugs such as aspirin or ibuprofen during the third trimester can cause constriction of the fetal



• **Fig. 47.3** Pathogenesis of persistent pulmonary hypertension of the newborn *(PPHN)*. *DA*, Ductus arteriosus; *NSAID*, nonsteroidal antiinflammatory drug; *PFO*, patent foramen ovale; *PVR*, pulmonary vascular resistance; *SSRI*, selective serotonin receptor inhibitor.

ductus arteriosus, which in turn can trigger pulmonary vascular remodeling and PPHN. Based on the findings of a recent epidemiologic study, the relationship is complex and may be dependent on the specific agent and the timing of exposure,[25] however aspirin use during late pregnancy remains a consistent risk factor for PPHN. Use of selective serotonin receptor inhibitors during the last half of pregnancy has been associated with an increased incidence of PPHN in several population studies, although the severity of PPHN has not been well described, and other studies have not found this association.[26–28] Maternal depression is a risk factor for adverse pregnancy outcomes, as such maternal physical and psychologic well-being remain the primary factors guiding antidepressant therapy during pregnancy and the postpartum period.

Several postnatal events can disrupt the perinatal transition and contribute to the pathogenesis of PPHN (see Fig. 47.3). Birth by elective cesarean delivery delays the decrease in pulmonary arterial pressure and increases the risk of PPHN,[29] and delivery before 39 weeks' gestation likely amplifies this effect. When compared with matched controls, infants with PPHN are more likely to have been born by cesarean delivery, born to diabetic mothers, mothers of advanced maternal age, mothers of Black race, or mothers with asthma or a high body mass index—in addition to being born before 37 weeks' gestation.[2,23,30] Perinatal asphyxia with resultant fetal hypoxemia, ischemia, acidosis, and cardiac ventricular dysfunction prevents the necessary perinatal adaptation, delays the normal decrease in PVR, and increases the risk of PPHN.[31] Acute perinatal asphyxia is associated with reversible pulmonary vasoconstriction, while chronic *in utero* asphyxia can induce vascular remodeling that is less responsive to acute vasodilation.[32]

The adoption of therapeutic hypothermia for management of neonatal encephalopathy in the term and near-term infant has led to concern that this therapy would increase risk of PPHN in asphyxiated infants. Deep levels of hypothermia (30°C to 32°C) have been shown to increase mean pulmonary arterial pressure in neonatal lambs[33] and have been associated with increased use of extracorporeal membrane oxygenation (ECMO) and inhaled nitric oxide (iNO) in human studies.[34] Furthermore, hypothermia shifts the oxygen dissociation curve to the left and decreases PaO_2 at a given peripheral capillary oxygen saturation (SpO_2). However, pooled analysis of randomized trials of standard therapeutic hypothermia (33.5°C) has not shown an increased incidence of PPHN (25% vs 20%) in treated infants.[35,36]

Patients with severe hypoxemia who are not responding as expected to conventional therapies should be evaluated for developmental lung diseases associated with refractory PPHN. Children with Down syndrome (trisomy 21) commonly develop pulmonary hypertension in association with structural heart defects but also have a 10-fold increased risk of idiopathic PPHN. In a Dutch cohort, PPHN was documented in 5.2% of Down syndrome infants,[37] and other studies have shown that Down syndrome infants are overrepresented in neonates requiring ECMO support.[38] Polymorphisms of genes for bone morphogenetic protein receptor or other transforming growth factor β receptors, other critical growth factors, or vasoactive enzymes (e.g., NO synthase, phosphodiesterase) have not been shown to increase the risk of neonatal PPHN. Additional systematic evaluation for genetic etiologies of refractory or prolonged PPHN should include: inherited surfactant dysfunction disorders, such as SP-B/C or ATP-binding cassette A3 gene (ABCA3) deficiency[39,40]; genetic disruption of distal lung development, such as TTF-1 and Nkx 2.1; FOXF1 mutations leading to alveolar capillary dysplasia (ACD),[41] T-Box transcription factor 4 gene (TBX4)[42]; genetic variants in corticotropin releasing hormone (CRH) receptor 1 and CRH-binding protein[43]; and inborn errors of metabolism, such as methylmalonic acidemia. Genotype analysis of neonates with PPHN have also identified polymorphisms in carbamoylphosphate synthase, a key urea cycle enzyme that maintains substrate availability for endogenous nitric oxide production[44,45] and genetic variants for cortisol signaling (*CRHR1* and *CRHBP*), as well as increased levels of 17-hydroxyprogesterone.[43]

Abnormal Pulmonary Vasoregulation in PPHN

Disruptions of the NO–cGMP, prostacyclin–cAMP, and endothelin signaling pathways play an important role in the vascular abnormalities associated with PPHN. The NO–cGMP pathway has been a topic of particularly intense investigation in the last two decades. Decreased expression and activity of endothelial nitric oxide synthase (eNOS) has been documented in lamb models of chronic intrauterine pulmonary hypertension,[46,47] and decreased eNOS expression has been found in umbilical venous endothelial cell cultures from human infants with meconium staining who developed PPHN.[48] These important findings were rapidly followed by clinical testing of iNO, leading to its adoption as the primary vasodilator therapy for PPHN. However, numerous other signaling abnormalities limit the effect of endogenous or exogenous NO. In PPHN, expression and activity of soluble guanylate cyclase is decreased, and cGMP phosphodiesterase activity is increased, leading to lower cGMP levels and limitations of NO-induced vasodilation. Oxidant stress associated with vascular dysfunction and/or exposure to hyperoxia can oxidize and reduce soluble guanylate cyclase activity and increase cGMP phosphodiesterase activity, accentuating these vascular abnormalities.

Prostacyclin is important in the normal pulmonary vascular transition, although less is known about abnormal prostacyclin–cAMP signaling in PPHN. Data from animal models suggest reductions in prostacyclin synthesis and downstream adenylate cyclase responses, analogous to the abnormalities reported for NO–cGMP signaling.[49,50] In addition, production of thromboxane, a vasoconstrictor arachidonic acid metabolite, plays a role in pulmonary hypertension produced by chronic hypoxia.[51] Elevated levels of circulating endothelin 1 (ET-1), a potent vasoconstrictor, have been demonstrated in lambs and newborns with PPHN.[52,53] Endothelin effects are mediated through two receptors: ET_A receptors on smooth muscle cells that mediate vasoconstriction and ET_B receptors on endothelial cells that mediate vasodilation. In PPHN, the balance of endothelin receptors is shifted to the vasoconstrictor (ET_A) pathways.[54] Endothelin may also affect vascular tone by increasing production of vasoconstrictor reactive oxygen species such as superoxide and hydrogen peroxide[55] and by decreasing expression and activity of peroxisome proliferator–activated receptor γ, which maintains the vasodilatory balance in the fetal lung.

Disorders Associated With Pulmonary Hypertension at Birth

Congenital Diaphragmatic Hernia

Worldwide, CDH affects approximately 2.3 in every 10,000 live births.[56,57] CDH includes abnormal diaphragm development, herniation of abdominal viscera into the chest, and a variable degree

of lung hypoplasia. Herniation occurs most often in the posterolateral segments of the diaphragm, and 80% of the defects occur on the left side. Severe CDH develops early in lung development and involves an arrest in the normal patterns of airway branching in both lungs, resulting in reduced lung volume and impaired alveolarization. Abnormal distal vascular and air space growth is likely because of the loss of stretch-induced stimulation of lung development from impaired fetal breathing movements due to disruption of the diaphragm. The pulmonary vascular findings include vascular remodeling superimposed on hypoplasia or pruning of the pulmonary vascular bed, producing increased vascular tone and altered vasoreactivity after birth.[58]

After birth, PVR often remains at suprasystemic levels, resulting in pulmonary hypertension, extrapulmonary right-to-left shunting across the foramen ovale and ductus arteriosus, and profound hypoxemia. High PVR in the newborn with CDH is related to multiple factors, including the small cross-sectional area of pulmonary arteries, structural vascular remodeling, and vasoconstriction with altered reactivity. The mediators of altered pulmonary vascular reactivity in CDH remain under investigation, with substantial evidence pointing to disruptions in NO–cGMP and ET-1 signaling.[59] The level of vascular endothelial growth factor, a key stimulator of angiogenesis, is increased in the lungs of infants with CDH who died; this has been interpreted as a compensatory mechanism for reduced eNOS expression and NO production.[58] In some infants, chronic pulmonary hypertension will persist for months or years, including structural and functional abnormalities of pulmonary circulation in the ipsilateral and contralateral lung. Cardiac catheterization of infants with prolonged pulmonary hypertension has revealed numerous vascular abnormalities, including left pulmonary artery hypoplasia or stenosis, pulmonary vein stenosis, and delayed venous return.[60]

In addition to pulmonary vascular disease, it is important to appreciate that structural and functional abnormalities of the left ventricle create a relative left ventricular hypoplasia that impairs filling and creates pulmonary venous hypertension. Often, newborns with severe CDH have severe pulmonary hypertension with right-to-left shunting at the ductus arteriosus but have left-to-right shunting at the atrial septum because of high left atrial pressure from left ventricular dysfunction.[61] This circulatory pattern will diminish the clinical response to iNO during the first few days after birth and produce significant pulmonary and systemic hemodynamic instability. Some infants may have exceptionally severe left ventricular dysfunction that leads to dependence on the right ventricle for systemic perfusion; this subset of patients may benefit from a management approach that maintains patency of the ductus arteriosus, reduces left ventricular afterload, and delays the use of iNO until left ventricular performance improves.[62,63] In addition to its role in the acute setting, left ventricular dysfunction can contribute to the severity of pulmonary hypertension during the late and chronic courses in infants with CDH.

Alveolar Capillary Dysplasia

Unlike pediatric and adult pulmonary hypertension, few genetic factors have been identified for PPHN. A notable exception is alveolar capillary dysplasia with misalignment of lung vessels, a rare but universally lethal cause of pulmonary hypertension in the newborn.[64] Shortly after birth, affected infants typically exhibit cyanosis, minimal parenchymal disease, and respiratory distress refractory to all known therapies, including extracorporeal support, although later presentations (at several weeks or months of age) have been recognized. More than 50% of infants exhibit other anomalies, most commonly affecting the genitourinary, cardiovascular, and gastrointestinal systems. The diagnosis is established by direct examination of lung tissue by lung biopsy or autopsy. Characteristic findings include simplification of lung architecture, widened and poorly cellular septa with a paucity of capillaries, and strikingly muscularized small arterioles. "Misaligned pulmonary veins" have been described within the same adventitial sheath as pulmonary arteries; these were recently identified to be congested bronchial veins that represent intrapulmonary vascular anastomoses creating right-to-left intrapulmonary shunts and profound refractory hypoxemia.[65] Approximately 10% of reported alveolar capillary dysplasia cases have a familial association, and mutations or deletions in the transcription factor forkhead box F1 gene (*FOXF1*) or deletions upstream of *FOXF1* are identified in 40% of infants with alveolar capillary dysplasia.[41] A murine model of forkhead box F1 deficiency has been studied, which demonstrates the importance of the forkhead box F1 in embryonic development of the pulmonary vasculature.[66]

Pulmonary Hypertension in Premature Infants

Birth at 23 to 26 weeks' gestational age is associated with high rates of PPHN,[3] usually secondary to hypoxemic respiratory failure related to significant lung pathology soon after birth such as RDS, PPROM, or oligohydramnios causing pulmonary hypoplasia. Moreover, data from the Neonatal Research Network of Japan suggest that the incidence of PPHN in extremely preterm infants is increasing. In 2003 they reported 54 cases which increased to 147 cases in 2012, with the annual increase seen mostly in infants born at 22 to 24 weeks' gestation.[67] In a large prospective study, echocardiographic findings suggestive of pulmonary hypertension, such as ventricular septal wall flattening and right ventricular dilation, were found in approximately 40% of extremely low-birth-weight-babies at 7 days of age and predicted bronchopulmonary dysplasia (BPD) and late pulmonary hypertension.[68] The off-label use of iNO in premature infants has increased dramatically in the past two decades, with the greatest increased use among infants born extremely preterm (23 to 26 weeks' gestation).[69] Observational studies have shown a favorable response to iNO therapy in preterm infants born after extreme preterm premature rupture of membranes (PPROM) and resultant oligohydramnios.[3,70]

BPD has emerged as an important cause of chronic pulmonary hypertension, with an incidence of 16% to 25% in infants with BPD.[68] Severe or prolonged pulmonary hypertension increases the risk of late morbidity, ICU readmission, and death to nearly 50%.[71] The pathogenesis of pulmonary hypertension associated with BPD involves complex interactions between prenatal factors, such as growth restriction, preeclampsia, oligohydramnios, or fetal inflammation, and postnatal injury due to ventilator-induced lung injury, hyperoxia, hemodynamic stress, and infection. The result is impaired angiogenesis, abnormal vascular signaling, and vascular pruning, resulting in a reduction in the alveolar–capillary surface area. Identifying pulmonary hypertension in infants with BPD requires a high index of suspicion and systematic longitudinal evaluation. Echocardiography remains the most practical screening tool and should be considered in infants who continue to have cyanotic spells or require oxygen supplementation at 36 weeks' corrected gestational age.

In infants with evidence of significant pulmonary hypertension by echocardiogram, cardiac catheterization may provide a more accurate quantification of the severity of disease, as well as allow vasoreactivity testing.[72]

Clinical Evaluation and Diagnosis

PPHN typically presents as respiratory distress and cyanosis within hours of birth and may be associated with a variety of lung and/or cardiac disorders (see Table 47.1). Clinically, PPHN is most often recognized in the term or near-term neonate but should also be considered in premature neonates who have cyanosis out of proportion to their parenchymal lung disease.[73] While PPHN is often associated with signs of perinatal distress, such as asphyxia, low Apgar scores, or meconium staining, idiopathic PPHN can present without signs of acute perinatal distress.

PPHN is characterized by labile hypoxemia that is poorly responsive to supplemental oxygen. An important goal for the initial clinical evaluation is to rule out cyanotic cardiac disease and to determine whether a hypoxemic infant has PPHN-type physiology. Because a patent foramen ovale and ductus arteriosus are normally present early in life, elevated PVR in the newborn will produce extrapulmonary shunting of blood through these fetal channels, leading to severe and potentially unresponsive hypoxemia. In the presence of right-to-left shunting across the patent ductus arteriosus, "differential cyanosis" is often present, which is difficult to observe by physical examination but may be detected by a gradient in PaO_2 and/or oxygen saturation between the right radial artery versus descending aorta sites.[20] Considering that the left subclavian artery may have either a preductal or a postductal origin from the aorta, the oximeter probe should be applied to a foot for postductal pulse oximetry monitoring. Saturation differences greater than 5% to 10% generally indicate the presence of PPHN, but it is important to remember that a similar pattern of postductal desaturation may be observed in ductus-dependent cardiac diseases, including hypoplastic left-sided heart syndrome, coarctation of the aorta, or interrupted aortic arch.

Not all newborns with hypoxemia have PPHN physiology. In many infants, an intrapulmonary shunt or ventilation–perfusion mismatch resulting from parenchymal lung disease is the predominant abnormality rather than shunting of blood flow across the patent ductus arteriosus and patent foramen ovale. In this setting, hypoxemia is the result of pulmonary arterial blood perfusing the nonaerated lung regions. Although PVR is often elevated in hypoxemic newborns without PPHN, high PVR does not contribute significantly to hypoxemia in these cases. Rapid improvement in response to supplemental oxygen or increased mean airway pressure suggests ventilation–perfusion mismatch due to primary lung disease, although this may not be obvious with severe parenchymal lung disease. Furthermore, most infants with PPHN have at least a transient improvement in oxygenation in response to interventions such as high inspired oxygen concentration therapy and/or mechanical ventilation. Therefore these clinical findings can only suggest and not confirm the diagnosis. At present, there is no single biochemical marker that has emerged with sufficient sensitivity and specificity for the diagnosis and management of PPHN.

Radiographic findings are variable, depending on the primary disease associated with PPHN. Classically, the lung on a chest x-ray in idiopathic PPHN is oligemic, normally or slightly hyperinflated, and lacks parenchymal infiltrates. In general, the degree of hypoxemia is disproportionate to the severity of radiographic evidence of lung disease. Laboratory findings may include hypoglycemia, hypocalcemia, polycythemia, and thrombocytopenia.

Echocardiography is the gold standard to confirm the diagnosis and is an important tool for monitoring the response to therapy. The initial echocardiographic evaluation rules out structural heart disease causing hypoxemia or ductal shunting (e.g., coarctation of the aorta or total anomalous pulmonary venous return), determines the predominant direction of shunting at the patent foramen ovale and patent ductus arteriosus, and assesses ventricular function. The diagnosis of PPHN is made with certainty if bidirectional or predominantly right-to-left shunting across the foramen ovale or ductus arteriosus is observed, although other signs such as flattening or left deviation of the intraventricular velocity, tricuspid regurgitant velocity, and increased right ventricular dilation also suggest the diagnosis. The echocardiogram is also critical for the evaluation of right and left ventricular function. In some infants, predominant right-to-left shunting at the ductus arteriosus associated with left-to-right shunt at the foramen ovale is observed, indicating a significant contribution of left ventricular dysfunction to the underlying pathophysiology. When severe left ventricular dysfunction accompanies pulmonary hypertension, pulmonary vasodilation alone may be ineffective in improving oxygenation and must be accompanied by targeted therapies to increase cardiac performance and decrease left ventricular afterload. Thus careful echocardiographic assessment provides invaluable information about the underlying pathophysiology and will help guide the course of treatment.

Traditional echocardiographic evaluations of the right ventricle (RV) in infants with PPHN focus on the tricuspid regurgitation jet (TRJV) and the PDA shunt direction. However, cardiac function can be significantly decreased in infants with PPHN secondary to persistent RV afterload or prolonged hypoxemia.[74] Thus, while TRJV can be elevated in infants with PPHN, it is not reliably measurable and its absence does not consistently correlate with clinical outcomes.[75,76] Malowitz and colleagues have also demonstrated that echocardiography measurements of tricuspid annular plane systolic excursion (TAPSE) and right ventricle global longitudinal peak strain (GLPS) in addition to a predominant right-to-left shunt across the PDA to be more predictive of progression to death or ECMO in infants with PPHN.[75]

General Management

In 2015, the American Heart Association and the American Thoracic Society published guidelines for management of pediatric pulmonary hypertension, including PPHN (Box 47.1).[63] The general management principles for PPHN include maintenance of normal temperature (except for those undergoing therapeutic hypothermia), electrolytes (particularly calcium), glucose, hemoglobin, and intravascular volume. Mechanical ventilation is usually required to optimize lung volumes, although few guidelines exist to standardize ventilator management. Some advocate a "gentle ventilation" approach, with avoidance of paralysis and blood gas goals consisting of PaO_2 of 50 to 70 mmHg and $PaCO_2$ of 40 to 60 mmHg. This strategy has never been rigorously tested[77] but may well be the optimal strategy for infants still in the early phases of disease. When significant parenchymal lung disease is present, lung recruitment strategies, such as high-frequency ventilation, improve lung expansion and amplify the response to iNO.[78,79] However, care should be taken to avoid settings that may cause lung overdistension, which can lead to inflammatory

• BOX 47.1 American Heart Association and American Thoracic Society Management Guidelines for Persistent Pulmonary Hypertension of the Newborn

1. Inhaled nitric oxide therapy is indicated to reduce the need for extracorporeal membrane oxygenation (ECMO) support in term and near-term infants with persistent pulmonary hypertension of the newborn (PPHN) or hypoxemic respiratory failure who have an oxygenation index that exceeds 25 (class I, level A).
2. Lung recruitment strategies can improve the efficacy of inhaled nitric oxide therapy and should be applied in patients with PPHN associated with parenchymal lung disease (class 1, level B).
3. ECMO support is indicated for term and near-term neonates with severe pulmonary hypertension and/or hypoxemia that is refractory to inhaled nitric oxide therapy and optimization of respiratory and cardiac function (class I, level A).
4. Evaluation for disorders of lung development, such as alveolar capillary dysplasia and genetic surfactant protein diseases, is reasonable for infants with severe PPHN whose condition fails to improve after vasodilator, lung recruitment, and/or ECMO therapy (class IIa, level B).
5. Sildenafil use is a reasonable adjunctive therapy for infants with PPHN who are refractory to inhaled nitric oxide therapy, especially with an oxygenation index that exceeds 25 (class IIa, level B).
6. Inhaled prostacyclin analogues may be considered as adjunctive therapy for infants with PPHN who are refractory to inhaled nitric oxide therapy and have an oxygenation index that exceeds 25 (class IIb, level B).
7. Intravenous administration of milrinone is reasonable therapy for infants with PPHN and signs of left ventricular dysfunction (class IIa, level B).
8. Inhaled nitric oxide can be beneficial for preterm infants with severe hypoxemia that is primarily due to PPHN physiology rather than parenchymal lung disease (class IIa, level B).
9. Inhaled nitric oxide therapy and other pulmonary arterial hypertension–targeted drug therapies should be used cautiously in individuals with congenital diaphragmatic hernia, especially in those with confirmed or suspected left ventricular dysfunction (class IIa, level B).

From Abman SH, Hansmann G, Archer SL, et al. Pediatric pulmonary hypertension: guidelines from the American Heart Association and American Thoracic Society. *Circulation*. 2015;132(21):2037–2099.

changes, pulmonary edema, and decreased lung compliance, all of which can exacerbate pulmonary hypertension.

The use of surfactant should be considered only for infants with parenchymal lung disease. Single-center trials have shown that surfactant improves oxygenation, reduces air leak, and reduces the need for ECMO in infants with meconium aspiration.[80] A multicenter trial showed benefit in infants with parenchymal lung diseases such as meconium aspiration syndrome and sepsis.[81] This trial also demonstrated the greatest benefit for infants with earlier or milder disease (oxygenation index 15 to 22) but no benefit in the subset of newborns with idiopathic PPHN. A recent systematic review and metanalyses of surfactant therapy for neonates with meconium aspiration syndrome showed that those who received bolus surfactant needed ECMO less often than controls. However, the limited number of studies and neonates enrolled led the authors to conclude that more studies are urgently needed to evaluate the efficacy and cost-effectiveness of this therapy.[82] A retrospective study of infants in the CDH study group registry suggested that surfactant therapy for infants with CDH who were on ECMO did not increase survival or decrease the length of ECMO, length of intubation, or subsequent need for supplemental oxygen.[83]

Acidosis is a pulmonary vasoconstrictor, particularly when combined with hypoxia,[84] and pH should be maintained above 7.30. However, alkalosis (pH > 7.45) is not recommended. The use of alkalosis was frequent before the approval of iNO therapy, based on studies that found transient increases in PaO_2 after acute hyperventilation.[85] However, the pulmonary vascular response to alkalosis is transient, and prolonged alkalosis may paradoxically worsen pulmonary vascular tone, reactivity, and permeability edema.[86] Furthermore, alkalosis produces cerebral vasoconstriction, reducing cerebral blood flow and oxygen delivery to the brain, and may be associated with worse neurodevelopmental outcomes.[87] Similarly, there is no evidence to suggest that the use of sodium bicarbonate infusions to induce alkalosis provides any short-term or long-term benefit.[1]

Systemic hemodynamics should be optimized, with use of intravascular volume to provide sufficient preload and cardiotonic therapy to enhance cardiac output and systemic O_2 transport. Systemic hypotension may worsen right-to-left shunting, impair oxygen delivery, and worsen gas exchange in patients with parenchymal lung disease. In addition, high right ventricular systolic pressure reduces systolic flow through the right coronary artery, and low systemic blood pressure leads to a decline in right coronary artery diastolic flow, so correction of both hemodynamic abnormalities is key to avoiding right ventricular ischemia.[88] However, the goal is more complex than simply increasing blood pressure, and careful attention should be paid to right and left ventricular function and the choice of medication. Frontline agents typically include dopamine (5 to 20 μg/kg/min) and milrinone (0.2 to 0.99 μg/kg/min). Milrinone, which inhibits cAMP phosphodiesterase 3 (PDE3) activity in cardiomyocytes and pulmonary and systemic arterial smooth muscle, is viewed as an "inodilator" that may be especially useful in the context of left ventricular dysfunction and pulmonary venous hypertension.[89] Anecdotal evidence also supports the use of arginine vasopressin for severe PPHN (Table 47.2), based on its ability to raise systemic pressure via V_1 receptors, and to reduce pulmonary arterial pressure via activation of NO synthase.[90]

Infants who fail to respond to medical treatment, as evidenced by failure to sustain improvement in oxygenation with good hemodynamic function, may require treatment with ECMO. ECMO is the only proven lifesaving rescue modality for severe PPHN, although it is also costly, labor-intensive, and associated with potential adverse effects, such as intracranial hemorrhage and ligation of the right common carotid artery. The oxygenation index (calculated as [mean airway pressure × FIO_2 × 100]/PaO_2) is used to gauge the severity of disease, with an oxygenation index greater than 40 used as an indication for transfer to an ECMO center. Even with all available therapies, the mortality for PPHN remains between 5% and 10%.[2]

Oxygen

Supplemental oxygen is a mainstay of PPHN therapy to maintain oxygen delivery to the brain and other tissues and for its pulmonary vasodilator properties. Alveolar hypoxia and hypoxemia should be avoided, as both are pulmonary vasoconstrictors and contribute to the pathophysiology of PPHN. However, the degree of hyperoxic ventilation needed for optimal pulmonary vasodilation remains under investigation. The use of oxygen concentrations greater than 60% has not been shown to provide any additional benefit in pulmonary vasodilation, and hyperoxic ventilation increases oxidant stress and lung injury which may paradoxically impair the

TABLE 47.2 Pharmacologic Management of Persistent Pulmonary Hypertension of the Newborn

Drug	Dose	Mechanism of Action	Adverse Reactions/Safety Monitoring	Indications
Inhaled nitric oxide	5–20 ppm	Activates soluble guanylate cyclase in vascular smooth muscle cells Selective pulmonary vasodilator Low doses (<10 ppm) enhance ventilation–perfusion matching with parenchymal lung disease ("microselective effects")	Rebound PH with rapid discontinuation of inhaled nitric oxide therapy Methemoglobinemia with higher doses	Hypoxemic respiratory failure with PPHN
Sildenafil (Revatio)	Intravenous: loading dose: 0.4 mg/kg in 3 h Maintenance dose 1.6 mg/kg/day	Selective PDE5 inhibitor that increases vascular cGMP levels and pulmonary vascular relaxation	Hypotension (particularly with rapid loading dose) Hypoxemia	PPHN refractory to inhaled nitric oxide therapy and other conventional therapies
Milrinone	Intravenous: loading dose: 0.75 μg/kg/min for 3 h (for infants with hypotension or severe illness, may choose to not use loading dose) Maintenance dose: 0.2–0.33 μg/kg/min. Increasing by 0.33 μg/kg/min to a maximum of 0.99 μg/kg/min	PDE3 inhibitor that increases cAMP levels Improves cardiac performance through reduced systemic afterload, enhanced contractility, and pulmonary vasodilation	Hypotension, thrombocytopenia, arrhythmias	Left ventricular dysfunction, poor cardiac output
Prostacyclin	Flolan: Inhaled: 20–100 ng/kg/min. Start at 2 ng/kg/min and increase to 20 ng/kg/min within 3 h Continuous intravenous infusion: starting dose: 1-2 ng/kg/min, incremental increases as tolerated, monitor newborn for tachyphylaxis Remodulin: Continuous infusion: 1.25 ng/kg/min to start, titrate up slowly to 10 ng/kg/min Can be delivered subcutaneously in the same dosing range	Activates adenylate cyclase in vascular smooth muscle cells Pulmonary vasodilator	Ventilation–perfusion mismatch may complicate use in the setting of lung disease Nonselective vasodilator, can cause systemic hypotension Risk of rebound PH with sudden withdrawal Flushing, diarrhea	PPHN refractory to iNO therapy and other conventional therapies
Bosentan	Enteral: 1–2 mg/kg every 12 h	Dual ET_A and ET_B receptor inhibitor	Hepatotoxicity, edema, anemia, teratogen Monitor monthly LFT results	PPHN refractory to iNO and other conventional therapies Generally used for chronic PH (CDH, BPD)
Vasopressin	0.0003–0.004 units/kg/min	Predominantly used for enhancement of systemic hemodynamics in severe PPHN May have pulmonary vasodilator effects via activation of eNOS	Hypotension, arrhythmias Hyponatremia: increases cAMP level in distal tubules, leading to decreased urine volume	Systemic hypotension refractory to catecholamines?

cAMP, Cyclic AMP; *BPD*, bronchopulmonary dysplasia; *CDH*, congenital diaphragmatic hernia; *cGMP*, cyclic AMP; *eNOS*, endothelial nitric oxide synthase; *iNO*, inhaled nitric oxide; *LFT*, liver function test; *PDE3*, phosphodiesterase 3; *PDE5*, phosphodiesterase 5; *PH*, pulmonary hypertension; *PPHN*, persistent pulmonary hypertension of the newborn.

vasodilator response to iNO. Therefore the oxygen concentration and PaO_2 should be titrated to maximize pulmonary vasodilation. Studies in newborn lambs suggest that the lowest PVR can be maintained with a preductal SpO_2 in the 91% to 97% range with preductal PaO_2 between 60 and 80 mmHg,[91,92] although randomized studies have not been performed in infants with PPHN. Overall, oxygen should be used like any other drug in the therapeutic context, considering its potential benefits and side effects.

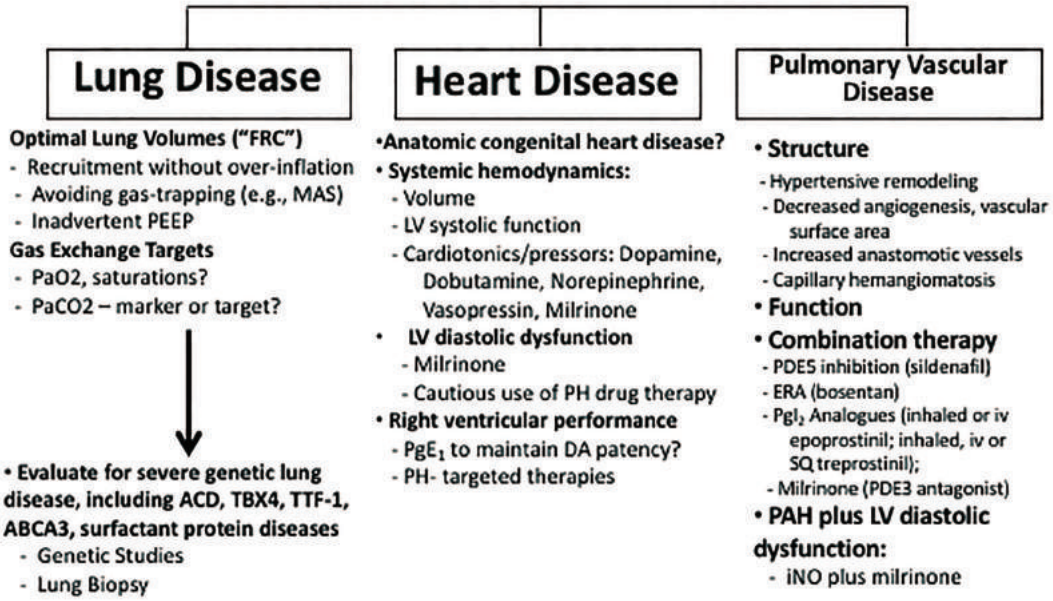
Inhaled Nitric Oxide

The primary goal of PPHN therapy is selective pulmonary vasodilation. Inhaled NO (iNO) is the only United States Food and Drug Administration (FDA) approved specific pulmonary vasodilator therapy for late preterm and term infants with PPHN, based on extensive safety and efficacy data from large placebo-controlled trials.[63] Endogenous NO is produced by endothelial cells and causes pulmonary vasodilation through the generation of cGMP. Studies have shown that inhaled NO diffuses from the alveolus into the smooth muscle cells and leads to selective pulmonary vasodilation. It is inactivated by hemoglobin in the circulation and hence has minimal systemic vasodilator effect.[79,93] Inhaled NO is a rapid and potent pulmonary vasodilator and because NO is a small gas molecule, it can be delivered as inhalation therapy to air spaces approximating the pulmonary vascular bed causing decreased intrapulmonary right-to-left shunting and improved V/Q matching.

Inhaled NO immediately improves oxygenation and decreases the need for ECMO support in newborns with PPHN who have an oxygenation index greater than 25.[94] The appropriate starting dose is 20 ppm; higher doses in nonresponders did not improve immediate responses or outcomes,[94] and treatment with high NO doses (80 ppm) increases the risk of methemoglobinemia.[95] The reported incidence of high levels of methemoglobinemia is very low with the use of an iNO dose less than 40 ppm[78,96,97] and data from a retrospective study of term and preterm infants indicated that high levels of methemoglobinemia (>2.5%) were observed in infants requiring FiO_2 greater than 60% at the time of methemoglobinemia measurement.[98] Similarly, to term infants, preterm infants with early PPHN, particularly after prolonged preterm rupture of membranes or oligohydramnios, will show marked improvement in oxygenation after treatment with iNO.[73] Recent reports indicate that iNO use ranges from 4% to 8% in extremely preterm infants.[99]

The optimal window for introduction of iNO therapy remains uncertain. The initial randomized trials studied term and near-term infants with severe PPHN and an oxygenation index of 25 to 40.[94,96] A subsequent large trial tested earlier use of iNO in infants with moderate respiratory failure (median oxygenation index of approximately 20) but did not report reductions in ECMO use/death relative to controls[5,100] (16.7% vs 19.5%). Once an infant has stabilized, weaning can generally be accomplished in 4 to 5 days. Prolonged need for iNO therapy without resolution of disease should lead to a more extensive evaluation to determine whether other underlying anatomic lung or cardiovascular disease is present, such as pulmonary venous stenosis, alveolar capillary dysplasia, and severe lung hypoplasia.

The response to iNO is both therapeutic and diagnostic (Fig. 47.4). Up to 40% of infants will not respond or sustain a response to iNO. Failure to respond to iNO is most commonly associated with inadequate lung recruitment. Thus, lung recruitment optimization with the use of PEEP to achieve and maintain functional residual capacity (FRC) (approximately 8 to 9 posterior rib expansion on anteroposterior chest x-ray) often ensures adequate lung recruitment and improves iNO responsiveness. Under- or over-inflation of the lung can increase PVR secondary to the mechanical compressional effects on the extra-alveolar and intra-alveolar pulmonary blood vessels. Atelectasis, under-inflation, increases intrapulmonary right-to-left shunting from inadequate ventilation and oxygenation leading to worsening hypoxia and hypercarbia. Lung over-inflation can impede venous return and cause systemic hypotension. In the true non-responders despite optimal lung recruitment, the clinician should carefully analyze the relative roles of parenchymal lung disease, pulmonary vascular disease, and cardiac dysfunction for each infant. For instance, if severe air space disease is associated with PPHN, strategies such as high-frequency ventilation that optimize lung expansion are likely to be effective, and the two therapies used together are more effective than either used



• **Fig. 47.4** An integrative approach to persistent pulmonary hypertension of the newborn *(PPHN)* newborns with poor responsiveness to iNO therapy. *FRC*, Functional reserve capacity; *MAS*, meconium aspiration syndrome; *LV*, left ventricle; *PH*, pulmonary hypertension; *PAH*, pulmonary arterial hypertension.

individually.[78] Considering iNO is usually delivered with high concentrations of oxygen, these therapies could interact and lead to enhanced production of reactive oxygen and reactive nitrogen metabolites, both of which may contribute to vasoconstriction and/or inadequate responses to iNO.

Other Therapeutic Agents

Phosphodiesterase Inhibitors

Increased activity of PDE5 has been consistently reported in experimental models of PPHN.[16,101] More recently, in vitro and in vivo studies have shown that even brief periods of hyperoxia independently increase the activity of PDE5, by a mechanism that appears to involve reactive oxygen species.[101–103] This finding could partially explain why some infants do not respond favorably to iNO.

Sildenafil is the best studied of the available phosphodiesterase inhibitors and can be administered by the enteral and intravenous routes. A small randomized trial found that enteral administration of sildenafil improved oxygenation and survival in term and late preterm neonates with PPHN.[104] In a subsequent dose-finding study, intravenous administration of sildenafil improved oxygenation in neonates with and without concurrent iNO treatment,[105] and may diminish the rebound pulmonary hypertension that can be seen during weaning of iNO. Sildenafil may be a useful adjunct in patients with partial or poorly sustained responses to iNO, but a recent multicenter trial did not find that intravenous sildenafil reduced treatment failure or time on iNO.[106] Sildenafil is generally well tolerated, although hypotension can occur, particularly if a loading dose is given too rapidly.[105] The availability of sildenafil as an enteral preparation also makes it feasible for long-term therapy for infants with chronic pulmonary hypertension associated with CDH[107] and BPD,[108] although this aspect of treatment needs more study and long-term follow-up. Other phosphodiesterase inhibitors include tadalafil, which has a longer half-life than sildenafil and has shown some benefit in PPHN refractory to iNO,[109,110] and vardenafil, which is being studied mostly in pre-clinical models at this time.

Prostanoids

Prostacyclin (PGI2) is a potent drug that induces vasodilation through activation of adenylate cyclase and increasing cAMP in pulmonary arterial smooth muscles. Unlike iNO, intravenously delivered PGI2 can potentially worsen ventilation-perfusion matching in the setting of lung disease. Epoprostenol was approved by the FDA in 1995 for the treatment of adult pulmonary arterial hypertension. While intravenously administered prostacyclin remains a mainstay of therapy for pulmonary hypertension in adults, rapid dosage escalation is often necessary for acute disease and can produce systemic hypotension. Its utility in the neonatal intensive care unit setting is also limited by the need for a dedicated central venous catheter, the potential to worsen ventilation–perfusion matching, and other systemic side effects including pain.

Aerosolized prostacyclin has been widely adopted in adult critical care units for the treatment of pulmonary hypertension.[111] While fewer reports describe its use in infants, case series indicate that continuous inhaled prostacyclin therapy (50 to 100 ng/kg/min) is well tolerated and improves oxygenation in infants with severe PPHN and inadequate response to iNO.[112,113] The risks include airway irritation from the alkaline solution needed to maintain drug stability, rebound pulmonary hypertension if use of the drug is abruptly discontinued, and inconsistent drug delivery due to drug loss into the circuit. New and more stable preparations are emerging that are specifically designed for intermittent nebulization, such as iloprost or treprostinil.[114–116] Treprostinil is particularly promising because it is also suitable for systemic administration, including by the subcutaneous route.[117]

Milrinone

Inhibition of PDE3, which metabolizes cAMP, might also enhance cAMP signaling in PPHN.[118] Milrinone, a PDE3 inhibitor, has been shown to decrease pulmonary arterial pressure and resistance and to act additively with iNO in animal studies.[119] In a 2006 report, the addition of intravenous milrinone therapy for neonates with severe PPHN and poor iNO responsiveness was associated with improvement in oxygenation without hemodynamic compromise.[120] As milrinone can improve both right and left ventricular function,[89] perhaps its greatest utility is in the setting of PPHN with LV dysfunction, in which lowering systemic vascular resistance with milrinone may improve LV performance and by lowering pulmonary venous pressure, can reduce right-to left-shunting. However, milrinone can reduce systemic blood pressure and cause systemic hypotension,[121] which may further impair myocardial perfusion and worsen cardiac function. Milrinone should be used with particular caution in preterm infants due to the risk for systemic hypotension and perhaps intraventricular hemorrhage.[122,123]

Endothelin Receptor Antagonists

ET-1 is a potent vasoconstrictor synthesized by vascular endothelial cells that acts through two receptors, ET_A and ET_B. The ET_A receptor plays a critical role in vasoconstriction, and selective blockade of the ET_A receptor causes fetal pulmonary vasodilation.[124] *ET-1* gene expression and ET-1 levels are increased in the lungs and pulmonary arterial endothelial cells in the fetal lamb model of PPHN.[125,126] Long-term intrauterine ET_A receptor blockade following ductal ligation decreases pulmonary arterial pressure and distal muscularization of small pulmonary arteries in utero, decreases right ventricular hypertrophy, and increases the fall in PVR at delivery in newborn lambs with PPHN.[127] Thus it is likely that ET-1, acting through the ET_A receptor, contributes to the pathogenesis and pathophysiology of PPHN. Use of bosentan, a nonspecific ET-1 receptor blocker, is an established therapy for pulmonary hypertension in adults. Two recent trials have shown that bosentan is well tolerated in neonates with PPHN. One single-center trial reported that bosentan led to substantial improvements in oxygenation in an iNO-naïve population of PPHN infants.[128] However, the FUTURE-4 multicenter trial found that bosentan as an adjunctive therapy with iNO therapy did not improve PPHN outcomes, reduce the time to receipt of iNO therapy, or reduce the time to extubation, possibly in part due to inconsistent intestinal absorption.[129]

Magnesium Sulfate

Other therapies that have been trialed for treatment of PPHN include intravenous (IV) magnesium sulfate ($MgSO_4$). A randomized controlled study comparing clinical efficacy of IV magnesium sulfate vs. oral sildenafil in infants with PPHN did not find $MgSO_4$ to be more effective than oral sildenafil in lowering

oxygen index (OI) and pulmonary artery pressure.[130] In addition, the duration of mechanical ventilation was longer and the need for inotropic support was greater with $MgSO_4$.

Outcomes

In the late 1980s and early 1990s, studies began reporting on the high risk of death and abnormal neurodevelopmental outcomes for survivors of PPHN.[131] One study showed that severity of oxygenation at presentation (measured by the alveolar–arterial oxygen difference) correlated well with survival but not with long-term outcome.[132] Other reports indicated that low Apgar scores and prolonged hypocarbia were predictive of adverse neurodevelopmental outcomes.[131,133]

The long-term outcome data for the largest iNO trials are summarized in Table 47.3, which also provides the most comprehensive outcome data for PPHN infants treated with and without iNO. Overall, these results highlight that neurodevelopmental impairment is frequent in this population (14% to 30%) and is not reduced by iNO therapy.[5,133,134] In each of these trials, a trend toward higher motor impairment was observed in the iNO group, and in the early iNO trial reported by Konduri et al. (2007),[5] the difference in the Bayley psychomotor developmental index was statistically significant. The Neonatal Inhaled Nitric Oxide Study Group (2000)[132] reported that the rate of disability was not affected by the need for ECMO, leading the authors to speculate that the timing of brain injury was most likely before ECMO.

Neurodevelopmental impairment is high even for those infants with moderate PPHN (oxygenation index of 15 to 25). The overall rates of neurodevelopmental impairment were similar to those of other trials that enrolled patients with more severe disease, and early iNO use did not translate into improved outcomes.[5] The proportion of infants with medical problems, such as the need for home medications, oxygen, and gastrostomy feedings, also tended to be higher in the early iNO group, although these differences were not statistically significant.

More than 33,400 newborns have been treated with ECMO for respiratory failure, with a cumulative overall survival to discharge of 73% (www.elso.org; September 2021). Neuroimaging abnormalities are identified in 10% to 20% of infants during or after ECMO,[135] and mild to major neurodevelopmental impairment is described in 15% to 25% of neonatal ECMO survivors. However, determining the role of ECMO versus other disease factors in the neurodevelopmental outcomes for ECMO survivors is difficult. From the pre-iNO era, the UK Collaborative Randomized Trial of ECMO provides the most direct comparison of outcomes after ECMO versus conventional treatment and included neurodevelopmental assessments at ages 1, 4, and 7 years. At age 1 year, a significant survival benefit (68% vs. 41%) was observed in the ECMO-treated group versus the conventionally treated group,[136] and survivors demonstrated similar rates of impairment or disability. At the 4-year follow-up, there continued to be a higher proportion of survivors without disability in the ECMO-treated group versus the conventionally treated group (50% vs. 37%),[137] although the rates of disability were similar at age 7 years (55% vs. 50%).[138] The high rate of neurodevelopmental impairment in both groups suggests that the underlying disease process, rather than the treatment, is the major influence on neurodevelopmental outcome. Several more recent studies also indicate that ECMO survivors may be at risk of academic problems,[139] so these children will benefit from detailed assessment as they reach school age. Recent data from the extracorporeal life support organization (ELSO) registry indicates that neonatal ECMO rates have decreased dramatically from 80% in 1990 to about 8% in 2017[140] which likely reflects improvements in medical management. However, there are still significant morbidities associated with ECMO therapy, likely reflecting infants that are more critically ill with significant comorbidities being managed with ECMO.[140]

TABLE 47.3 Neurodevelopmental Outcomes for the Major Inhaled Nitric Oxide Clinical Trials

Study	Number of Participants	Initial OI	Age at Follow-Up (Months)	NDI (%)		Bayley Mental Developmental Index		Bayley Psychomotor Developmental Index		Cerebral Palsy (%)	
				Control	iNO	Control	iNO	Control	iNO	Control	iNO
Neonatal Inhaled Nitric Oxide Study Group[134] (2000)	235	44	18–24	30	35	87	85	93.6	85.7	10.3	11.8
Clark et al.[136] (2003)	248	39	12	14	19	95	95	85	92	1.4	4
Davidson (2002)*	155	24.7	12	20	18	†	†	‡	†	6	8
Konduri et al.[5] (2007)	299	19.2	18–24	25	28	86.1	83.3	98	89	6.3	8.2

*The reference reporting the outcomes for the Davidson trial is entered as Lipkin et al. (2002).
†Seventy-one percent of the control group and 69% of the inhaled nitric oxide (iNO) group were reported as normal (mental developmental index ≥85).
‡Eighty-two percent of the control group and 76% of the iNO group were reported as normal (psychomotor developmental index ≥85).
NDI, Neurodevelopmental impairment; *OI*, oxygenation index.

Summary

Persistent pulmonary hypertension complicates the course of up to 10% of neonates with respiratory failure. It is associated with a diverse set of cardiopulmonary conditions, and its pathophysiologic mechanisms are characterized by vascular dysfunction, injury, and remodeling that occur before and after birth. In the last 20 years, experimental work on the basic mechanisms of vascular regulation of the developing pulmonary circulation has improved the range of therapeutic approaches for neonates with PPHN. In particular, iNO has proven to be a selective and effective pulmonary vasodilator for infants with PPHN, although successful clinical management requires meticulous care for all aspects of the associated lung and cardiac disease. Current research is focused on developing a better understanding of cellular responses in the remodeled vasculature and will likely elucidate additional signaling pathways and lead to new therapeutic strategies. More work is needed to further reduce mortality and improve neurodevelopmental outcomes of sick newborns with pulmonary hypertension, especially in patients with lung hypoplasia and advanced structural vascular disease.

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Patent Ductus Arteriosus in the Preterm Infant

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KEY POINTS

- Clinical signs of a symptomatic patent ductus arteriosus (PDA) usually appear later than echocardiographic signs and are related to the degree of left-to-right ductal shunting.
- Factors known to play a prominent role in regulation of ductal patency involve those that promote constriction (oxygen, endothelin, calcium channels, catecholamines, and Rho kinase) and those that oppose it (intraluminal pressure, prostaglandins, nitric oxide, carbon monoxide, potassium channels, and cyclic adenosine monophosphate and guanosine monophosphate).
- Closure of the ductus arteriosus occurs in two phases: (1) "functional" closure of the lumen by smooth muscle constriction, within hours after birth and (2) "anatomic" occlusion of the lumen over the next several days, due to neointimal thickening and loss of smooth muscle cells from the inner muscle media.
- The most important mechanism that prevents the preterm ductus from constricting after birth is its increased sensitivity to the vasodilating effects of prostaglandin E2. Inhibitors of prostaglandin production (e.g., indomethacin, ibuprofen, acetaminophen) are usually effective in promoting ductus closure in preterm infants.
- Early surgical ligation, while eliminating the detrimental effects of a PDA on lung development, may create its own set of problems for the preterm infant that counteract those benefits (e.g., postligation hypotension, bronchopulmonary dysplasia, vocal cord paralysis, neurodevelopmental abnormalities).
- While there may be general consensus on the efficacy of cyclooxygenase inhibitors for treatment of a PDA, questions about proper dosage, treatment duration, optimal timing, and treatment criteria remain controversial.
- Although a moderate-to-large PDA increases pulmonary blood flow and edema and decreases systemic blood pressure, it is not clear which is preferable: (1) to close the PDA (surgically or pharmacologically) or (2) to deal with the pulmonary edema and hypotension through other means, while awaiting spontaneous ductal closure.
- Further investigation is needed to determine which preterm infants are most likely to benefit from ductal closure and which might be best left untreated.

Introduction

The ductus arteriosus represents a persistence of the terminal portion of the sixth branchial arch. During fetal life, the ductus arteriosus serves to divert blood away from the fluid-filled lungs toward the descending aorta and placenta. After birth, constriction of the ductus arteriosus and obliteration of its lumen separate the pulmonary and systemic circulations. In full-term infants, obliteration of the ductus arteriosus takes place through a process of vasoconstriction and anatomic remodeling. In the preterm, the ductus arteriosus frequently fails to close. The clinical consequences of a patent ductus arteriosus (PDA) are related to the degree of left-to-right PDA shunt with its associated change in blood flow to the lungs, kidneys, and intestine.

Diagnosis

Phase contrast magnetic resonance imaging offers the most accurate measurements of ductal shunt volume, and the effects of a PDA on left ventricular and systemic blood flow volumes.[1] Unfortunately, these measurements are difficult to obtain in extremely immature, sick preterm infants. Two-dimensional echocardiography and color Doppler flow mapping is the current standard for assessing the presence, magnitude, and direction of PDA shunting. Ductus diameter ≥1.5 mm (or >50% of the diameter of the left pulmonary artery), left atrial-to–aortic root (LA/Ao) ratio ≥1.5, reversal of forward blood flow in the descending aorta during diastole, and end diastolic flow velocity in the left pulmonary artery ≥0.20 m/s are signs consistent with a moderate-to-large PDA shunt.[1,2] Unfortunately, the interobserver repeatability of all echocardiographic parameters is relatively poor.[3]

Clinical signs of a PDA (systolic murmur, hyperdynamic precordial impulse, full pulses, widened pulse pressure, and/or worsening respiratory status) usually appear later than echocardiographic signs and are less sensitive in determining the degree of left-to-right shunt. Certain signs such as continuous murmur or hyperactive left ventricular impulse are relatively specific for a PDA but lack sensitivity; conversely, worsening respiratory status, while a sensitive indicator, is relatively nonspecific for a PDA. Tachycardia is not a useful or reliable indicator of a PDA in preterm infants. Infants with large left-to-right shunts may have evidence of cardiomegaly and increased pulmonary arterial markings on their chest x-rays; however, in general, the chest x-ray and electrocardiogram are not useful in diagnosing a PDA. Although elevated plasma concentrations of brain natriuretic peptide (BNP) and N-terminal pro-BNP (NTpBNP) have been found to

correlate with the presence of a moderate sized left-to-right PDA shunt, changes in BNP and N-terminal pro-BNP concentrations have poor sensitivity and specificity in predicting increases or decreases in PDA shunt magnitude and cannot be used to replace echocardiography in the management of PDA shunts.[4,5] Although there has been little consensus in the past about what constitutes a clinically important PDA, recent studies have shown that moderate and large PDA shunts are associated with significant neonatal morbidities, whereas small PDA shunts have similar outcomes as no PDA shunts.[6–8] Although PDA shunt magnitude plays a significant role in creating its hemodynamic significance, equally important are the duration of shunt exposure, infant's gestational age, ventricular diastolic function, and need for invasive respiratory support.[7,9–12]

Incidence (Table 48.1)

Functional closure of the ductus occurs in almost 50% of full-term infants by 24 hours, in 90% by 48 hours, and in all by 72 hours. The rate of ductus closure is delayed in preterm infants; however, essentially all preterm infants who are ≥30 weeks' gestation (including those with respiratory distress syndrome) will close their ductus by the fourth day after birth. Infants born at less than 30 weeks' gestation have a 65% incidence of persistent ductus patency beyond day 4. Even among the most immature infants (≤27 weeks' gestation), spontaneous closure can occur during the neonatal period. However, when it does occur, it usually occurs late during the NICU hospitalization (average age = 61 ± 37 days).[13–19] Among preterm infants discharged from the hospital with a persistent PDA, 86% will achieve PDA closure by 1 year of age. The remainder will require continued observation or device closure.[13–19]

Factors like surfactant administration, infection, being small for gestational age at 26 to 29 weeks, and excessive fluid administration increase the likelihood of developing a symptomatic PDA.[13,20–27] On the other hand, being small for gestational age at 23 to 24 weeks, nonwhite, or having received antenatal glucocorticoids reduce risk of PDA.[28–34]

Regulation of Ductus Patency—Vasoconstriction and Vasorelaxation

In Utero Regulation

Ductus arteriosus patency is determined by the balance between dilating and constricting forces. Smooth muscle tone in the ductus arteriosus is determined by the phosphorylation and dephosphorylation of myosin light chains. The fetal ductus has a high level of intrinsic tone due to elevated levels of intracellular calcium (Ca^{2+}) which activate myosin light chain kinase, producing myosin light chain phosphorylation and smooth muscle constriction.[35] Extracellular Ca^{2+} enters the smooth muscle cytosol primarily through voltage-operated Ca^{2+} channels (Ca_L and T-type channels) and transient receptor potential (TRP) channels in the plasma membrane.[36,37] Calcium is also released from intracellular stores in the sarcoplasmic and endoplasmic reticulum (SR and ER) through ryanodine receptors and inositol 1,4,5-trisphosphate receptors (IP3Rs).[37,38]

The contractile proteins in the fetal ductus (smooth muscle myosin, calponin, and caldesmon) are more differentiated and more sensitive to the contractile effects of Ca^{2+} than they are the aorta and the pulmonary artery.[39–42] Increased Rho kinase activity in the ductus increases smooth muscle sensitivity to Ca^{2+} by inhibiting myosin light chain dephosphorylation. Endothelin-1 plays a role in the elevated basal tone of the fetal ductus arteriosus by inducing IP3 and releasing Ca^{2+} from the sarcoplasmic reticulum.[43,44] Recently, a role for serotonin in promoting fetal ductus tone has been suggested since selective serotonin reuptake inhibitors constrict the ductus in utero.[45]

The factors that oppose ductus arteriosus constriction in utero are better understood. The elevated vascular pressure within the ductus lumen (due to the high resistance in the constricted pulmonary vascular bed) plays an important role in opposing ductus constriction.[46]

K^+ channels buffer ductus tone by regulating the rate of extracellular Ca^{2+} entry into the smooth muscle cells. When K^+ channels are open, K^+ exits the cell turning the membrane potential more negative (i.e., hyperpolarizing the cell). Hyperpolarization

TABLE 48.1 Incidence of Patent Ductus Arteriosus Among Infants Less Than 30 Weeks' Gestation

PRESENCE OF PDA (ANY SIZE) (%)						
Gestation (wk)	**Day 4**	**Day 7**	**Day 20**	**Day 40**	**Day 60**	**Day 80**
28–29	55	33	20	10	8	
26–27	84	68	48	38	27	27
24–25	96	87	75	72	56	38
PRESENCE OF HEMODYNAMICALLY SIGNIFICANT PDA (%)*						
Gestation (wk)	**Day 4**	**Day 7**	**Day 20**	**Day 40**	**Day 60**	**Day 80**
27–28		21	13	5	1	0
25–26		64	50	22	3	0
23–24		93	88	58	33	14

*Ductus diameter ≥2 mm on echocardiography *plus* need for ventilator support
Data from references. 13–15,17–19.

inhibits extracellular Ca^{2+} influx through voltage-gated L-type Ca^{2+} (CaL) channels. Several K^+ channels [voltage-gated (Kv), Ca^{2+}-activated (K_{Ca}), and ATP-dependent (K_{ATP})] K^+ channels are present in fetal ductus smooth muscle cells. Their relative contribution to resting membrane potential depends on the animal species and the stage of development.

The fetal ductus also produces vasodilators that help to maintain ductus patency. Prostaglandins (PGs) are the dominant vasodilators that oppose ductus constriction in the later part of gestation.[47] Inhibitors of PG synthesis constrict the fetal ductus. PGE_2 is the most potent PG produced by the ductus and appears to be the most important prostanoid to regulate ductus patency.[48,49] The ductus is extraordinarily sensitive to the vasodilating effects of PGE_2. In the ductus, all three of the PGE receptors (EP2, EP3, and EP4) participate in vasodilation by activating adenylate cyclase and increasing ductus smooth muscle cyclic adenosine monophosphate (AMP) (which inhibits myosin light chain kinase and myosin light chain phosphorylation, thereby inhibiting the sensitivity of the contractile proteins to calcium).[42,50] Low levels of phosphodiesterase (the enzyme that degrades cyclic AMP) in the fetal ductus account for the vessel's increased sensitivity to PGE2.[51,52] PGE_2, acting through EP3 receptors (in lamb) and EP4 receptors (in rabbit), also activates the K_{ATP} and Kv channels, respectively, increasing outward K^+ current, hyperpolarizing the cell, and further inhibiting Ca^{2+} influx.[50,53]

Both isoforms of the enzyme responsible for synthesizing PGs (cyclooxygenase [COX]-1 and COX-2) are expressed in the fetal ductus.[54] In the fetal mouse, COX-2 appears to be the COX isoform responsible PGE2 production, whereas in the fetal sheep, both COX-1 and COX-2 play a role.[54,55] High circulating PGE_2, originating from the placenta, also regulates fetal ductus behavior,[56] due to low in utero pulmonary clearance.[57,58]

Nitric oxide, formed mainly by eNOS, is made by the fetal ductus arteriosus and appears to play an important role in maintaining ductus patency in rodent fetuses early in gestation.[47] NO activates soluble guanylyl cyclase increasing ductus smooth muscle cyclic GMP (cGMP) and cGMP-dependent protein kinase (PKG). PKG decreases intracellular Ca^{2+} by inhibiting Ca^{2+} influx, stimulating its removal, and by inhibiting the Rho-kinase pathway. PGE_2 and NO are coupled for reciprocal compensation since cyclooxygenase inhibition upregulates NO.[59] The importance of NO in maintaining in utero patency in larger species has not been conclusively demonstrated (see below).[60]

Carbon monoxide (CO) relaxes the ductus arteriosus.[61,62] Under physiologic conditions the amount of CO made by the ductus does not seem to affect ductus tone; however, in circumstances where its synthesis is upregulated, for example, endotoxemia, CO may exert a relaxing influence on the ductus.[63,64] Hydrogen sulfide (made by ductus endothelial and smooth muscle cells) also has been identified as another endogenous factor that inhibits fetal ductus tone by opening K_{ATP} channels.[65,66]

Chronic Inhibition of Prostaglandin Signaling In Utero

Short term, pharmacologic inhibition of PG signaling produces ductus constriction, in utero, however, chronic inhibition produces the opposite effect in mice: a persistent PDA in utero and a persistent PDA after birth.[55,67–69] It is now clear that, in addition to its role in maintaining fetal ductus patency, PGE2 is essential for vascular homeostasis and the induction of pathways necessary for postnatal closure.[70,71] Depletion of the PG EP4 receptor decreases the expression of several genes that control postnatal oxygen-induced ductus constriction (see below). Chronic inhibition of PG synthesis decreases Ca_L- and K^+-channel genes (Ca_Lalpha1c, Ca_Lbeta2, Kir6.1, and Kv1.5), which regulate Ca^{2+} entry and phosphodiesterase expression, which increases the ductus's sensitivity to cAMP- or cGMP-dependent vasodilators.[71] Both ablation of the PG EP4 receptor or its chronic blockade produce marked reductions in α-SM actin, SM22α, myosin heavy chain, and serum response factor by altering Wnt/β-catenin signaling.[70,72] Global deletion of the EP4 receptor blocks postnatal ductus closure. However, vascular smooth muscle-specific EP4-gene deletion does not have the same effect which suggests that vascular smooth muscle EP4 alone may not be sufficient for mediating DA closure.[70]

Similar to its effects during rodent gestation, pharmacologic inhibition of PG synthesis by indomethacin during human pregnancy also is associated with an increased incidence of PDA after birth.[73] However, in the human newborn, this appears to be due to indomethacin's ability to produce ductus constriction in utero. Constriction in utero produces ischemic hypoxia, increased NO production, and smooth muscle cell death which prevent the ductus from constricting after birth and make it resistant to the constrictive effects of postnatal indomethacin.[74,75] These changes are similar to the events that occur postnatally (see below). Although paracetamol (acetaminophen) also inhibits fetal ductus PGE_2 production, it rarely causes fetal ductus closure in pregnant women.[76]

Postnatal Regulation (Box 48.1)

In the full-term newborn, closure of the ductus arteriosus occurs in two phases: (1) "functional" closure of the lumen within the first hours after birth by smooth muscle constriction, and (2) "anatomic" remodeling of the vessel over the next days by extensive neointimal thickening and loss of smooth muscle cells from the inner muscle media.

Several factors promote ductus constriction in the full-term newborn following delivery: (1) an increase in arterial PO_2, (2) a decrease in blood pressure within the ductus lumen (due to the postnatal decrease in pulmonary vascular resistance), (3) a decrease in circulating PGE_2 (due to loss of placental PG production and increased removal by the lung), and (4) a decrease in the number of PGE_2 receptors in the ductus wall.[50,57,77] Although the newborn ductus continues to be sensitive to the vasodilating effects of NO, it loses its ability to respond to PGE_2.[78,79]

The postnatal increase in arterial PaO_2 plays an important role in postnatal ductus constriction. Infants born at higher elevations have an increased incidence of PDA.[80] Oxygen-induced contraction can be demonstrated in the presence of inhibitors of PG, NO, and endothelin signaling and in the absence of endothelial cells. This suggests that these endothelial-derived vasoactive substances are not essential for normoxic constriction. In most species, oxygen appears to constrict the ductus arteriosus through mechanisms that are both dependent and independent of smooth muscle membrane depolarization.[36] Oxygen depolarizes ductus smooth muscle cells by inhibiting K^+ channels.[81,82] Following depolarization Ca^{2+} enters the ductus smooth muscle through L-type and T-type voltage dependent, Ca^{2+} channels.[43,83–85] Several O_2 sensitive K^+ channels have been found in the fetal ductus (including Kv1.5 and Kv2.1). These vary with species and gestational age and may account for the differing sensitivity of the ductus to oxygen.[86,87] Increased oxygen tensions also inhibit ductus K_{ATP} channels (by increasing mitochondrial ATP production), have a

direct effect on the Ca^{2+} L-channels, voltage-dependent T-type Ca^{2+} channels, and store-operated Ca^{2+} channels, and inhibit the forward mode of the Na^{+}/Ca^{2+} exchanger.[37,38,85,88,89] Oxygen also increases smooth muscle sensitivity to Ca^{2+} by activating Rho kinase mediated pathways.[36–38,90,91]

Mitochondria in the ductus smooth muscle cells are important upstream oxygen sensors. Elevated PO_2 causes mitochondrial fission, mediated by dynamin-related protein-1, which increases electron transport chain complex I activity and the production of reactive oxygen species (superoxide and H_2O_2).[81,91–95] Inhibition of mitochondrial fission selectively inhibits O_2-induced H_2O_2 production and ductus constriction, without altering constriction to other agonists.[92,93] In most species H_2O_2 appears to stimulate vasoconstriction by increasing Ca^{2+} influx and Ca^{2+} sensitization by regulating K^{+} channels, Ca^{2+} channels, and Rho kinase. However, in some species H_2O_2 acts only as a vasodilator, or as both a vasodilator and a vasoconstrictor (depending on its concentration).[96,97]

In the chicken ductus, reactive oxygen species induce their own amplification pathway by activating neutral sphingomyelinase and increasing ceramide production. Ceramide, which activates NADPH oxidase, amplifies the formation of reactive oxygen species by transferring electrons from NADPH to oxygen.[98,99]

• BOX 48.1 Changes That Lead to Ductus Closure After Birth

- Decreased ductus intraluminal blood pressure after postnatal pulmonary vasodilation
- Removal of placenta as a source for PGE_2 production
- Increased pulmonary clearance of circulating PGE_2
- Decreased circulating PGE_2 concentrations
- Decreased prostaglandin receptors (EP4) after birth
- Decreased intracellular cAMP and cGMP concentrations
- Increased arterial oxygen tension
- Increased H_2O_2 release by mitochondria
- Oxygen responsive K^{+} channels close resulting in smooth muscle cell depolarization
- Smooth muscle cell depolarization increases extracellular calcium entry into cytoplasm through voltage dependent calcium L-and T-channels
- Increased calcium release from sarcoplasmic and endoplasmic reticulum
- Increased Rho kinase activity increases smooth muscle calcium sensitization
- Increased cytochrome p450 3A13 activity
- Increased isoprostane production
- Increased endothelin production
- Decreased serum osmolality after birth activates TRPM3 receptors
- Increased myosin light chain kinase activity
- Decreased myosin light chain kinase phosphatase
- Profound hypoxia develops in muscle media as vessel constricts
- Increased VEGF and PDGF production occurs as a result of ductus constriction
- Mononuclear cells and platelets attach to luminal endothelial cells
- Endothelial cells detach from basement membrane and proliferate
- Fragmentation of internal elastic membrane
- Hyaluronic acid, fibronectin, fibulin-1, and chondroitin sulfate accumulate in subendothelial space and in muscle media
- Smooth muscle cells from the muscle media migrate into neointima forming mounds that occlude residual ductus lumen
- Anoxia of the muscle media causes smooth muscle cells to die

Studies in animals suggest that a cytochrome P450 hemoprotein also appears to be involved with oxygen-induced constriction.[100–103] This mechanism may have parallels in preterm human ductus since cytochrome P450 inhibitors increase the incidence of PDA.[104,105] Oxygen also activates the epidermal growth factor receptor (EGFR) in ductus smooth muscle cells. EGFR inhibition attenuates oxygen-induced constriction.[92]

During the transition from fetus to newborn isoprostanes are formed nonenzymatically by free radical-mediated peroxidation of phospholipid-bound arachidonic acid. In mice, isoprostanes have both contractile and vasodilatory effects on the ductus by activating both thromboxane and EP4 receptors, respectively. With advancing gestation, the balance shifts in favor of the contractile effects of thromboxane stimulation.[106]

Oxygen also increases the potent vasoconstrictor, endothelin-1.[107] The role of endothelin-1 in postnatal ductus closure is still unclear due to the marked species variation in its contribution to oxygen-induced ductus constriction.[44,82,108–110] The postnatal increase in PaO_2 also has profound modulatory effects on other vasoactive systems.[111] Elevated oxygen tensions can increase the ductus's contractile response to neural mediators and can decrease the formation of vasodilator PGs.[35,112] Although the contractile effects of oxygen play an important role in postnatal ductus constriction, they are not essential for postnatal ductus closure. For example, mice lacking the endothelin A receptor have diminished oxygen-induced ductus constriction, however, their ductus closes normally after birth.[44]

Developmental Regulation (Box 48.2)

In contrast with the full-term ductus, the premature ductus is less likely to constrict after birth. The intrinsic tone of the extremely immature ductus is decreased compared to the ductus at term.[35] This may be due to the presence of immature smooth muscle myosin isoforms, with a weaker contractile capacity, and to decreased Rho kinase expression and activity.[39,41,43,90,91] Ca^{2+} entry through L-type Ca^{2+} channels appears to be impaired in the

• BOX 48.2 Factors Contributing to Ductal Patency in Preterm Neonates

- Immature smooth muscle isoforms
- Decreased calcium entry through calcium L-channels
- Decreased Rho kinase activity (decreased calcium sensitization)
- Increased potassium K_{Ca} channels (which are not regulated by oxygen)
- Decreased potassium K_v channels (which are regulated by oxygen)
- Decreased endothelin production
- Increased circulating PGE_2 concentrations
- Decreased pulmonary clearance of circulating prostaglandin E2
- Increased prostaglandin receptors (EP4) after birth
- Increased EP4 receptor coupling with adenyl cyclase
- Increased cyclic AMP production
- Decreased cyclic AMP degradation by phosphodiesterases
- Increased postnatal PGE_2, nitric oxide, and other vasodilator production
- Decreased antenatal exposure to cortisol
- Increased serum osmolality after birth
- Decreased ductus wall thickness
- Decreased postnatal constriction
- Both decreased ductus wall thickness and decreased postnatal constriction protect premature ductus from developing profound postnatal ductus muscle media hypoxia and prevent anatomic changes needed for permanent closure

immature ductus (especially under hypoxic conditions).[43,88,91] The potassium channels that promote ductus relaxation also change during gestation (switching from K_{Ca} channels, which are not regulated by PaO_2, to Kv channels, which can be inhibited by increased PaO_2).[86,113,114] Reduced expression and function of the putative oxygen-sensing Kv channels appear to contribute to ductus patency in the preterm rabbit, sheep, baboon, mouse, and chicken.[91,113,114] In contrast, a decrease in Kv channel expression occurs with advancing gestation in the rat, which suggests that in that species DA closure may occur by eliminating Kv channels.[86]

Premature infants have elevated circulating concentrations of PGE_2, which may play a significant role in maintaining ductus patency during the first days after birth. This is due to the decreased ability of the premature lung to clear circulating PGE_2.[57,77] Probably the most important mechanism preventing the preterm ductus from constricting after birth is its increased sensitivity to the vasodilating effects of PGE_2. The increased sensitivity to PGE_2 is due to both increased cyclic AMP production (from enhanced receptor coupling with adenyl cyclase) and decreased cyclic AMP degradation by phosphodiesterase.[51,115]

PGs may be the most important factor opposing ductus constriction in utero and immediately after preterm birth, but by the end of the first week there is enhanced production of NO and other vasodilatory inflammatory molecules within the ductus wall. Smooth muscle glucose, glycogen, and ATP concentrations also become depleted within the ductus smooth muscle, making it more difficult to maintain active constriction.[116–118] As a result, inhibitors of PG production become less effective toward the end of the first postnatal week.[119–121] Inhibitors of NO production have been used successfully as adjunctive therapy to close the PDA in situations where indomethacin was ineffective in constricting the ductus by itself.[122,123]

PGs may also contribute to persistent ductus patency in full-term infants since indomethacin can produce a substantial degree of ductus constriction even in full-term infants.[124,125] However, this is more likely to occur in those born at 37 weeks' rather than at 42 weeks' gestation.

Several other prenatal and postnatal factors also affect the rate of ductus constriction in preterm newborns. Circulating concentrations of thyroid hormones and cortisol increase with advancing fetal gestation and play a role in ductus arteriosus maturation. Preterm infants treated with thyroid hormone have a lower incidence of PDA.[126,127] Similarly, full-term infants with congenital hypothyroidism have an increased incidence of PDA that appears to respond to thyroid hormone replacement therapy.[128] Elevated fetal cortisol concentrations also foster ductus maturation by increasing its sensitivity to oxygen.[34,129] Both prenatal and postnatal glucocorticoid exposure reduces the incidence of PDA in premature humans and animals.[29,34,130–135] However, caution must be used when administering postnatal glucocorticoids since the risk of necrotizing enterocolitis is increased when administered concurrently with indomethacin or ibuprofen.[134,136]

Prenatal administration of vitamin A has been shown to increase both the intracellular Ca^{2+} and contractile response of the preterm ductus to oxygen in addition to stimulating PDGF-mediated smooth muscle cell migration, proliferation, and hyaluronic acid and fibronectin production.[137–139] Although vitamin A did not increase the rate of PDA closure in two randomized controlled trials (RCTs), the need for ductus ligation among AGA infants was significantly decreased.[140,141]

Other factors include serum hyperosmolality and brain natriuretic peptide (both of which have direct effects on the DA), caffeine (which is associated with decreased PDA but has no direct effect on the DA), and glutamate (however, glutamine supplementation in RCTs does not alter the incidence of PDA).[142–148] Although phototherapy has occasionally been associated with PDA, it has not been found to alter the incidence of PDA in RCTs.[149,150]

Anatomic Closure-Histologic Changes

Anatomic remodeling of the ductus arteriosus is essential for permanent luminal closure. Fragmentation of the internal elastic lamina and progressive intimal thickening start in the second half of gestation and accelerate after postnatal constriction. As the intima increases in size, it occludes the already constricted lumen. The increase in intimal thickening is due (1) to migration of smooth muscle cells from the muscle media into the neointima and (2) to proliferation of luminal endothelial cells. The process of intimal cushion formation starts with accumulation of hyaluronan (HA) below the luminal endothelial cells, loss of laminin and collagen IV from their basement membrane, and separation from the internal elastic lamina. The hygroscopic properties of HA create an environment well suited for cell migration.[151] The endothelial and smooth muscle cells of the ductus arteriosus differ from those of the adjacent vessels in their sensitivity to transforming growth factor β (TGFβ) which increase their HA production.[152] PGs, acting through the EP4 receptor, play a stimulatory role in ductus HA production and neointimal thickening by activating pathways that involve cyclic AMP, cAMP-protein kinase A, Epac (exchange protein activated by cAMP), phospholipase C, alpha1G T-type voltage dependent channels, fibulin-1, cysteine-rich protein CCN3, and Wnt/β-catenin.[85,153–158]

Accompanying the increase in HA in the neointimal space is an increase in fibronectin (FN) (which facilitates ductus smooth muscle cell migration) and chondroitin sulfate (CS) (which interferes with elastin assembly).[151,159,160] Intimal cushion formation is associated with disruption of the internal elastic laminae (IEL) underlying the ductus luminal endothelial cells. Ductus endothelial cells have high levels of tissue-type plasminogen activator which activates the elastolytic enzyme matrix metalloproteinases 2 at sites of IEL disruption.[161] In the aorta, well-developed elastic laminae surround smooth muscle cells and prevent the vascular wall from collapsing. In contrast, smooth muscle cells in the ductus are surrounded by thin, fragmented elastin fibers that do not prevent it from collapsing during vasoconstriction.[162] The disruption of elastin fiber assembly around ductus smooth muscle cells is not due to increased elastase activity or decreased tropoelastin production. Rather, it appears to be due to developmental mechanisms that reduce insolubilization of elastin and prevent formation of intact elastic laminae. PGE_2 also plays a critical role (through an EP4-cSrc-PLCγ-signaling pathway) in inhibiting thick elastin fiber formation in the ductus. Lysyl oxidase, which catalyzes elastin cross-linking, is degraded when the EP4 receptor is stimulated.[163] Impaired assembly of thick elastic laminae facilitates smooth muscle cell migration by removing a physical barrier. In addition, the accumulation of soluble, truncated tropoelastin acts as a smooth muscle cell chemoattractant.[164] It is interesting to note that when EP4 expression is decreased in the EP4 knockout mouse and Brown-Norway rat, the DA's elastic laminae appear abnormally well developed, similar to the aorta, and intimal cushions fail to develop.[162,163,165]

Antenatal betamethasone and the postnatal increase in PaO_2 also stimulate ductus smooth muscle proliferation and migration

by increasing ADP-ribosyltransferase 3 expression and activating the RhoA-ROCK-PTEN pathway, respectively.[166,167]

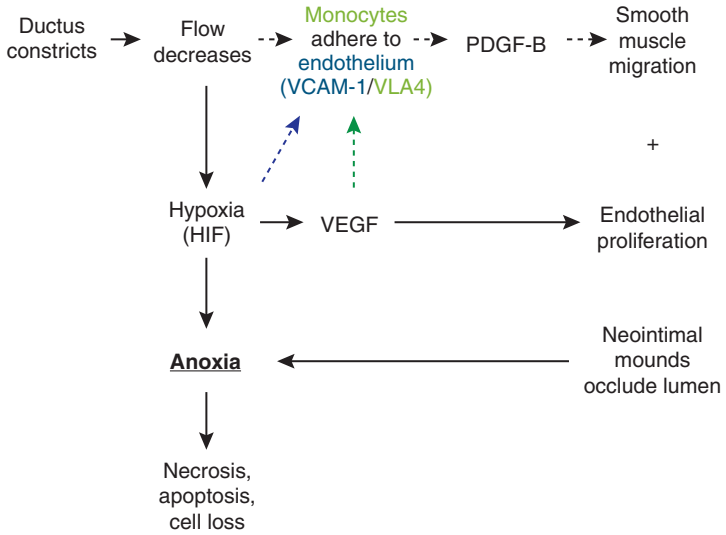
Relationship Between Vasoconstriction and Anatomic Closure (Fig. 48.1)

In full-term animals, both the loss of vasodilator regulation and the anatomic events that lead to permanent closure appear to be controlled by the degree of ductus smooth muscle constriction. Constriction produces ischemic hypoxia of the vessel wall.[168] Experimental models that alter the ability of the ductus to constrict at term prevent the normal histologic changes that occur after birth.[46,55,69,169–171] Ductus luminal blood flow provides oxygen and nutrients to the inner half of the ductus wall. Because of the thickness of the term ductus, intramural vasa vasorum are needed to provide oxygen and nutrients to the outer half of its wall. These collapsible, intramural vasa vasorum provide the ductus with a unique mechanism for controlling the maximal diffusion distance for oxygen and nutrients across its wall. Ductus constriction obliterates vasa vasorum flow to the outer muscle media, which turns the entire thickness of the muscle media into a virtual avascular zone.[172] The profound ischemic hypoxia that follows the constriction inhibits local production of PGE_2 and NO, induces local production of hypoxia-inducible factors like HIF1α and vascular endothelial growth factor (VEGF), and produces smooth muscle apoptosis in the ductus wall. VEGF plays a critical role in the migration of the ductus smooth muscle cells into the neointima and in the proliferation of intramural vasa vasorum.[173] The hypoxic muscle media and shift from laminar to turbulent/stagnant flow in the constricted lumen induce expression of genes known to be essential for remodeling (HIF1α, vascular cell adhesion molecule 1 [VCAM-1], E-selectin, IL-8, MCSF-1, CD154, IFNγ, IL-6, and TNFα).[117,174] Circulating mononuclear cells adhere to newly expressed VCAM-1 and E-selectin adhesion molecules and become activated monocytes/macrophages that produce PDGF. PDGF stimulates smooth muscle migration and proliferation into the neointima.[117] The inflammatory response following postnatal ductus constriction appears to be necessary for neointimal remodeling since the extent of remodeling is determined by the degree of mononuclear cell adhesion.[117] In mice, platelets play an essential role in ductus luminal closure by forming a platelet plug to fill the small residual lumen that persists after postnatal constriction.[175]

In preterm infants, the ductus frequently remains open for many days after birth. Even when it does constrict, the premature ductus frequently fails to develop profound hypoxia and anatomic remodeling. The preterm infant requires a greater degree of ductal constriction than the term infant to develop a comparable degree of hypoxia. In contrast with the full-term ductus, where vasa vasorum are needed to provide oxygen and nutrients to the outer two-thirds of the ductus wall, intramural vasa vasorum are absent from the ductus wall of infants born before 26 weeks' gestation where luminal blood flow can provide all the oxygen and nutrients the thin-wall ductus requires. The absence of intramural vasa vasorum leaves the preterm ductus without a mechanism to rapidly increase the diffusion distance across its wall during postnatal constriction. As long as any degree of luminal patency exists, the thin-wall preterm ductus fails to become profoundly hypoxic and fails to undergo anatomic remodeling after birth. As a result, the preterm ductus requires complete cessation of luminal flow before it can develop the same degree of hypoxia as found at term. Once the preterm ductus develops the same degree of hypoxic ischemia as the term ductus, most of the anatomic changes seen at term will occur.[35,122] However, if the premature ductus does not develop the degree of ischemic hypoxia needed to induce cell death and anatomic remodeling, it will continue to be responsive to vasodilators and continue to be susceptible to vessel reopening.

Genetic Regulation

Recent studies have used genome-wide transcriptome analysis to examine differences in gene expression between the ductus and



• **Fig. 48.1** Ductus Remodeling: Role of Constriction, Hypoxia, and Mononuclear Cell Adhesion in Neointima Formation and Smooth Muscle Cell Death. Postnatal constriction produces hypoxia in the ductus wall. The hypoxic smooth muscle and endothelial cells increase their expression of vascular endothelial growth factor *(VEGF)* and vascular cell adhesion molecule 1 (VCAM-1), respectively. VEGF is required for endothelial cell proliferation. VEGF also attracts circulating mononuclear cells (expressing very late antigen-4 [VLA-4]) to the endothelial cell surface. Under very low flow conditions, the weakly adherent VLA-4$^+$ mononuclear cells attach to VCAM-1 on the endothelial cell surface and release PDGF and MMP-9 (which promote smooth muscle migration into the neointima). Tight constriction and loss of luminal flow are essential for VEGF expression, mononuclear cell adhesion, neointimal formation, and luminal occlusion.

the aorta (as well as the effects of oxygen and preterm birth on ductus gene expression). Genes related to neural crest migration (e.g., TFAP2b), TGF-beta signaling, matrix molecules (like fibronectin and lysyl oxidase), actin-myosin interactions, potassium, and Ca^{2+} ion signaling, as well as PG, endothelin, and angiotensin II signaling have increased expression in the ductus compared with the aorta. Mutations in several of these ductus-dominant genes/pathways (examined in either mouse knockout models or human genetic syndromes) are associated with persistent ductus patency in term neonates.[44,55,72,175–215] Single nucleotide polymorphisms (SNPs) have also been associated with isolated, nonsyndromic, persistent ductus patency in term infants (e.g., in TGFBR2).[216]

Although several pathways appear to be commonly involved in DA regulation among different species or different genetic backgrounds, the relative expression and importance of single orthologous genes frequently differ.[176,177,217–222] For example, endothelin receptor stimulation may account for 44% of the oxygen induced contraction in the rat, whereas it only contributes to 13% of the contraction in the rabbit, and has a negligible role in the human ductus.[82,110] Oxygen depolarizes the ductus smooth muscle cells by inhibiting K^+ channels.[81,82] In the rabbit, an increase in the expression of Kv channels appears to be responsible for the developmental increase in the oxygen-induced contraction.[114] In contrast, in the rat, Kv channel expression decreases with advancing gestation.[86] Ductus patency is critically dependent on vasodilator PGs in most species; however, notable exceptions exist in the guinea pig, chicken, and emu ductus, where locally derived PGs do not appear to play a significant role in ductus patency.[223–226]

Genetic and familial factors also play a role in persistent ductus patency in preterm infants.[227,228] Single nucleotide polymorphisms (SNPs) in several genes have been associated with preterm PDA (ATR type 1, IFNγ, estrogen receptor-alpha PvuII, TFAP2B, PGI synthase, TRAF1) and CYP2C9.[229–233] There is a growing body of evidence to suggest that SNPs in TFAP2B may be responsible for some of the PDAs that occur in preterm infants (especially those that are unresponsive to indomethacin).[232] TFAP2B regulates genes that are important in ductus smooth muscle development.[234] However, race, ethnicity, and ancestral background appear to be important modifiers of TFAP2B's effects. TFAP2B polymorphisms are associated with important changes in DA gene expression and persistent PDA in preterm infants but only when present in infants with European ancestry.[235,236]

Hemodynamic and Pulmonary Alterations

The pathophysiologic features of a PDA depend both on the magnitude of the left-to-right shunt and on the cardiac and pulmonary responses to the shunt. The PDA left-to-right shunt lowers systemic blood pressures (both systolic and diastolic) and adds to the incidence of inotrope-dependent hypotension often observed among preterm infants (born before 28 weeks' gestation) at the end of the first week.[237,238]

There are important differences between immature and mature infants in the heart's ability to handle the increased volume load from the PDA. The immature fetal ventricles have less cardiac sympathetic innervation and are less distensible than at term and generate less force per gram of myocardium.[239] The relative lack of left ventricular distensibility in immature infants is more a function of the ventricle's tissue constituents than of poor muscle function. As a result, left ventricular distension secondary to a large left-to-right PDA shunt may produce a higher left ventricular end-diastolic pressure at smaller ventricular volumes. The increase in left ventricular pressure increases pulmonary venous pressure and causes pulmonary congestion.

Studies in preterm animal and human newborns have shown that despite these limitations, preterm newborns are able to increase left ventricular output and maintain their "effective" systemic blood flow, even with left-to-right PDA shunts equal to 50% of left ventricular output. With shunts greater than 50% of left ventricular output, "effective" systemic blood flow falls, despite a continued increase in left ventricular output.[237,240] The increase in left ventricular output associated with a PDA is accomplished not by an increase in heart rate, but by an increase in stroke volume.[237,240] The presence of a PDA does not affect myocardial contractility.[241] Instead, stroke volume increases primarily as a result of a simultaneous decrease in afterload resistance and increase in left ventricular preload. Several weeks of PDA exposure causes the left heart to remodel to a larger, more spherical shape with an increase in wall thickness. This returns to normal values following PDA closure.[241]

Despite the ability of the left ventricle to increase its output in the face of a left-to-right ductus shunt, blood flow distribution is significantly rearranged. This redistribution of systemic blood flow occurs even with small shunts.[237] Blood flow to the gastrointestinal tract and kidneys is decreased due to a combination of decreased perfusion pressure and localized vasoconstriction. Mesenteric blood flow is decreased in both fasting and fed states in the presence of a PDA as is mesenteric regional oxygen saturation, although the latter has not been universally observed.[242–244] Significant decreases in organ blood flow and oxygenation may occur long before there are any signs of left ventricular compromise and may contribute to the decreased glomerular filtration rate that has been observed with ductus patency.[240,245–248] Cerebral blood flow and oxygenation are also compromised in the presence of a moderate-to-large left-to-right PDA shunt due to the limited ability of the preterm newborn to autoregulate cerebral blood flow.[249,250] Small-for-gestational age infants are at even greater risk for having low cerebral oxygen saturations due to an increased incidence of impaired cerebrovascular autoregulation.[251,252] Recent evidence suggests that any negative impact of a PDA on neonatal brain growth may have more to do with its effects on cerebral oxygen saturation than on the magnitude of the shunt or the treatments used to close it.[253] In contrast with the effects of a PDA on mesenteric and renal oxygen saturations, cerebral oxygen saturations may be preserved in the presence of a moderate-to-large PDA if cerebral autoregulation remains intact.[254] Therefore, as long as continuous cerebral oxygen monitoring demonstrates that cerebral oxygenation is not compromised, it may be reasonable to delay treatments to close the PDA while awaiting spontaneous closure.[253]

The decreased ability of the preterm infant to maintain active pulmonary vasoconstriction may be responsible in part for early development of a "large" left-to-right PDA shunt.[255–257] Randomized, controlled trials have shown that the presence of a PDA increases the incidence of early hemorrhagic pulmonary edema/pulmonary hemorrhage.[258–261] Therapeutic maneuvers or prenatal conditions that lead to a rapid postnatal drop in pulmonary vascular resistance, like surfactant replacement or intrauterine growth retardation, can exacerbate the amount of left-to-right shunt and lead to pulmonary hemorrhage.[25,262,263]

In premature animals, a wide-open PDA increases the hydraulic pressures in the pulmonary vasculature on both the arterial and venous sides of the capillary bed. In addition, the increased pulmonary blood flow and immature precapillary arterial tone cause the

pulmonary vascular hydraulic pressure head to shift downstream toward the capillary fluid filtration sites increasing the rate of fluid transudation into the pulmonary interstitium.[264,265] Extremely premature infants (gestational age ≤28 weeks) have both increased alveolar capillary permeability and lower plasma oncotic pressures compared with more mature infants.[266] As a result, any increase in microvascular perfusion pressure increases the rate of fluid and protein transudation. If plasma proteins enter the alveolar space, surfactant function is inhibited and alveolar surface tension is increased.[267] The increased FiO_2 and mean airway pressures required to overcome these early changes in compliance may play a role in the development of chronic lung disease.[248,268,269] Depending on the gestational age and the species examined, changes in pulmonary mechanics may occur as early as 1 day after birth or not before several days of exposure to a PDA left-to-right shunt.[264,270]

Although preterm animals with a PDA have increased fluid and protein transudation into the lung interstitium, a simultaneous increase in lung lymph flow appears to eliminate the excess fluid and protein flux into the lung.[265] This compensatory increase in lung lymph acts as an "edema safety factor," inhibiting fluid accumulation in the lungs. As a result, during the first days after birth there is no net increase in water or protein accumulation in the lung and there is no change in pulmonary mechanics.[21,248,264,271,272] This delicate balance between the PDA-induced fluid filtration and lymphatic reabsorption is consistent with the observation, made in human infants, that closure of the ductus arteriosus, within the first 24 hours after birth, has no effect on the course of the newborn's hyaline membrane disease. However, after several days of mechanical ventilation, there is a decrease in pulmonary capillary surface area which increases both the pulmonary microvascular pressure and rate of hydraulic fluid filtration.[273] As a result, it is not uncommon for infants with a persistent PDA to develop pulmonary edema and alterations in pulmonary mechanics at 6 to 10 days after birth. In these infants, improvement in lung compliance occurs following closure of the PDA.[248,274–278]

Preterm baboon newborns, delivered at 67% term gestation and mechanically ventilated for 2 weeks, have been used to examine the effects of a PDA on pulmonary mechanics.[270] Baboons were either treated with a cyclooxygenase inhibitor to close the ductus or allowed to have a moderate-size PDA persist for the 2-week experiment. Exposure to a persistent PDA did not alter surfactant secretion, pulmonary epithelial protein permeability, or presence of surfactant inhibitory proteins, nor did it alter the expression of pro-inflammatory or tissue remodeling genes. In contrast with full-term baboons, which mobilize lung fluid rapidly after birth, preterm newborns (with both an open and closed ductus) mobilize lung fluid much more slowly.[270] A persistent PDA led to a small but significant increase in lung water at 2 weeks after delivery. The indomethacin or ibuprofen used to constrict the PDA increased expression of alveolar epithelial sodium channels, which facilitate fluid removal from the alveolar compartment. This finding may account for the decreased incidence of pulmonary edema and hemorrhage observed in infants treated with prophylactic indomethacin or ibuprofen.[258–261]

Pharmacologic closure of the PDA was associated with improved lung development in the preterm baboons. In contrast to the animals with an open ductus, where an arrest in alveolar development (the hallmark of the new "BPD") was noticeable by 2 weeks after birth, pharmacological closure of the PDA led to improved alveolarization.[270] Whether the improvement in alveolarization is due to the closure of the ductus or the pharmacologic agents (indomethacin/ibuprofen) used to close it is unknown at this time. Neutrophil counts are elevated in the tracheal fluid of human infants with a PDA and indomethacin can decrease tracheal neutrophil accumulation.[279] However, the decreased neutrophil accumulation occurs in both those who close and in those who fail to close their PDA after indomethacin.[280] The same improvements in pulmonary mechanics and alveolar surface area have not been observed after surgical ligation of the PDA. Early surgical ligation increases the expression of genes involved with pulmonary inflammation and decreases the expression of pulmonary epithelial sodium channels (that are critical for alveolar water clearance).[281] These changes may contribute to the lack of improvement in pulmonary mechanics after PDA ligation. In addition, early surgical ligation impedes lung growth.[270,282,283] These findings raise the possibility that early ductus ligation, while eliminating the detrimental effects of a PDA on lung development, may create its own set of problems that counteract many of the benefits derived from ductus closure.[284,285]

Not all of the changes associated with a PDA are necessarily detrimental to the immature infant with respiratory distress syndrome. Persistence of the left-to-right shunt maintains an elevated Pao_2 in the presence of atelectasis. This phenomenon is due to recirculation of oxygenated arterial blood through lungs that are not fully expanded.[237,286] Decreases in systemic arterial O_2 content have been observed after PDA closure, despite the absence of any alterations in pulmonary mechanics.

Treatment

Treatment Options for Closing a Patent Ductus Arteriosus

Open surgical ligation produces definitive ductus arteriosus closure, however, it is associated with several important morbidities: thoracotomy, exposure to surgical anesthesia, pneumothorax, chylothorax, scoliosis, and infection.[287] In addition, following PDA ligation, between 20% and 60% of ELBW infants will develop unilateral vocal cord paralysis (which increases the requirements for tube feedings and respiratory support and persists well beyond the neonatal period).[288–292] Post-ligation cardiac syndrome and profound hypotension during the postoperative period is another complication that affects 30% to 50% of infants with birthweights ≤1000 g.[293] The incidence of profound postoperative hypotension is inversely related to the infant's corrected age and appears to be due to impaired adrenal stimulation, low cortisol release, and decreased vascular tone.[294,295] Early surgical ligation also contributes to the development of bronchopulmonary dysplasia.[284,285] Neurodevelopmental abnormalities are also more frequent following early PDA ligations.[296] Therefore, avoiding or delaying ligation appears to be beneficial since many of the morbidities associated with ligation (post-ligation hypotension, vocal cord paralysis, bronchopulmonary dysplasia) and abnormal neurocognitive development appear to be reduced when ligation is delayed.[284,288,296–299] In a recent study of preterm infants undergoing PDA ligation, short-term respiratory improvement was only observed in infants with the worst lung disease prior to ligation, that is, those requiring high-frequency ventilation. Those managed with conventional ventilation prior to ligation had no respiratory improvement at 7 days after ligation and required higher respiratory support in the early postoperative period.[300]

In full-term infants, transcatheter PDA device closure (TCPC) has replaced surgical ligation as the treatment of choice. With the approval of the Piccolo device, the use of TCPC in the premature population (even for infants with birthweights <1000 g) has become a feasible alternative to surgical ligation.[301–304] TCPC may decrease overall exposure to mechanical ventilation and the risks of post-ligation cardiac syndrome, however, no RCTs have compared TCPC outcomes with approaches that rely on pharmacologic treatment or conservative management/nontreatment of the PDA.[305,306] Although infrequent, major adverse events (5% to 10%) and death (2%) related to the procedure do occur, and, as expected, their incidence increases as the size of the infant and the center's experience with the procedure decreases.[304,307,308]

Inhibition of PG synthesis with nonselective inhibitors of COX-1 and COX-2 (e.g., indomethacin and ibuprofen) appears to be an effective alternative to surgical ligation.[257] However, both drugs have been associated with several potential adverse effects in the newborn. Indomethacin produces significant reductions in renal, mesenteric, and cerebral blood flow.[309–318] Indomethacin also has noxious effects on gut mucosa and reduces cerebral oxygenation.[318–320] Alterations in creatinine clearance and oliguria (that are minimally responsive to dopamine or furosemide therapy) are common problems after indomethacin. Furosemide, when used in combination with indomethacin, actually increases the incidence of acute renal failure.[321–324] Renal function returns to normal after the initial doses of indomethacin.[325] There are some reports that COX inhibitors may affect glomerular development in neonatal animals; however, these finding have not been consistently observed.[326–328] Indomethacin's action on these organ systems may not be due entirely to its inhibition of PG synthesis.[329–331] Indomethacin also has effects on lipoxygenase activity, and histamine and endothelin release, although the relevance of these effects to any neonatal morbidities is still unknown.[332–334]

Although indomethacin produces significant physiologic alterations, and has been associated with several morbidities, none of the controlled, randomized trials that have examined the relationship between indomethacin and neonatal morbidity have found an increase in the incidence of necrotizing enterocolitis, gastrointestinal perforation, retinopathy of prematurity (ROP), chronic lung disease, or cerebral white matter injury following indomethacin treatment.[335] Neither indomethacin by itself, nor in addition to enteral feeding, has been shown to increase the incidence of gastrointestinal perforations. In fact, infants receiving small "trophic" enteral feedings during indomethacin treatment attain full enteral feedings at a faster rate than those kept *nil per os* during treatment.[336] On the other hand, the combination of indomethacin *and* postnatal steroids does increase the incidence of gastrointestinal perforations and necrotizing enterocolitis.[135,136,337]

Indomethacin's cerebral vasoconstrictive effects are frequently cited as a concern for neonatologists; however, a Cochrane systematic review found that indomethacin prophylaxis is more likely to decrease rather than increase the incidence of periventricular leukomalacia.[317,335,338] There is no evidence that prophylactic indomethacin has any adverse effect on neurodevelopmental outcome at 18 months; in fact, there is evidence that it may have long-term benefits at 4.5 and 8 years especially in preterm male infants.[339–342]

While there may be general consensus on the efficacy of indomethacin for treatment of a PDA, questions about proper dosage, treatment duration, and optimal timing of treatment remain quite controversial. Indomethacin's plasma clearance depends on postnatal age.[343–347] Therefore, a dosage regime recommended for infants at the end of the first week (when the half-life of the drug is 21 hours) may lead to elevated and prolonged plasma concentrations when used in infants on day 1 (when the half-life is 71 hours).[346,347] Conversely, a single loading dose of indomethacin (0.2 mg/kg), without subsequent maintenance doses, can be effective in closing a PDA when administered within the first 24 hours following delivery.[348,349]

Many variations in dosage regimens have been evaluated.[350,351] A prolonged low-dose course of indomethacin (0.1 mg/kg every 24 hours for 5 to 7 days) may increase the rate of permanent closure especially in infants who still have residual ductus flow after completing the standard short course (2 to 3 doses over 24 hours), and may decrease indomethacin's impairment of renal function.[352–355] This dosage regimen still needs further evaluation since a higher NEC rate was observed in the infants receiving prolonged maintenance indomethacin.[350] Although some have suggested that the dose of indomethacin be increased when conventional dosing fails to produce ductus closure, a randomized controlled trial examining this approach found that the rate of ductus closure was not substantially improved despite a nearly 3-fold increase in serum indomethacin concentrations.[356–358] More worrisome was the fact that the higher indomethacin concentrations produced significant increases in the incidence of moderate/severe ROP and late renal dysfunction.[359]

The postnatal age at which indomethacin is administered plays an important role in determining its effectiveness. With advancing postnatal age, dilator PGs play less of a role in maintaining ductus patency (see above).[30,32] Even when indomethacin concentrations have been maintained in the "desired" range, the drug's ability to produce complete ductus closure declines during the first days after birth.[121,309,343,345,360] Despite its decreased effectiveness, it may still be advisable to attempt PDA closure with indomethacin before proceeding to surgical ligation or TCPC even in cases of advanced postnatal age.[361]

Recurrence of a symptomatic PDA can occur after initial successful treatment. The rate of reopening, which is greatest among the most immature infants, appears to be related to the timing and completeness of ductus closure after the treatment course.[121,248] Permanent anatomic closure requires tight constriction of the ductus lumen and the development of ductus wall hypoxia (see above). Among infants delivered before 28 weeks' gestation, 80% of those that closed their ductus clinically following indomethacin treatment, will reopen their ductus and develop clinical symptoms if there is any evidence of luminal patency on the Doppler examination performed at the end of indomethacin treatment.[121] The thinner the ductus wall, the less likely it is that profound hypoxia and anatomic remodeling will occur even with tight constriction. Only 9% of infants delivered between 26 and 27 weeks' gestation will reopen their PDA if the ductus is found to be closed by Doppler/echocardiography; in contrast, 20% of those delivered before 26 weeks are likely to reopen their PDA despite evidence of ductus closure on a prior echocardiogram.[362] Early treatment produces a tighter degree of ductus constriction and as a result produces higher rates of ductus wall hypoxia and permanent closure.[121] Recurrence of a PDA can also occur after bacterial infections when circulating PGE_2 concentrations reach the pharmacologic range.[26] Bacterial endotoxin increases NO production and tumor necrosis factor α (but not PGE_2) in the ductus wall which may explain why indomethacin is less effective in closing the PDA after infection.[363]

Ibuprofen, another nonselective cyclooxygenase inhibitor, appears to be as effective as indomethacin in producing PDA closure in very low-birth-weight infants (at least in infants

with a mean gestational age of 28 weeks).[364,365] Compared with indomethacin, it has a reduced rate of NEC and renal insufficiency.[366,367] Oro-gastric administration of ibuprofen appears to be as effective as IV administration.[366] In contrast with indomethacin, ibuprofen does not appear to affect mesenteric blood flow and has less of an effect on renal perfusion, oliguria, and cerebral blood flow.[310,318,329,331,368,369] Unfortunately, most of the studies that have compared ibuprofen with indomethacin have not included extremely low-birth-weight infants (≤25 weeks' gestation), which is the group that is most resistant to pharmacologic ductus closure and where the optimal age-appropriate dosing schedule for ibuprofen is still under consideration.[370,371] Some of the higher ibuprofen dose options are concerning because of ibuprofen's effects on total and free serum bilirubin concentrations.[372,373] Ibuprofen does not have the same effects as indomethacin on cerebral autoregulation. As a result, prophylactic ibuprofen does not appear to have the same intracranial hemorrhage sparing effect that is seen with indomethacin (see below). In contrast to indomethacin, ibuprofen alters pathways regulating intestinal microbial colonization, mucus production, and epithelial defense.[374]

Paracetamol (acetaminophen) also appears to be an effective agent for inducing PDA closure. Acetaminophen's vasoconstrictive effect appears to be mediated through inhibition of the peroxidase moiety of PGH2 synthetase.[375,376] Numerous randomized clinical trials (RCTs) and subsequent meta-analyses suggest that acetaminophen is as effective as indomethacin and ibuprofen.[377–390] However, only one of the RCTs exclusively enrolled infants that were at high risk for having a persistent PDA (i.e., those born before 28 weeks' gestation). In one multi-center trial enrolling infants less than 28 weeks' gestation who still had a moderate-to-large PDA at the end of the first week, indomethacin had the greatest rate of constriction (when compared with the rate of spontaneous PDA constriction [20%] during the 7 to 10 days after enrollment: relative risk [95% CI] = 3.21 [2.05 to 5.01]), followed by ibuprofen = 2.03 (1.05 to 3.91), and acetaminophen = 1.33 (0.55 to 3.24).[391] Recent concern has been raised about acetaminophen's safety profile based on reports of neurocognitive impairment after prenatal exposure and of hypotension following intravenous administration.[392,393]

Indomethacin and Intracranial Hemorrhage

Previous RCTs have shown that indomethacin can decrease the incidence of intracranial hemorrhage (ICH) in preterm infants and experimental animals.[339,394–396] This has not been the case with ibuprofen or paracetamol. The effects of indomethacin on ICH do not appear to be due to its effects on ductus patency.[394,395] Indomethacin decreases cerebral blood flow, decreases reactive postasphyxia cerebral hyperemia, and accelerates maturation of the germinal matrix microvasculature.[397–401] Since most intracranial hemorrhages occur within the first 3 days after birth, the beneficial effects of indomethacin have only been seen when it has been given prophylactically, during the first 18 hours after birth.[402] The RCTs that demonstrate a beneficial effect of prophylactic indomethacin on ICH were performed prior to the year 2000. During the last 21 years the overall rate of ICH in the neonatal population has been steadily decreasing and more recent studies have found a more limited effect of prophylactic indomethacin on the rate of ICH.[403–406] In addition, infants who are coagulopathic or have platelet counts below 100×10^9/L may be at increased risk for intracerebral bleeding when COX inhibitors are administered.[407]

Patent Ductus Arteriosus and Neonatal Morbidity: To Close or Not to Close the Patent Ductus Arteriosus

Although a prolonged, persistent left-to-right shunt through a PDA shortens the life span of animals and humans, there is controversy about whether or not the PDA needs to be closed during the neonatal period.[55,408–415] Since preterm infants have a high rate of spontaneous PDA closure during the first 2 years, routine early treatment runs the risk of exposing infants to drugs or procedures they might not need. At this time it is not clear which is preferable: (1) to close the PDA (either pharmacologically or surgically) in the preterm neonate, or (2) to deal with the PDA-induced pulmonary edema and hypotension more "conservatively" while awaiting spontaneous PDA closure. A "conservative," expectant management approach might include: dopamine, which decreases PDA shunting by increasing pulmonary vascular resistance; fluid restriction, which minimizes PDA symptoms but fails to reduce the PDA shunt and is associated with decreased systemic blood flow; and increased serum creatinine, diuretics, and/or increased end-expiratory pressure, which improves pulmonary edema and compliance but has only a small effect on the left-to-right PDA shunt.[27,416–421]

RCTs, which initiated "prophylactic" pharmacologic PDA treatment within 12 hours of birth in either all infants or just those with echocardiographic evidence of a large PDA reported a decrease in a number of short-term morbidities ([1] severe early pulmonary hemorrhage, [2] severe grades of IVH [but only when indomethacin was the treatment used], [3] presence of a symptomatic PDA, [4] dopamine-dependent hypotension, [5] need for higher levels of ventilator support, and [6] need for surgical PDA ligation) compared with postponing treatment until later in the first postnatal week.[258,259,261,335,339,422,423] However, there were no differences in the incidence of long-term morbidities like BPD, NEC, or neurodevelopmental impairment. In addition, 40% to 50% of infants in the prophylactic treatment group were treated unnecessarily since their PDA would have closed spontaneously by 7 days.[14]

Little information exists about the consequences of exposure to a persistent moderate-large PDA shunt for greater than 1 week. Two small RCTs, performed more than 40 years ago, found that a persistent untreated symptomatic PDA prolonged the need for respiratory support and increased pulmonary morbidity.[269,424] Whether these findings still are applicable in the setting of modern neonatal respiratory care (with "gentle ventilation," acceptance of elevated arterial PCO_2, and less tracheal intubation) is a question starting to be addressed by RCTs. In contrast with the earlier RCTs described above, more recent trials have focused on the most immature infants (<28 to 29 weeks' gestation) with moderate-large PDA shunts which are likely to persist for several weeks. The PDA-TOLERATE RCT and the "Nonintervention versus Oral Ibuprofen" RCT enrolled infants with moderate-large PDAs that persisted beyond the first week and compared early routine PDA treatment (starting at 8 days) with a conservative, expectant management approach.[425,426] Neither trial observed a difference in the incidence of BPD, NEC, or mortality. However, both trials suffered from having a low rate of PDA closure in the early intervention arm of the study resulting in prolonged PDA exposure (>14 days) in both groups. Similar problems (either unexpectedly low rates of drug-induced PDA closure or higher rates of spontaneous closure) existed in two additional pilot RCTs that enrolled infants within 72 hours of birth.[427,428] As a result, little difference existed

in the duration of PDA exposure between the study treatment arms for more than 50% of the study population. More effective PDA closure strategies will need to be devised for future RCTs if we are ever to learn whether early routine closure of the PDA will or will not decrease the incidence of BPD or late morbidity.

Another problem for future RCTs is knowing which infants to enroll, that is, determining who actually is at risk for increased morbidity when a PDA is present. An association between PDA and BPD or late morbidity only exists when *both* moderate-to-large shunts persist beyond 7 to 14 days *and* mechanical ventilation/tracheal intubation is required for ≥10 days.[6–8,10–12,429] Infants requiring shorter intubation durations have the same incidence of BPD whether or not a PDA persists for several weeks after birth.[10–12] This suggests that routine early PDA treatment may have no benefit, as far as the outcome BPD is concerned, for infants requiring intubation for less than 10 days. What about infants who are intubated for ≥10 days? Might routine PDA treatment be justified in this group of infants? Unfortunately, none of the reported RCTs have focused on this group of infants. All RCT populations have been diluted with infants for whom closing the PDA had little or no consequence. Early screening tools need to be developed (like the one proposed by El-Khuffash et al.[9]) to discriminate between infants who are the most appropriate candidates for enrollment in future trials and those who should be excluded.

In the absence of current RCTs several cohort-controlled observational studies have examined whether exposure to moderate-to-large PDA shunts for greater than 8 days increases morbidity.[405,430] These studies found no increase in the incidence of NEC, ROP, or death but did find an increased incidence of BPD and BPD/death among infants that were exposed to a moderate-to-large PDA for greater than 2 weeks. In addition, the longer infants were exposed to a PDA before PDA treatment was initiated, the less likely PDA treatment was associated with a reduction in BPD and BPD/death.[405,430] These findings are consistent with several recent observations: (1) during the past decade, as the use of PDA treatment has decreased, there has been a significant increase in adjusted local mortality among infants weighing less than 750 g; (2) neonatal centers that do not treat the PDA or consider PDA treatment only after the first postnatal week report significantly higher rates of BPD and BPD/death than centers that regularly screen and treat the PDA as part of their admission care.[403,431–437]

Whether exposure to a moderate-to-large PDA shunt for more than the first 7 to 14 days actually contributes to the development of BPD and BPD/death is a question that awaits testing in appropriately designed RCTs. At this time what we do know is that neither a delay of 2-to-5 days before starting PDA treatment, nor treatment that starts only after infants have been exposed to a moderate-to-large shunt for more than 2 weeks appear to affect the incidence of BPD and BPD/death. Further investigations will be needed to determine which infants are most likely to benefit from PDA treatment and which infants might best be left untreated.

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Perinatal Arrhythmias

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KEY POINTS

- The term *supraventricular tachycardia* (SVT) encompasses several different arrhythmias that may have different diagnostic and therapeutic requirements.
- Tachycardia mechanisms may be caused by conduction reentry, enhanced automaticity, or triggered automaticity.
- Isolated atrial and ventricular ectopy is common in the fetus and neonate and usually does not require any therapy.
- Congenital forms of complete heart block may result in slow heart rates but may not require pacemaker implantation.
- Fetal arrhythmias are rare but, when present, can result in significant hemodynamic compromise.
- Management of fetal arrhythmias is complex and involves evaluation of multiple factors, including (1) severity of rhythm abnormality, (2) fetal comorbidities and signs of heart failure, (3) maternal comorbidities, and (4) gestational age at diagnosis.

The cardiac conduction system in the normal human heart enables a synchronized and orderly activation of the myocardium and chambers to provide optimal cardiac output. This sequence begins even in the earliest stages of cardiac development. However, disorders of cardiac rhythm can occur at any stage of life, from the fetus to the infant to the adult. These can range from transient and mild disturbances in rhythm to recurrent or persistent arrhythmias that can have significant hemodynamic consequences. Some abnormalities, such as isolated atrial or ventricular ectopy, can be more bothersome to the care provider than to the patient. Other disturbances, such as incessant tachyarrhythmias, can progress to heart failure and hemodynamic collapse. While the incidence of cardiac arrhythmias is increased in the setting of congenital heart disease (CHD), many of these abnormalities are commonly seen in the setting of an otherwise structurally normal heart. Knowledge of the spectrum of cardiac rhythm disturbances is important for prompt recognition, referral, and management. In this chapter, the etiology and common mechanisms of various arrhythmias, as well as differential diagnosis and treatment strategies, will be discussed in separate sections. The unique issues surrounding the diagnosis and management of arrhythmias in the fetus will also be addressed.

Conduction System of the Human Heart

To properly understand the mechanisms of arrhythmogenesis, it is important to recognize the core elements of the normal cardiac conduction system. The embryogenesis of the heart and the conduction system are beyond the scope of this chapter. However, the components of the cardiac conduction system are relatively set by the second trimester of gestation and provide the substrate for many of the observed arrhythmias.[1]

Sinus Node

The sinus node resides in the area between the vena cava and the right atrium and is predominantly located near the superior vena cava to the right-atrial junction.[2] However, there is no anatomic feature that is visible on gross inspection. Functional electrophysiology testing studies and animal studies have demonstrated that the nodal tissue can span the lateral wall of the right atrium, extending from the superior vena cava to the inferior vena cava.

The sinoatrial nodal tissue is histologically distinct from the atrial myocardium and has no contractile components. Electrical impulses are generated by automatic action potential behavior. Activation of the adjacent atrial myocardium by the sinus nodal tissue results in a wave of depolarization that propagates by cell-to-cell activation in a superior-to-inferior and a right-toward-left (in the structurally normal heart) fashion.

Atrioventricular Node

The atrioventricular (AV) node is a more organized area of conduction in the right atrium near the crux of the heart. More well-defined than the sinus node, the compact AV node is located in the posterior portion of the interatrial septum just anterior to the tricuspid valve and (in most cases) provides a singular conduction channel into the bundle of His and the distal conduction system.[1]

The majority of the atrial propagation wave depolarizes the mass of the atria. However, impulses that reach the area of the AV node enter the transition zone of conduction "input" into the AV node. Overall conduction velocity is slowed through the compact AV node but then exits the node into the specialized conduction fibers of the His–Purkinje system.

His–Purkinje System

In the normal heart, the atrial and ventricular myocardium are electrically isolated from each other by the AV rings (tricuspid and mitral annuli). The penetrating bundle of His is usually the only conductive tissue that traverses the AV rings into the ventricles. The bundle of His is insulated from the ventricular myocardium until it gives off branches. The first branch from the His bundle enables activation of the septum. Next, the bundle bifurcates into the right- and left-bundle branches. The left bundle divides into anterior and posterior fascicles. At the terminus of each of these

bundle branches, the specialized conduction tracts fan into an intricate network of short Purkinje fibers that insert into numerous sites on the ventricular myocardium. Electrical impulses that are conducted through the His–Purkinje system result in activation of the ventricular myocardium in a highly organized and synchronized fashion, translating into a mechanically efficient contraction of both left and right ventricles almost simultaneously.

Neonatal Arrhythmias

Abnormalities in Cardiac Conduction

When the normal sequence of cardiac activation is disturbed, the result can be an irregularity in the cardiac rhythm. However, in many cases, these disturbances will only be detected on electrocardiography or rhythm monitoring and have no manifestations on physical examination or otherwise. AV block can occur at any level of the conduction pathway if anything impedes the propagation of the impulse through that conduction segment. This can be functional (because of negative vagal stimulation) or anatomic (as can be seen following cardiac surgery).

First-Degree Atrioventricular Block

Any conduction delay that prolongs the PR interval beyond the normal range for age is considered a first-degree AV block. By definition, impulses must still be conducted such that a 1:1 AV relationship persists. In the neonate, the normal PR interval is generally between 80 and 120 ms, up to 140 ms in the first few months of life, and up to 160 ms in the first 6 months of life.[3] In general, first-degree AV block is benign and does not require any special treatment. It can be a normal variant or may be the result of influences that prolong the overall conduction time through the AV node and His–Purkinje system. Increased vagal tone is a common cause of PR prolongation. In the newborn, this can be a manifestation of vagal stimulation caused by a nasogastric or orogastric tube stimulating the oropharynx. More pathologic causes of PR prolongation can include neonatal lupus syndrome or myocarditis. Although there is evidence that significant PR prolongation has a negative impact on cardiac output in the adult heart, there is no indication that this is true in the neonate with a structurally and functionally normal heart. Even when the PR interval is extremely prolonged, there is usually no hemodynamic impact, and the cardiac examination remains essentially unchanged.

Second-Degree Atrioventricular Block

Second-degree AV block is ascribed when there is incomplete conduction from the atrium to the ventricle; that is to say, not every P wave is conducted to a QRS complex. This usually manifests as a skipped beat on physical examination or on cardiac monitoring. Mobitz type I conduction block, also known as the *Wenckebach pattern*, describes a pattern wherein the AV conduction becomes progressively prolonged with each successive beat. The PR interval becomes gradually longer, and after a number of beats (usually two or three, although this can certainly be longer), there is failure to conduct for a single beat. The subsequent P wave is then conducted with a normalized PR interval, and the cycle begins anew. Mobitz type II conduction block is present when there is a cyclical lack of conduction; however, the progressive PR prolongation seen in the Wenckebach pattern is lacking. Mobitz type I conduction can be seen in conditions of increased vagal tone (as described previously), whereas Mobitz type II is practically unheard of in the neonate in the absence of any other heart disease.

Third-Degree Atrioventricular Block

Third-degree, or complete AV block, occurs when there is a complete lack of conduction between the atria and the ventricles. In most situations of complete AV block, there is an escape depolarization mechanism, either junctional or ventricular in origin, which ensures that cardiac output is maintained. Complete AV block can be congenital or acquired. Congenital complete AV block is a particularly challenging entity and is described in a subsequent section. The most common causes of acquired complete heart block are infections or neonatal myocarditis. In addition, acquired complete heart block can occur as a complication of neonatal cardiac surgery in 1% to 3% of cases that involve surgical intervention near the interventricular septum.[4]

Ventricular Preexcitation

Ventricular preexcitation occurs when the ventricular myocardium is activated abnormally, usually by an accessory bypass tract. The ventricular depolarization pattern is thus a combination (fusion) of early activation of a portion of the ventricles through an accessory pathway and the remainder of activation occurring via the normal His–Purkinje system. In general, these bypass tracts bridge the AV groove that typically separates the atria from the ventricles and result in an anomalous electrical connection to the ventricles.

There are three electrocardiographic features that define this "Wolff–Parkinson–White" pattern: (1) short PR interval, (2) a slurred "delta wave," and (3) widened QRS duration. How evident this pattern is on electrocardiogram (ECG) can vary depending on the location of the accessory pathway (and thus how early the preexcited portion is activated) and how quickly the ventricles are also activated by the His–Purkinje system. In the newborn heart, the rapid transit time through the AV node and His–Purkinje system can result in a very minimal amount of preexcitation being evident, and appreciation of the presence of the Wolff–Parkinson–White pattern can be delayed, sometimes for years.[5] Usually, this diagnosis is made only when the newborn patient experiences a tachyarrhythmia (as discussed later).

Abnormalities in Cardiac Rhythm

Alterations in the normal cardiac rhythm can occur by one of several mechanisms and sometimes in combination. These include: (1) enhanced automaticity, (2) reentry mechanisms, and (3) triggered automaticity.

Certain areas of the heart have a tendency to exhibit spontaneous automaticity, such as the sinus node and the AV junction. Sometimes an abnormal cluster of cells can have a particular tendency toward this behavior. Gradual depolarization of the tissue during phase 0 of the action potential (electrical diastole) eventually crosses the action potential threshold, resulting in the depolarization–repolarization sequence, after which it returns to electrical diastole until the gradual depolarization occurs again.[1] After the action potential begins in the abnormal ectopic focus, the adjacent myocardium is also depolarized, which then propagates a wavefront outwards from that locus. Automatic rhythms tend to exhibit "warm-up" and "cool-down" behavior, gradually (although sometimes briskly) accelerating and then decelerating back to normal. Even when persistent, there tends to be beat-to-beat variability, and the rate can also be influenced by external influences such as autonomic tone, metabolic states, or hormonal influences. In addition, automatic rhythms can be "overdrive" suppressed when driven by a faster rhythm from another source.

Triggered automaticity occurs when excitable tissue spontaneously depolarizes to the activation threshold, beginning an action potential in that cell or cells that are then perpetuated to the adjacent myocardium. This can occur in fairly normal tissue but can be particularly enhanced by conditions of acidosis, mechanical stimulation, myocardial injury, or inflammation. Increased automaticity is often observed in the presence of certain drugs, such as inotropic medications like dopamine or epinephrine or stimulant medications like caffeine.

Reentry rhythms are some of the more instantly recognizable tachyarrhythmias in neonates and young infants. In contrast to the gradual nature of automatic rhythms, reentry has a very abrupt onset and termination. During the rhythm, the rate tends to be fairly stereotyped and consistent. Reentrant rhythms require several prerequisites to perpetuate. First, there must be an arrhythmia "circuit" present with both antegrade conducting and retrograde conducting limbs. Next, there must be differential conduction between the limbs of the arrhythmia circuit, whereupon one limb must exhibit slowing or unidirectional block. Finally, all elements of the arrhythmia circuit must be able to support a repetitive rhythm at a fixed rate.

Ectopic Beats

Premature Atrial Complexes

Isolated atrial ectopy is commonly seen in children and, in particular, during the newborn period. There are no ethnic predilections toward ectopy. Premature atrial complexes can be observed during fetal monitoring and have been reported to occur in up to 25% to 50% of normal newborns;[6] however, in the vast majority of cases, this resolves within the first few months of life. Premature atrial complexes are caused by an early triggered depolarization of the atria from an ectopic focus that is separate from the sinus node. On surface ECG this manifests as a P wave that is earlier than would be expected from the preceding rhythm, and the ectopic P wave has a very distinct axis and morphology from the normal sinus P wave. In this manner, one can differentiate the premature atrial complex as originating from a location separate from the sinus node.

Most atrial ectopy in the neonate is conducted normally, meaning that the premature atrial complex is followed by a normal-appearing QRS complex. The PR interval may be measured as different from normal, which is more a reflection of the atrial depolarization beginning in an abnormal location (and thus either closer or further than the sinus node) rather than indicating any defect in AV conduction. When a premature atrial complex is closely coupled to the preceding beat, the conducted QRS complex may have an abnormal appearance. This "aberrant conduction" may just be slightly wider than normal, or it may have the appearance of a complete bundle branch block. This is caused by the early impulse failing to conduct, as the refractory period for that segment has been exceeded. Aberrantly conducted premature atrial complexes are often mistaken for premature complexes because of their wider appearance but can be distinguished by the presence of a preceding P wave. Blocked premature atrial complexes can occur if the ectopic beat occurs early enough after the preceding beat. While this effect is more pronounced where this is impaired AV conduction, it is most commonly observed in the setting of normal nodal conduction and results from the early atrial impulse failing to conduct because of the refractory period of either the AV node or the His–Purkinje system. Blocked premature atrial complexes are identified by the presence of the ectopic P wave (usually with the T wave of the preceding beat) that has no QRS complex following. Often, there will be a sinus pause before the next normally conducted sinus return beat. In most instances, isolated premature atrial complexes in the newborn do not require any treatment.

Premature Ventricular Complexes

Isolated ventricular ectopy is also commonly observed in the normal neonate, although to a lesser extent than atrial ectopy.[6] Ventricular ectopic beats are usually distinguished by a widened QRS complex with a T-wave axis that is different when compared with the normal QRS–T complex. Importantly, there is no P wave that precedes the premature QRS complex. In isolation, ventricular ectopy has no pathologic implications for infants who have no other signs or symptoms to suggest any cardiac pathology.

Tachyarrhythmias

While a number of tachyarrhythmias can be observed during childhood, only a subset of distinct arrhythmias is observed with any frequency in the fetus and neonate (Table 49.1). The most common of these are orthodromic reciprocating tachycardia (ORT), atrial ectopic tachycardia, and atrial flutter.[7]

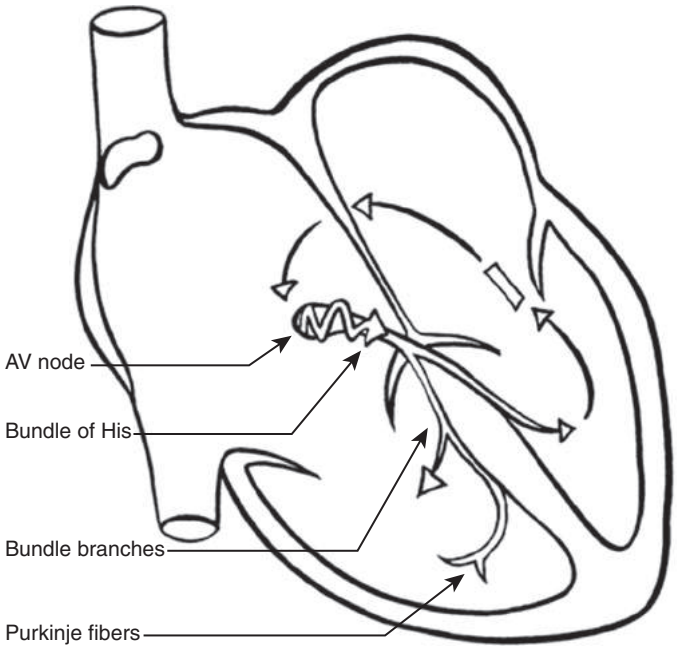
Orthodromic Reciprocating Tachycardia

ORT is the most common form of supraventricular tachycardia (SVT) in the fetus and the neonate.[8] It is the arrhythmia mechanism most commonly associated with SVT, so the terms are often used interchangeably. The tachycardia circuit for this reentrant arrhythmia utilizes the normal conduction system as the antegrade limb, and an accessory pathway provides the retrograde limb for the tachycardia (Fig. 49.1). When conditions are appropriate, impulses conducted through the His–Purkinje system to the ventricles are then carried in a retrograde fashion over an accessory pathway to the atria, which then reaches the AV node to start the cycle again. If not in their refractory periods, the atria, AV node and His–Purkinje system, ventricles, and accessory pathway all perpetuate a rapid repetitive SVT rhythm. In the fetal and neonatal heart, this form of SVT will manifest with very fixed rates that typically range between 240 and 300 beats per minute (bpm).

TABLE 49.1 Differentiating Neonatal Tachyarrhythmias

VA Relationship	Differential Diagnosis
A > V	Atrial ectopic tachycardia
	Atrial flutter
Short VA (VA < AV)	ORT (SVT)
	JET (VA-associated)
Long VA (VA ≥ AV)	Sinus tachycardia
	Atrial ectopic tachycardia
	PJRT
V > A	Ventricular tachycardia
	JET (VA-dissociated)

A, Atrial activity; *AV*, atrioventricular; *JET*, junctional ectopic tachycardia; *ORT*, orthodromic reciprocating tachycardia; *PJRT*, permanent form of junctional reciprocating tachycardia; *SVT*, supraventricular tachycardia; *V*, ventricular activity; *VA*, ventriculoatrial.



• **Fig. 49.1** Mechanism for Orthodromic Reciprocating Tachycardia. Antegrade conduction through His–Purkinje system depolarizes ventricles. Retrograde conduction from ventricles, over an accessory pathway, to atria.

So-called *retrograde* P waves may be visible on the ST segment or the T wave as evidence of the conduction time between the activation of the ventricles, passage through the accessory pathway, and subsequent reactivation of the atria. The measured RP interval can be used to aid in the differential diagnosis of the various forms of SVT, which can consequently be used to help guide specific management and prognosis.

When an accessory pathway only conducts in a retrograde direction, it is called a *concealed* accessory pathway; that is to say, there is no evidence of pathway conduction during normal sinus rhythm conditions, and the pathway only conducts from ventricle to atrium during reciprocating tachycardia. A manifest accessory pathway is one whose presence is known during normal sinus rhythm. These are the pathways that cause the ventricular preexcitation of Wolff–Parkinson–White syndrome (discussed previously). Manifest pathways are usually conducted bidirectionally; antegrade conduction results in preexcitation on resting ECG, and retrograde conduction is utilized during reciprocating tachycardia, resulting in a narrow QRS complex without preexcitation (Fig. 49.2).

For the neonate on continuous heart rate monitoring in the intensive care unit, such episodes of SVT are usually quite apparent by the abrupt onset and spontaneous termination of a rapid heart rate. In the absence of any monitoring, the development of SVT may not be as obvious. In contrast to older children who are able to report complaints of palpitations, neonates and young infants rarely give any indication of the tachyarrhythmia occurring within. Sometimes, pallor, diaphoresis, or a change in respiratory pattern may be evident to the outside observer. However, despite the rapid heart rates, SVT is generally well tolerated. Most episodes of ORT are self-limited, persisting only for seconds or minutes at a time; sometimes, episodes can last 30 to 60 minutes or more before terminating spontaneously. In rare instances, the SVT can be incessant, lasting for hours or sometimes a day or more. In these cases, delay in recognition and diagnosis can lead to a tachycardia-induced dilated cardiomyopathy and can possibly result in eventual cardiovascular collapse.

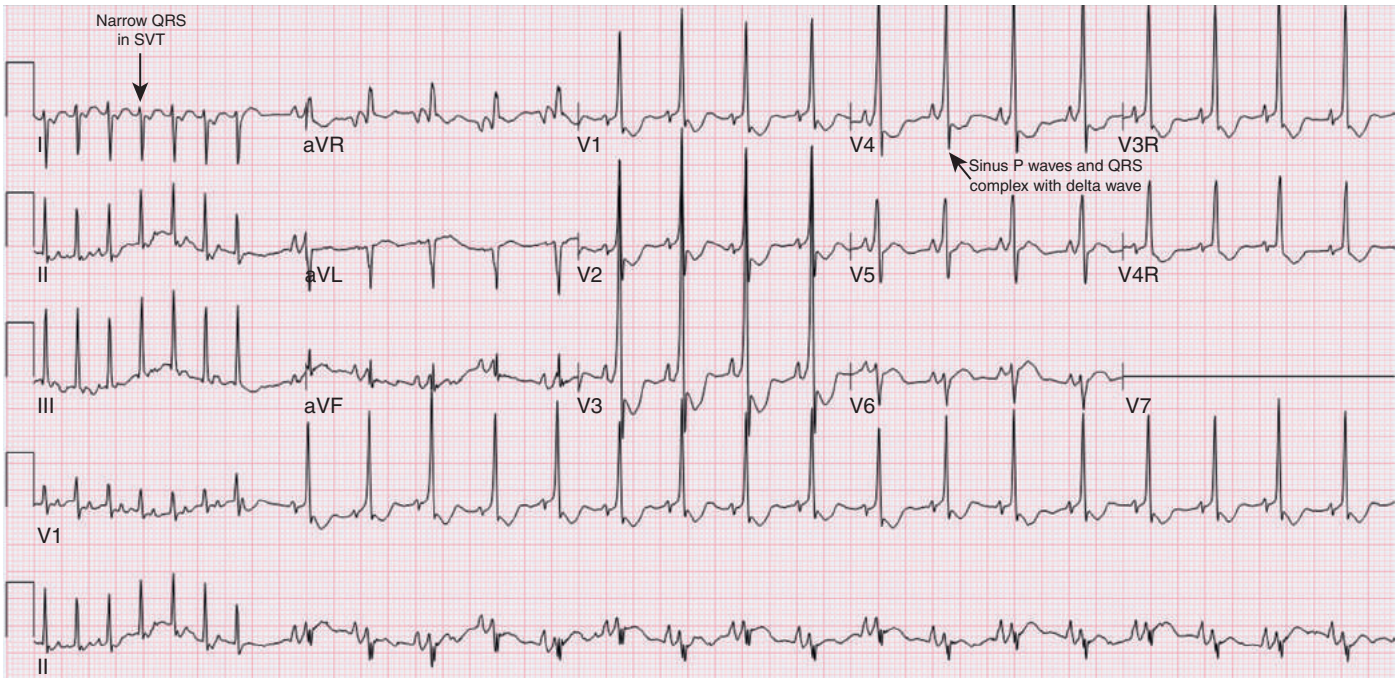
Acute management of ORT is directed at terminating the tachycardia and restoration of sinus rhythm. Slowing or transient blockade of AV nodal conduction is the mechanism by which most therapies work and includes vagal maneuvers (such as knee-chest position or diving reflex) and administration of adenosine. Initial adenosine doses of 0.05 to 0.1 mg/kg by rapid intravenous (IV) bolus are recommended for the neonate, with escalating doses as necessary. Direct current cardioversion for immediate arrhythmia termination can also be considered for the infant with impending collapse, although the risks versus benefits of this approach must be considered. For chronic medical therapy in infants prone to recurrent episodes of SVT, β-blockade in the form of propranolol is often used as a first-line agent. In some institutions, digoxin is used alone or in combination with propranolol. While calcium channel blockers are readily used in older children and adults for this indication, this class is generally avoided in a young infant due to the potential for significant myocardial depression. Second-line agents include sotalol, flecainide, and amiodarone. These medications are discussed further in the Management Considerations for Neonatal Tachyarrhythmias section later.

Permanent Form of Junctional Reciprocating Tachycardia

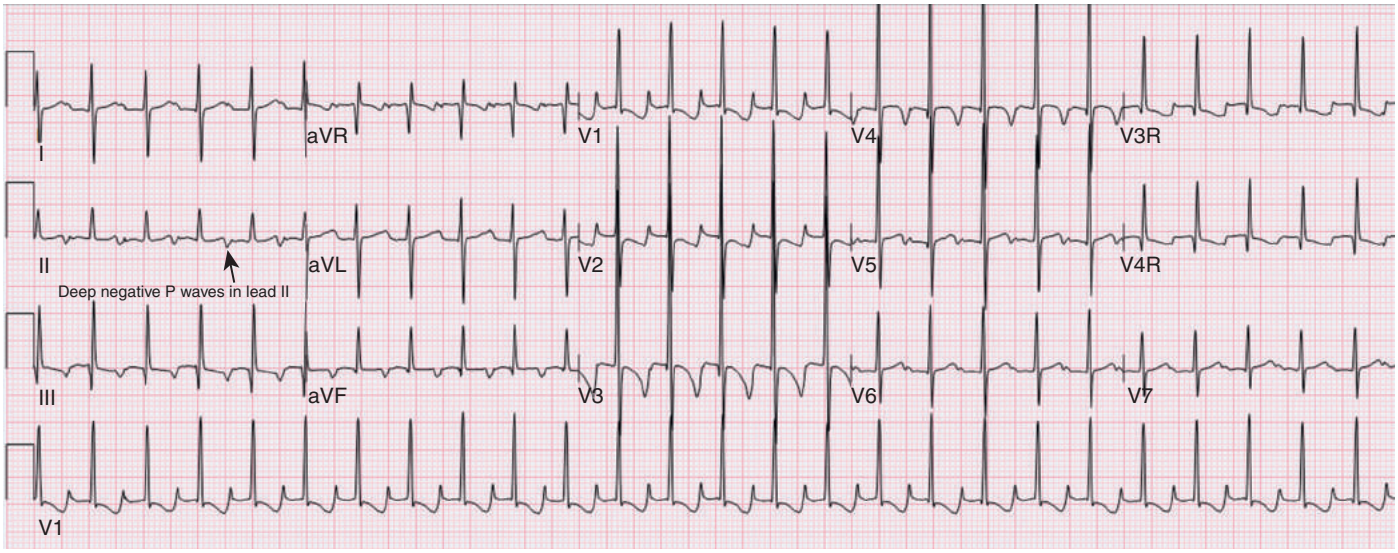
An unusual but particular vexing variant of ORT is the permanent form of junctional reciprocating tachycardia (PJRT). This is a reentry form of SVT that has rates that are usually far slower than the usual form of ORT, sometimes seemingly at the upper limits of the normal range for the newborn, around 180 to 200 bpm. However, PJRT has a tendency toward being an incessant arrhythmia, often spontaneously reinitiating immediately after tachycardia termination. The mechanism for PJRT is usually a concealed accessory pathway, typically located in the posterior septum or posterior tricuspid annulus. The conduction velocity of this retrograde limb is slower than in most other types of accessory pathways, resulting in a very long RP interval. Another hallmark feature is the presence of strongly negative retrograde P waves in the inferior leads, particularly lead II (Fig. 49.3). These infants often escape early detection as the tachycardia rates are not dramatically elevated, which can lead to delayed diagnosis and presentation with tachycardia-induced cardiomyopathy or congestive heart failure.[9] Treatment can be challenging as the arrhythmia has a tendency toward spontaneous reinitiation, even after successful termination of tachycardia. Medical therapy includes the drugs described above, although most infants with PJRT will require multiple agents for successful control of this arrhythmia.

Atrial Ectopic Tachycardia

Atrial ectopic tachycardia (AET) is a less common cause of SVT than ORT but is still well-represented among perinatal tachyarrhythmias. This is also known by other monikers, such as *ectopic atrial tachycardia* or *focal atrial tachycardia*. In contrast to reciprocating tachycardias, AET is not caused by a bypass tract but rather by a focus of cells in the atria that are more excitable than the sinus node and exhibit enhanced or triggered automaticity. As atrial activation originates from a location other than the sinus node, the P waves on surface ECG are usually quite distinctly different from normal (Fig. 49.4). The result is paroxysmal bursts of tachycardia, although prolonged and even incessant tachycardia can also be



• **Fig. 49.2** Wolff–Parkinson–White Syndrome. Spontaneous termination of orthodromic reciprocating tachycardia. Note change from narrow QRS in tachycardia, then wide preexcited QRS during subsequent sinus rhythm.

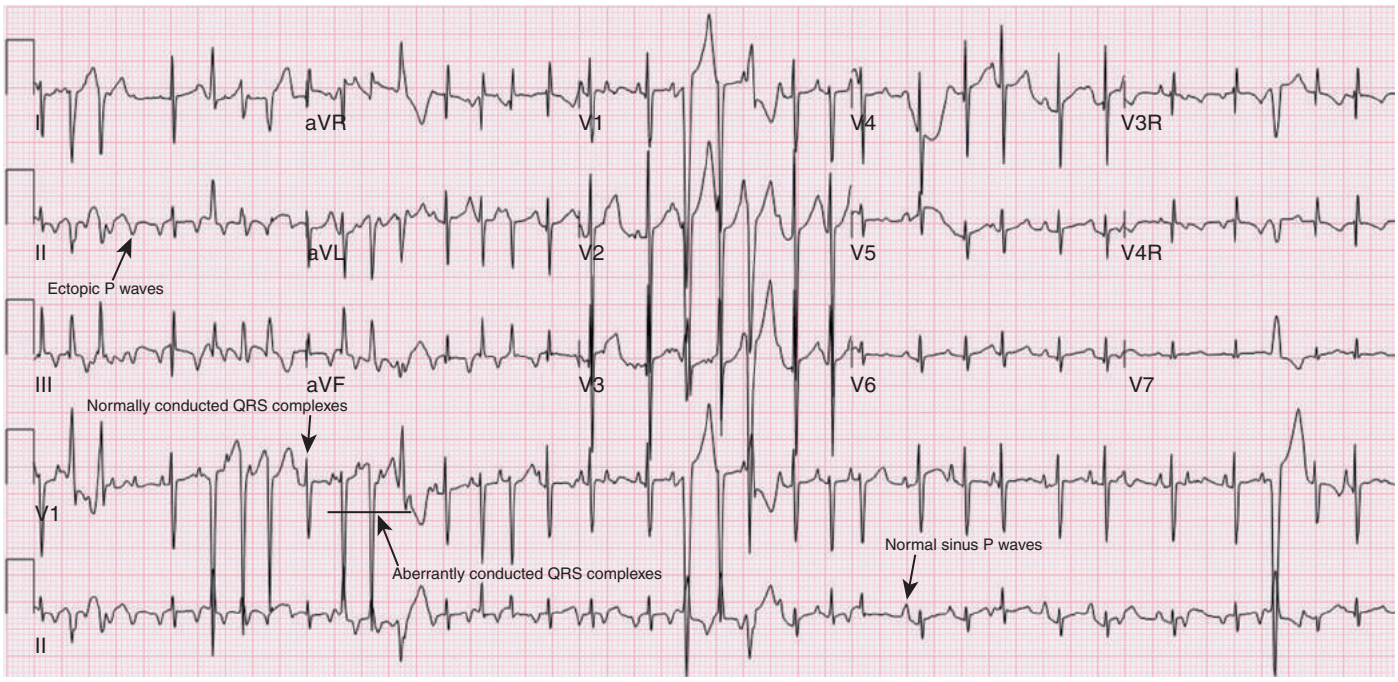


• **Fig. 49.3** Permanent Form of Junctional Reciprocating Tachycardia. Typical appearance of permanent junctional reciprocating tachycardia, with long ventriculoatrial time (ventriculoatrial > atrioventricular) and strongly negative P waves in inferior lead II, III, and aVF.

seen, which can lead to the development of tachycardia-induced cardiomyopathy.[10] This arrhythmia is often catecholamine-sensitive, with increased clinical signs during periods of activity or stimulation. Heart rates often vary during sustained tachycardia, and the rates can range from slightly faster than sinus rhythm to well over 300 bpm at times. Since the tachycardia does not depend on intact AV nodal conduction, intermittent AV block (such as 2:1 conduction or Wenckebach pattern) can sometimes be seen, and this feature is often used to aid in the correct diagnosis. By the same token, nodal blockade does little to affect this arrhythmia. Adenosine administration usually only transiently blocks the AV nodal conduction while the atrial arrhythmia continues unabated. While direct current cardioversion may interrupt the arrhythmia, it is likely to reinitiate spontaneously because of its paroxysmal nature.

Junctional Ectopic Tachycardia

Another automatic supraventricular arrhythmia of childhood is junctional ectopic tachycardia (JET). This is caused by increased automaticity of the cells around the AV junction resulting in direct activation of the His–Purkinje system. Heart rates range from



• **Fig. 49.4** Atrial Ectopic Tachycardia. P waves with unusual axis "march through" the tracing, with more P waves than QRS complexes on tracing. Wide aberrantly conducted QRS complexes are caused by "Ashman phenomenon" of conduction.

170 to 210 bpm and demonstrate the usual warm-up and cool-down behavior. The atrial activity may be conducted retrograde from the junctional activity or may be completely dissociated from it. JET can be either acquired or congenital. The acquired form can occur in up to 20% of patients following cardiopulmonary bypass for certain congenital cardiac surgeries[11] and can cause hemodynamic embarrassment because of the inherently unstable nature of those patients, even though the arrhythmia itself is fairly self-limited. The congenital form of JET is quite rare but, like atrial tachycardia, can be an incessant arrhythmia. Such patients can present with tachycardia-induced cardiomyopathy.[10]

Neonatal Atrial Flutter

Atrial flutter is a macro-reentrant arrhythmia that occurs entirely within the atria, with a depolarization wavefront that typically runs in a counterclockwise direction around the tricuspid valve annulus. The atrial rate is usually 300 bpm or greater, and the continuous atrial activation gives it a characteristic "sawtooth" pattern in the inferior leads, particularly lead II and III (Fig. 49.5). The atrial rate is exceedingly consistent and entirely independent of the ventricles, although varying degrees of AV conduction can be observed. While older infants and children might conduct every other (2:1) or every third (3:1) atrial impulse, the neonatal AV node has brisker conduction capability, so it may conduct each impulse (1:1), resulting in a very rapid ventricular rate. Administration of adenosine can be diagnostic by blocking AV nodal conduction while the flutter waves continue uninterrupted.

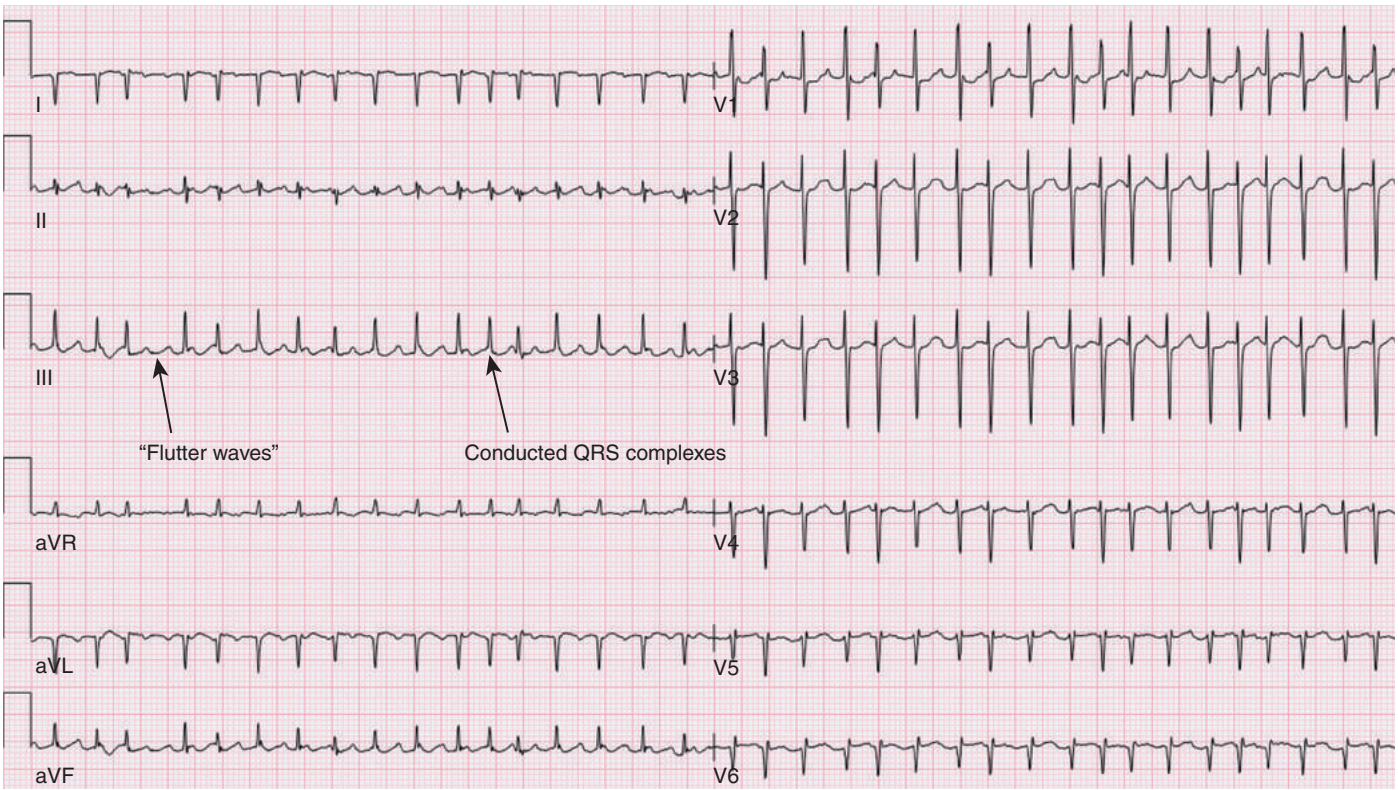
Medical therapy may be used to slow the ventricular rate. However, in most cases, direct current cardioversion is required; this is highly effective, and the recurrence risk is low.[12] In the fetus, there is often 1:1 conduction with a very rapid ventricular rate. When undetected and untreated, this can result in congestive heart failure, which manifests as hydrops fetalis. Detection and diagnosis may be difficult as it is unusual to be able to obtain a rhythm recording or ECG. A fetal echocardiogram may be able to demonstrate rapidly contracting atria (as discussed later). When necessary, fetal flutter may be treated by administering antiarrhythmic medications to the mother, although high maternal doses are often required to achieve adequate transplacental transfer to the fetus.

Ventricular Tachycardia

Ventricular tachycardia (VT) is a fairly uncommon arrhythmia in the perinatal period. It can take varied forms, ranging from a benign accelerated ventricular rhythm to a potentially life-threatening polymorphic VT. The latter is rarely encountered and occurs only in the setting of severe metabolic derangements or electrolyte disturbances or in the setting of an underlying congenital primary arrhythmia syndrome. VT may be suspected on the basis of a wide QRS complex tachycardia, although the differential diagnosis of such a rhythm includes a supraventricular rhythm conducted aberrantly, such as with a preexcitation pattern (as discussed earlier) or when conducted with a bundle branch block. However, in most instances, monomorphic VT is generally a benign entity in infancy and rarely requires intervention other than medical therapy.[13]

Accelerated idioventricular rhythm (AIVR) is a benign arrhythmia. Sometimes considered as "slow VT," AIVR is caused by enhanced automaticity of the ventricles and is usually due to intrinsic catecholamine states, electrolyte disturbances, or other conditions that predispose to increased automaticity. The hallmark is a monomorphic rhythm that originates in the ventricles but at rates that are only slightly faster than the normal sinus rates. This is generally well tolerated hemodynamically unless the infant is otherwise extremely compromised by other medical factors.

In contrast to the stability of monomorphic ventricular arrhythmias, polymorphic VT is a much more disorganized and potentially life-threatening arrhythmia and must be recognized



• **Fig. 49.5** Atrial Flutter. The atrial rate is approximately 360 beats per minute, with "flutter waves" visible in leads II, III, and aVF. Note that not all flutter P waves conduct equally to the number of QRS complexes.

and managed emergently. The ECG in polymorphic VT exhibits a rapid wide QRS complex, and the QRS morphology varies from beat to beat, indicating generally disordered ventricular depolarization. Cardiac output can be severely reduced. A particular variant of polymorphic VT is known as *torsades de pointes* (TdP). Literally "twisting about a point," the amplitude and axis of the QRS morphology rotate and undulate and can eventually degenerate into a more chaotic ventricular fibrillation. It is rarely seen in the neonate, except in the setting of primary arrhythmia genetic syndromes that are associated with sudden cardiac death, such as the long QT syndrome (LQTS) (as discussed later).

Management Considerations for Neonatal Tachyarrhythmias

Appropriate treatment of neonatal tachyarrhythmias depends largely upon prompt recognition and accurate diagnosis. The most common substrates for tachycardia in this subset are ORT and AET, in addition to neonatal atrial flutter. Orthodromic tachycardia can acutely respond to vagal maneuvers and (more reliably) to IV adenosine administration, although these methods will have little to no effect on either AET or neonatal flutter. Because of the increased likelihood of recurrent reentry tachycardia during early infancy, medical therapy is commonly utilized to decrease this possibility. Many infants can show no outward signs that they are experiencing tachycardia until incessant tachyarrhythmias result in dilated cardiomyopathy and cardiovascular collapse. Older infants and children will usually give more outward signs of being in a tachyarrhythmia, so they are less likely to present with cardiomyopathy and cardiovascular collapse.

Medical therapy for neonatal tachycardia varies considerably across institutions.[14] Digoxin selectively inhibits the sodium-potassium adenosine triphosphatase channel, which causes an increased intracellular sodium concentration. Other cardiac effects include a positive inotropic effect and an increased vagotonic effect. It is this latter effect that is suspected of contributing to the antiarrhythmic properties of the drug. In many institutions, digoxin was historically used most commonly for acute and chronic management of SVT, although its utilization varies greatly.[15]

Propranolol (Vaughan-Williams class II) is a nonspecific β-blocker medication that binds β-adrenergic receptors. This decreases overall sensitivity to adrenergic stimulation and has some direct effects on myocyte membrane potential. While the overall success of propranolol versus digoxin is comparable,[16] digoxin was less likely to succeed initially as monotherapy. In addition, digoxin is contraindicated in the Wolff–Parkinson–White syndrome because of enhancement of accessory pathway conduction properties, so ventricular preexcitation must be excluded before initiation of digoxin. The drug is also generally ineffective for AET. Enteral propranolol is increasingly used as first-line therapy for neonatal SVT, preferentially in high-volume treatment centers.[15] Particularly in the preterm newborn, the risk of β-blocker–associated hypoglycemia exists, so monitoring of blood glucose during initiation of propranolol therapy is recommended in this population.

Second-line agents include flecainide and sotalol, as well as amiodarone, and are highly effective in controlling the arrhythmia in incessant forms of neonatal SVT.[17] These drugs require closer monitoring because of their proarrhythmic effects. All three can potentially prolong the QT interval and potentially provoke ventricular arrhythmias. Flecainide (Vaughan-Williams class Ic)

inhibits the fast inward sodium current of the myocardial action potential and prolongs conduction through all cardiac tissues, particularly those of the His–Purkinje system and ventricular myocardium. This can result in a decreased ability of the conduction system to perpetuate SVT and is a very effective drug for treating SVT. Flecainide is bound by milk protein, and significant fluctuation in drug serum levels has been reported in neonates transitioning to oral feeds;[18] it can also prolong the QRS complex.

Vaughan-Williams class III medications used to treat perinatal SVT include sotalol and amiodarone. This class of drugs prolongs the action potential duration by extending the repolarization phase of the myocardium, thus increasing the overall refractory period of the tissue. This decreases the ability of the myocardium to support the repeated depolarizations necessary to support SVT. Sotalol exhibits β-blocker effects (class II) in lower doses and in higher doses exhibit more class III effects. Amiodarone is highly effective for acute and chronic management of tachyarrhythmias in the neonate; however, it must be judiciously used as it can have an effect on hypotension, bradycardia, heart block, cardiovascular collapse, and hypothyroidism. It has been reported to cause "neonatal gasping syndrome" and to leach plasticizers from polyvinyl chloride containers and syringes.[19] Because of the pharmacokinetics of amiodarone, it is usually administered by loading doses (in both IV and enteral dosing) for 7 to 10 days, after which the dose is decreased to a maintenance dose.

While commonly performed in older children, percutaneous catheter ablation for supraventricular arrhythmias is rarely performed in neonates and small infants. Technical issues and catheter size limit conventional mapping and ablation techniques. Late complications and deaths caused by injury to adjacent coronary artery structures have also been reported.[20]

While most ventricular arrhythmias in neonates are controllable and self-limited, certain conditions may predispose them to more life-threatening forms of VT. Particularly in cases in which defibrillation must be performed, for hemodynamically unstable VT or ventricular fibrillation, additional therapies must be considered. Reversible causes of TdP, such as electrolyte disturbances or drug-induced TdP, should be addressed by aggressive correction of the imbalance or removal of the offending agent(s). However, when no such cause can be identified, the etiology is often found to be a congenital arrhythmia syndrome, commonly LQTS (as discussed later). In more-affected infants, treatment with β-blocker medications may be used, and in severe cases with recurrent episodes of TdP or ventricular fibrillation in which the anticipated risk of sudden cardiac arrest is excessive, implantation of a pacemaker or a defibrillator might be considered; however, this must be weighed against the acute implantation issues and longer-term complications.

Bradyarrhythmias

Neonatal forms of bradycardia are few. In general, bradycardia is defined as sustained heart rates less than 100 bpm. This can be the result of normal physiologic causes or secondary to arrhythmias or abnormalities in conduction. Sinus bradycardia represents the most common mechanism in the neonate. This can be a normal physiologic variant (it is not uncommon to encounter a normal term neonate with a sinus rate of 90 bpm in the first 24 to 48 hours after birth, especially during sleep) or secondary to other causes (such as therapeutic hypothermia). Another common mechanism is vagal stimulation, which can be provoked by the presence of oral or nasal feeding tubes, endotracheal tubes, or episodes of gastroesophageal reflux.

Blocked Premature Atrial Complex

Atrial ectopy is commonly encountered in the fetus and newborn (discussed previously). In most instances, the premature atrial complex (PAC) normally conducts to a normal-appearing QRS complex. However, PACs that occur earlier in relation to the preceding QRS complex may find the conduction system or ventricular myocardium refractory to stimulation. Thus the PAC is not followed by any ventricular depolarization. This is described as a "blocked PAC." Most often, this is observed when a PAC occurs in the early portion of the preceding T wave, such that the ventricles are still in their refractory period and cannot depolarize. Conditions that affect the refractoriness of either the AV nodal conduction or myocardial refractoriness, such as increased vagal tone or metabolic acidosis, can also increase the likelihood that a PAC is blocked. Following the premature atrial depolarization, there is typically a delay before the subsequent sinus return beat. When atrial ectopy is frequent, as in blocked atrial bigeminy, the net effect is a functional "halving" of the sinus rate (Fig. 49.6).

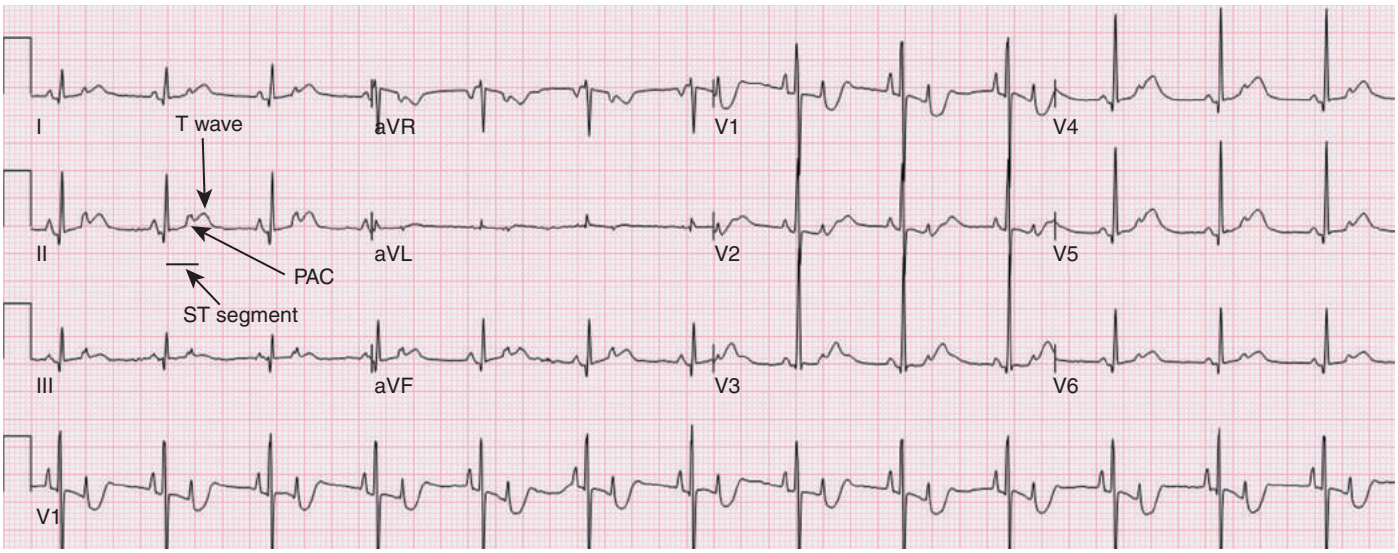
Long QT Syndrome

In severe forms of congenital LQTS, the QT prolongation can result in bradycardia in the fetus or the newborn. When the repolarization phase becomes significantly prolonged, the subsequent sinus P wave may fail to conduct normally or at all as the ventricular myocardium is still refractory to stimulation. As a result, the functional heart rate is halved since the ventricular rate is conducted 2:1 from the sinus rate. This can have significant implications as the bradycardia may not be tolerated by the patient. Chronotropic medications for this condition may do little to improve the functional heart rate as increasing the frequency of sinus P waves does not shorten the refractory period of the ventricular myocardium. Permanent pacemaker implantation may be necessary. In addition, the functional nature of this "2:1" heart block can result in irregularities in the conduction pattern (Fig. 49.7). This can result in profound QT prolongation as well as triggering VT and TdP.

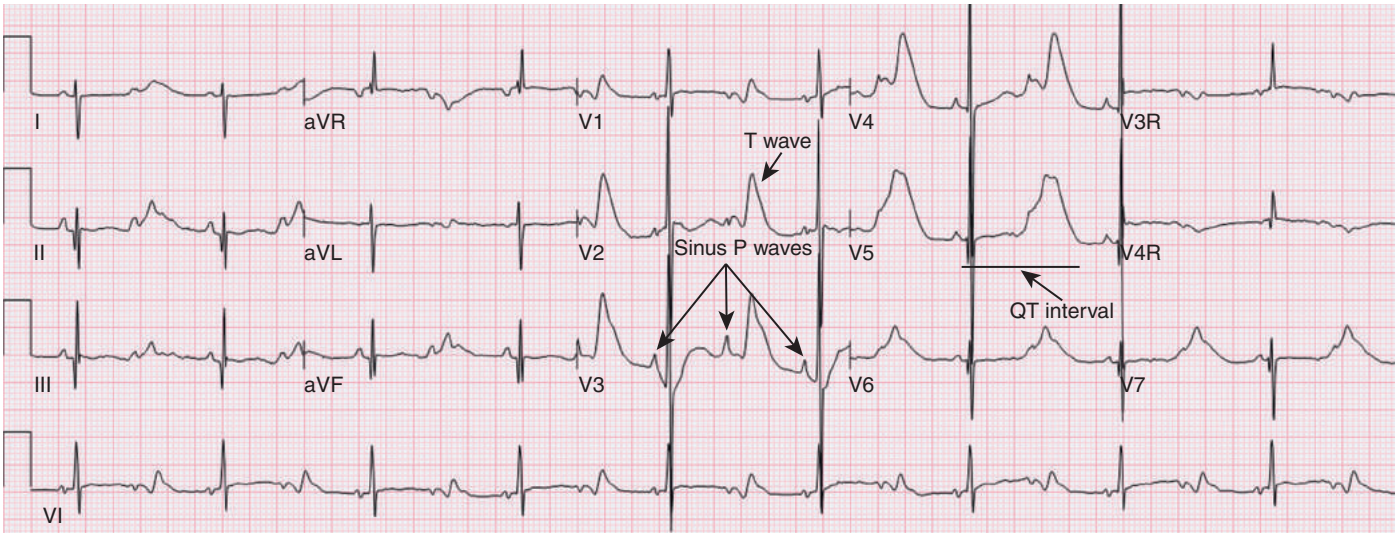
Congenital Complete Atrioventricular Block

Congenital complete AV block (CCAVB) is the absence of AV conduction. This is rather uncommon, occurring in only 1 in 15,000 to 1 in 20,000 births.[21] It is commonly associated with maternal lupus, although it also occurs in the absence of any known maternal disease. Fetal exposure to maternal autoantibodies (most commonly anti-SSA/Ro and anti-SSB/La antibodies) results in fibrosis of the AV nodal structures, leading to a progressive destructive process. There is some evidence that early treatment with high-dose maternal steroids could delay or even arrest this process;[22] however, the reported response rate to therapy is highly variable,[23–25] so the risk-benefit ratio of this therapy must be weighed on an individual basis. When detected, the fetus is monitored frequently for the development of hydrops fetalis, a sign of congestive heart failure in the fetus.

Complete heart block can also occur in the fetus or newborn in the setting of CHD, most commonly those defects that involve "L-transposition of the great arteries" or "ventricular inversion." In these conditions, fetal looping of the heart results in the His bundle being a far more superficial structure than normal and therefore prone to fibrosis. Development of complete heart block



• **Fig. 49.6** Premature Atrial Complexes. Premature atrial complexes (PAC) occurring on ST segments fail to conduct to ventricles resulting in "blocked atrial bigeminy."

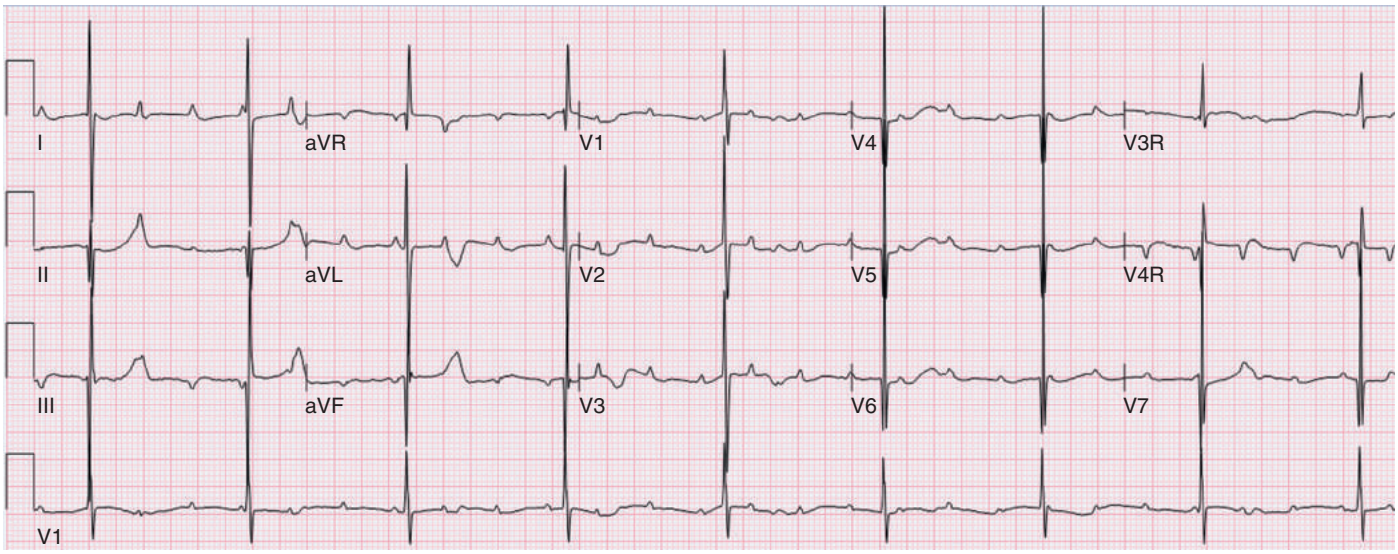


• **Fig. 49.7** Long QT Syndrome With 2:1 Functional Heart Block. The sinus rate is 110 bpm. The QT interval is profoundly prolonged (QTc = 751 ms), so every other sinus P wave cannot conduct because of the ventricular refractory period.

in this setting can occur at any stage of development and often does not occur until later childhood or adulthood.

In most cases of CCAVB, the region around the bundle of His remains intact and can exhibit normal properties of automaticity. This "junctional escape rhythm" typically results in a normal narrow QRS complex and ventricular contractility, despite the lack of AV conduction. It has the appearance of a normally conducted sinus beat but is completely dissociated from the sinus nodal P waves occurring above (Fig. 49.8). The escape rate in the fetus and neonate is often 60 to 80 bpm and can provide adequate cardiac output. There are many instances in which CCAVB is completely undetected in the newborn period and remains undetected for many years. The QRS complex may be wide (greater than 70 ms in the newborn) if there is extensive damage to the junctional region, implying that the escape rhythm arises from a location lower on the septum or from the ventricular myocardium itself.

However, a slower junctional escape rhythm, usually when the rate is below 50 bpm, is inadequate to support normal cardiac output. Signs of congestive heart failure may become manifest. In the fetal circulation, where the pulmonary vascular bed is largely bypassed by the ductus arteriosus, this will manifest as hydrops fetalis or intrauterine fetal demise. Management of the fetus with inadequate junctional escape rate and signs of poor cardiac output are addressed in the Fetal Rhythms section of this chapter. For the postnatal patient, signs of low cardiac output would include respiratory distress (with pulmonary congestion), poor perfusion, metabolic acidosis, low urine output, and frank edema. This can present a clinical challenge. Heart rate and blood pressure may be augmented by inotropic and chronotropic drip medications such as isoproterenol, epinephrine, and dobutamine. In some centers, temporary transvenous pacing may be utilized for emergent rescue, but this can be challenging because of the size disparity between



• **Fig. 49.8** Congenital Complete Atrioventricular Block. The sinus nodal rate is approximately 150 bpm, but there is complete atrioventricular dissociation with a junctional escape rate of 50 bpm.

commercially available temporary pacing leads and the small vessel size in this population.[26] Permanent pacemaker implantation is indicated if there is evidence of ventricular dysfunction or low cardiac output in the neonate with a heart rate less than 55 bpm (or less than 70 bpm in the setting of concurrent CHD) or if there is evidence of a wide QRS complex escape rhythm.[27,28] Box 49.1 summarizes the absolute and relative indications for pacemaker implantation in the neonate with CCAVB.

Fetal Arrhythmias

Fetal arrhythmias occur in 1% to 2% of pregnancies, with resultant hemodynamic compromise, hydrops fetalis, and fetal demise occurring in 10% of cases.[29,30] The arrhythmias may develop late in the second or third trimester; this is particularly true for premature contractions, atrial tachycardias, and VTs, which often do not manifest before 25 to 26 weeks' gestation and, in some cases, only in the third trimester.[31,32] Fetal arrhythmias require close monitoring, as even benign rhythms may have a small risk of complications. The standard modality for prenatal evaluation of fetal arrhythmias is via fetal echocardiography, focusing on the mechanical atrial and ventricular systoles and their relationship to one another (Fig. 49.9A and B) as a surrogate for AV synchrony/dyssynchrony.[33–35] Investigation of the fetus referred for an abnormal rhythm should focus on causes of impaired AV conduction and causes of abnormal atrial or ventricular rhythms, including varying degrees of AV block, congenital LQTS, myocarditis, intracardiac tumors, and structural CHD. Differentiation between types of arrhythmia mechanisms is helpful in determining the most optimal therapy and the likelihood of success of arrhythmia treatment.

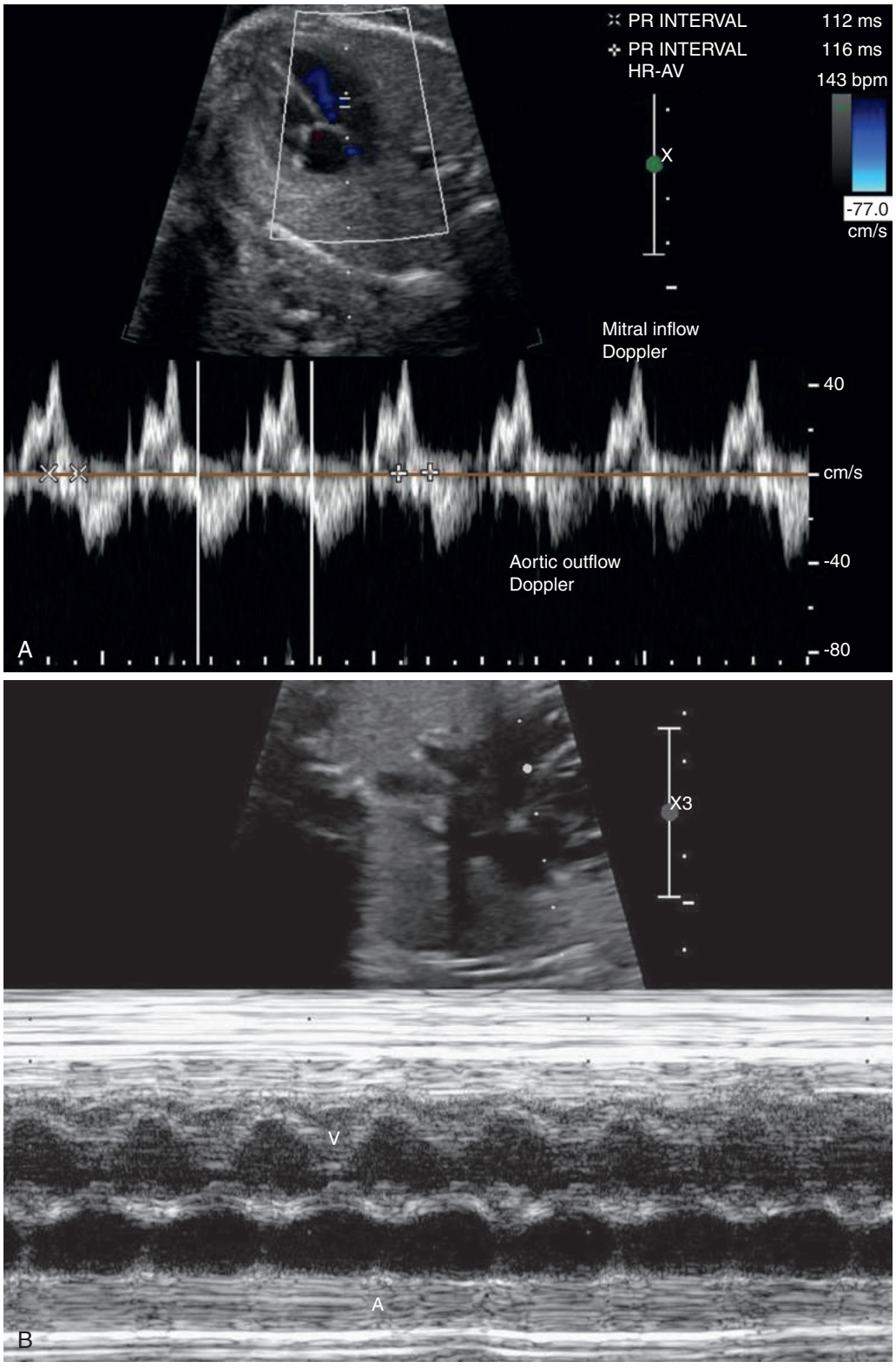
Benign Arrhythmias

One of the most common reasons for referral to the fetal cardiologist is irregular fetal heart rhythms, noted in 1% to 3% of pregnancies.[36] The causes of these irregular rhythms are often isolated premature atrial contractions.[37] Rarely, they may be ventricular in origin. The vast majority of these atrial ectopic beats are benign; premature atrial contractions have a small risk (0.5% to 1%) of developing into a fetal tachycardia.[38] However, 2% of cases may be associated with fetal LQTS, atrial flutter, and second-degree AV block.[39]

• BOX 49.1 Indications for Permanent Pacing in Congenital Complete Atrioventricular Block

- Permanent pacemaker implantation is indicated for patients with CCAVB with symptomatic bradycardia.
- Permanent pacemaker implantation is indicated for patients with CCAVB with a wide QRS escape rhythm, complex ventricular ectopy, or ventricular dysfunction.
- Permanent pacemaker implantation is indicated for CCAVB in asymptomatic neonates or infants when the mean ventricular rate is ≤50 bpm or lower. Ventricular rate alone should not be used as implant criteria, as symptoms due to low cardiac output may occur at faster heart rates.
- Permanent pacemaker implantation is indicated for CCAVB in neonates or infants with complex CHD when bradycardia is associated with hemodynamic compromise or when the mean ventricular rate is <60–70 bpm.
- Permanent pacemaker implantation is reasonable for asymptomatic CCAVB beyond the first year of life when the mean ventricular rate is ≤50 bpm or there are prolonged pauses in ventricular rate.
- Permanent pacemaker implantation is reasonable for CCAVB with left ventricular dilation (z score ≥3) associated with significant mitral insufficiency or systolic dysfunction.
- Permanent pacemaker implantation may be considered for CCAVB in asymptomatic adolescents with an acceptable ventricular rate, a narrow QRS complex, and normal ventricular function, based on individualized consideration of the risk/benefit ratio.

CCAVB, congenital complete atrioventricular block; *bpm*, beats per minute.
From Writing Committee Members, Shah MJ, Silka MJ, et al. 2021 PACES expert consensus statement on the indications and management of cardiovascular implantable electronic devices in pediatric patients. *Heart Rhythm*. 2021;18(11):P1925–1950.



• **Fig. 49.9** Fetal echocardiogram performed from a coronal view of the fetus demonstrating a four-chamber view of the heart with normal fetal heart rate (146 bpm) with 1:1 atrioventricular conduction via (A) normal mitral valve inflow and aortic valve outflow spectral Doppler. The measurement between the inflow and outflow is the PR interval (XX and ++ = 116 ms). (B) M-mode diagonally across the four-chamber heart measures the movement of the atrial (A) and ventricular (V) free wall. *bpm*, Beats per minute; *HR-AV*, heart rate measured from aortic valve Doppler.

Management of Benign Arrhythmias

The 2014 American Heart Association scientific statement for the diagnosis and treatment of fetuses with irregular rhythms[40] recommends that fetuses with frequent ectopic beats (bigeminy, trigeminy, or more than every 3 to 5 beats on average) should have a baseline fetal echocardiogram to assess cardiac structure and function and to determine the mechanism of the arrhythmia (Fig. 49.10). Medical treatment is not recommended for premature atrial contractions. If frequent ectopy continues, weekly fetal heart rate monitoring to assess for progression to tachycardia should be performed until it resolves. In fetuses with less frequent extrasystoles, a fetal echocardiogram is indicated if the irregular rhythm persists beyond 1 to 2 weeks or if there is difficulty differentiating a benign rhythm from a pathologic one.[40] A postnatal ECG should be performed if an irregular rhythm is auscultated after birth.

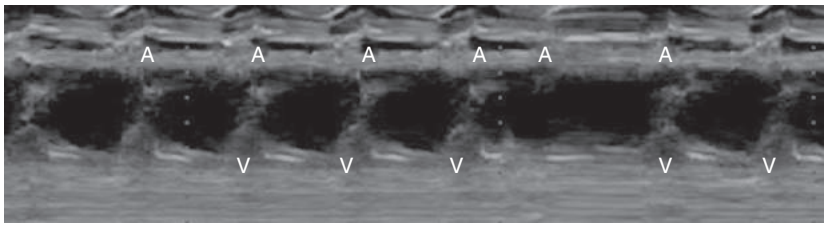
Fetal Tachycardias

Fetal tachycardia constitutes a rare but important cause of perinatal morbidity and mortality. Sustained SVT includes ORT (70%), atrial flutter (30%), and rare tachyarrhythmias and usually occurs at rates greater than 220 bpm.[36] The type of SVT can be determined using the mechanical PR interval derived from either the spectral Doppler of the mitral inflow and aortic outflow or the superior vena cava and aorta. Management for a given fetus can then be assessed based on the type of suspected SVT (Table 49.2). VT is a much less common cause of fetal tachycardia as are chaotic or multifocal atrial tachycardia.

In general, the goal of in utero treatment of fetal tachycardias is not conversion to 100% sinus rhythm but instead to establish sufficient sinus rhythm to allow resolution of hydrops and ventricular dysfunction. Management depends on the gestational age at presentation, degree of fetal compromise, maternal conditions, and potential risks to the mother from therapy.[40] Emergent delivery should be reserved for the hydropic fetus who exhibits persistent tachycardia refractory to medical management.[37] Delivery can be considered as a primary management strategy if the fetus is near term and if delivery incurs no significantly increased risk to the neonate.[40]

Orthodromic Reentrant Tachycardia

ORT accounts for approximately 70% of fetal tachycardias.[36] It usually presents with intermittent or sustained heart rates in the range of 240 to 260 bpm and is identified on fetal echocardiogram by the short RP interval (ventriculoatrial [VA] activation time),



• **Fig. 49.10** M-mode of the Fetal Heart Demonstrating an Isolated Premature Atrial Contraction. There is normal atrioventricular conduction at a normal rate until the fifth labeled atrial beat (A) that is early (ectopic) and results in a blocked ventricular beat (V).

TABLE 49.2 Fetal Supraventricular Tachycardia (SVT)

SVT Category	Gestational Age	Typical Rates	Atrioventricular Relationship	Onset/Termination	Transplacental Therapy
Short VA SVT • AVRT, ORT, AVNRT • Accounts for 60%–70%	>18 weeks	>180 bpm (210–320 bpm)	• 1:1 • VA interval less than ½ of the VV interval • VA/AV ratio <1	• Sudden onset/offset • Blocks in the AV node, terminates with non-conducted atrial beat	• First line: digoxin • Second line: sotalol or flecainide (± digoxin) • Third line: amiodarone
Long VA SVT • EAT, PJRT	>12 weeks	170–220 bpm	• 1:1 but may be variable (EAT) • VA interval greater than ½ of the VV interval • VA/AV ratio >1	• Gradual onset and offset (EAT) • Terminates with a nonconducted ventricular beat	• First line: flecainide • Second line: sotalol • Third line: amiodarone
Atrial flutter • Accounts for 25%–30%	>28 weeks	Atrial rate: 300–550 bpm Ventricular rate: 180–240 bpm	• Variable AV conduction (primarily 2:1 or 3:1) • Fixed ventricular rate		• First line: sotalol or flecainide • Second line: add digoxin • Third line: switch to amiodarone

In the setting of hydrops, may require combination therapy (digoxin+sotalol or digoxin+flecainide).
VA, ventriculoatrial; *AV*, atrioventricular; *VV*, ventriculoventricular.

1:1 AV relationship, and fast ventricular rate (Fig. 49.11). This rhythm results from conduction down the AV node to the ventricles and retrogrades through an accessory pathway to the atrium. Generally, reentrant ORT presents between 28 and 33 weeks' gestation.[41] Progression to hydrops is not uncommon, with risk factors being tachycardia duration, fetal immaturity, and concurrent structural heart disease.[29,41]

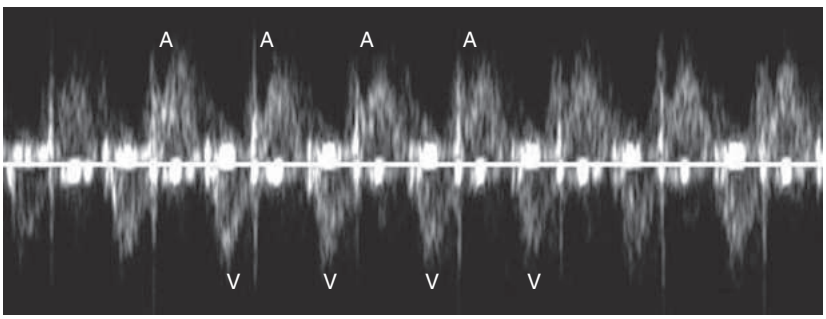
Intrauterine fetal treatment is recommended if delivery does not offer a lower risk. The first-line and second-line antiarrhythmic therapy choices are controversial, as are the management strategies after initial treatment failure. The use of combination therapies presents a greater risk for maternal and/or fetal complications, and thus monotherapy is recommended. Digoxin, flecainide, and sotalol as monotherapy have all been used successfully as first-line therapies for reentrant SVT,[42–44] though the use of digoxin is controversial because of the very small risk of atrial fibrillation with rapid antegrade conduction across an accessory pathway.[45] There is no study to date that supports one as the superior treatment option. All three of these medications, as well as amiodarone, have also been used as second-line therapy.[40] Amiodarone has a more significant toxicity profile for the mother and fetus and should be reserved as a third-line treatment for life-threatening arrhythmias. It should be discontinued once hydrops resolves. Verapamil and procainamide are no longer used to treat fetal tachyarrhythmias.[40] Transplacental transfer of drugs is reduced in the setting of hydrops; thus, direct fetal treatment (to the fetal buttock/thigh or intracordal) concomitantly with transplacental therapy has been described for restoration of sinus rhythm. These strategies should be limited to severely hydropic fetuses who cannot be delivered because of early gestational age, as there is an associated risk of fetal death.[46,47]

After delivery, up to 50% of reentrant SVT cases will not have postnatal recurrence.[29] Thus, medical treatment must be reassessed relative to the length of time since the last occurrence and the mechanism of clinical tachycardia.

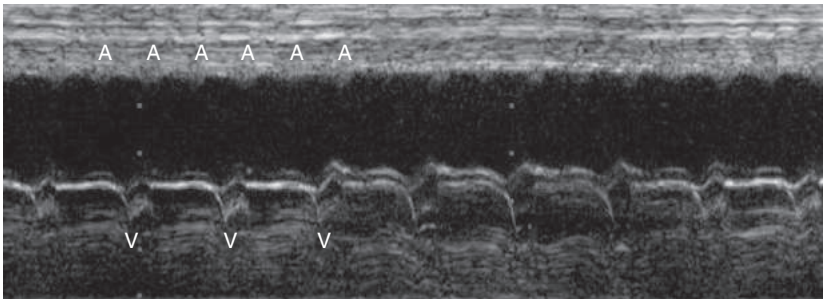
Atrial Flutter

Atrial flutter accounts for approximately 30% of fetal tachycardias.[36] It is associated with fetal myocarditis, structural CHD, and in utero exposure to SSA/SSB antibodies. It is typically characterized by atrial rates of 300 to 500 bpm and slower ventricular rates (150 to 170 bpm) because of a physiologic block in the AV node. It is best identified on fetal echocardiogram using an M-mode across an atrial free wall, interventricular septum, and ventricular free wall (Fig. 49.12). Atrial flutter is more likely to present later in pregnancy than reentrant SVT, at around 31 to 34 weeks gestation. It is generally a well-tolerated rhythm because of the lower ventricular rates.[33,36] However, the rhythm can remain undiagnosed prenatally because of the relatively normal fetal ventricular rates.[34] The lack of variability in the heart rate provides a clue to the diagnosis. Atrial flutter can co-occur with reentrant SVT in 12% to 33% of affected fetuses.[12]

Sotalol has been shown to be effective in converting 50% to 80% of fetuses with atrial flutter without mortality[44] and is considered first-line therapy. Digoxin is also recommended as first-line therapy, and amiodarone may be considered; however, procainamide is contraindicated.[48] Transesophageal pacing of synchronized cardioversion is recommended after delivery to restore sinus rhythm. Sinus node suppression may rarely occur due to in utero therapy, and backup external pacing should be available after cardioversion. Postnatal medical treatment should be reassessed given that atrial flutter may not recur.[40]



• **Fig. 49.11** Fetal Mitral Inflow and Aortic Outflow Spectral Doppler Demonstrating Reentrant Supraventricular Tachycardia. There is 1:1 atrioventricular conduction (A and V labels) at a steady rate of 238 beats per minute. The RP interval is 100 ms.



• **Fig. 49.12** M-mode of the fetal heart demonstrating 2:1 atrial flutter with an atrial rate of 120 beats per minute and a ventricular rate of 260 beats per minute. *A*, Atrial flutter waves; *V*, ventricular activation.

Sustained Ventricular Tachycardia

Fetal VT is associated with AV block, cardiac tumors, myocarditis, and ion channelopathies. LQTS should be suspected when tachyarrhythmia and bradyarrhythmia coexist.[49] Fetuses with LQTS can develop rapid TdP and monomorphic VT with subsequent development of ventricular dysfunction, AV valve insufficiency, and hydrops. Fetal magnetocardiography or ECG can confirm the diagnosis by identifying a prolonged QTc interval.[50–52] Intrauterine IV magnesium is recommended as first-line therapy for VT greater than 200 bpm, and treatment should be limited to less than 48 hours. If maternal magnesium levels are less than 6 mEq/L and there are no signs of toxicity, redosing can be considered. IV lidocaine, oral propranolol, or mexiletine may also be considered in conjunction with magnesium, especially in the setting of hydrops. If LQTS can be excluded, then sotalol, flecainide, and amiodarone are alternative therapies and have been shown to be successful in terminating fetal VT. Dexamethasone and IV infusion of immunoglobulin (IVIG) have been used in the setting of antibody-mediated or myocarditis-associated VT.[40]

Rare Tachycardias

Less common tachycardias such as AET and PJRT can be differentiated by the long RP interval (VA time) on fetal echocardiogram. The two can be differentiated by the gradual warm-up phase and variable AV nodal block at faster rates characteristic of AET and the sudden onset and consistent heart rate of PJRT. AET generally occurs at rates of 180 to 250 bpm and is most refractory to treatment both before and after birth.[36] These fetal tachycardias occur in the late second or third trimester. Multifocal atrial tachycardia is rare (associated with Costello syndrome) and usually occurs in the last weeks of pregnancy.[53] JET is commonly associated with SSA antibody exposure in the fetus and can be seen in the presence or absence of AV block. For AET and multifocal atrial tachycardia, treatment is recommended for average heart rates greater than 200 bpm with normal cardiac function or greater than 160 bpm with cardiac dysfunction.[40] Digoxin is recommended as the first-line therapy, though flecainide and sotalol may be considered. Flecainide or sotalol are recommended for PJRT or rapid AET. Similar therapies can be used for the medical management of fetal JET, though amiodarone has also been used. Fetuses that develop JET in the setting of anti-SSA antibody exposure can be treated with dexamethasone as well. After the delivery of a fetus with these rare tachycardias, continued medical treatment is usually required.

Sinus Tachycardia

Sinus tachycardia at rates of 180 to 190 bpm may mimic other pathologic tachycardias; however, ventricular dysfunction and hydrops are uncommon. Sinus tachycardia is associated with maternal infection, anemia, drug/medication use, hyperthyroidism, or trauma. Treatment of the underlying cause is recommended.[40]

Arrhythmia Medications

Relatively high doses of antiarrhythmic agents must be administered during pregnancy because maternal circulating blood volume and renal clearance are both increased. Thus, timing and strategies for fetal arrhythmia management must be carefully considered (Table 49.3). In most cases, treatment should be initiated in the hospital with close observation of maternal and fetal well-being. A baseline maternal ECG and electrolytes, as well as a cardiology consultation, should be obtained before the initiation of medications to assess for maternal risk factors such as preexcitation or LQTS. Serial maternal ECG, electrolyte concentrations, and drug levels should be monitored throughout the duration of therapy. Oral medication administration is recommended, except for IV magnesium and lidocaine and for digoxin loading doses.[40] Most arrhythmia medications demonstrate diminished transplacental transfer in the setting of hydrops, with the efficacy of digoxin decreasing to 25%.[37] However, flecainide and sotalol have been shown to be efficacious in the setting of hydrops.[40] If the maternal PR, QRS, or QT intervals are noted to prolong during therapy with digoxin, flecainide, or sotalol, respectively, doses should be decreased, and close observation is recommended. Serious maternal adverse reactions are rare and have resolved with discontinuation of therapy.[40]

Fetal Bradycardia

Fetal bradycardia is defined as a persistent heart rate of less than 120 bpm. Causes include sinus bradycardia secondary to sinus node dysfunction, channelopathies, maternal exposures/conditions, fetal central nervous system involvement, and blocked atrial bigeminy, which is associated with a 10% risk for conversion to SVT. The most common cause of fetal bradycardia is congenital AV block, occurring in approximately 1:20,000 live births.[54] Approximately 50% of fetal AV block is secondary to structural heart disease, and 40% is due to immune-mediated mechanisms,[55,56] and an additional 10% is idiopathic. Fetal congenital heart block at low heart rates is associated with significant morbidity and mortality, especially when associated with maternal autoimmune disorders. Management of fetal bradycardia is focused on frequent fetal echocardiographic evaluations to assess for the development of ventricular dysfunction, hydrops, or fetal heart rate less than 55 bpm. Treatment of nonimmune-mediated heart block is directed at augmenting fetal ventricular rates. For immune-mediated AV block, several therapies may be considered and are controversial.[22]

Benign Fetal Bradycardia

Maternal treatment with β-blockers, sedatives, and other medications has been associated with sinus node suppression. Fetuses with exposure to maternal anti-SSA/SSB antibodies or those with myocarditis may develop inflammation and fibrosis of the sinus node. Finally, fetuses with heterotaxy syndrome may develop

TABLE 49.3 Fetal Tachyarrhythmia Treatment Strategies

Tachycardia Duration	Treatment Strategy
Intermittent SVT OR Sustained* SVT <200 bpm without hydrops or hemodynamic compromise	• Observe without therapy • At least weekly fetal heart rate monitoring • Periodic fetal echocardiogram
Sustained* SVT ≥200 bpm with/without hydrops OR Intermittent SVT with hydrops or hemodynamic compromise	• Gestational age <37 weeks: transplacental antiarrhythmic therapy • Gestational age >37 weeks: deliver

*Sustained: >12 hours in a 24-hour monitoring period or >50% of echo scan time.
SVT, Supraventricular tachycardia.

bradycardia secondary to a low-atrial rhythm in left-atrial isomerism or dual sinoatrial nodes in right-atrial isomerism. In these conditions, fetal heart rates range between 90 and 130 bpm. No treatment is recommended.[40]

Blocked atrial bigeminy may result in fetal heart rates between 75 and 90 bpm with 2:1 AV conduction and can be mistaken for second-degree AV block. There is an increased risk of the development of fetal SVT, occurring in approximately 10% of fetuses with blocked atrial bigeminy.[40] Fetal echocardiography may demonstrate normal AV conduction followed by an early atrial beat coupled to the preceding ventricular beat, in which case the AV node is refractory. This results in failure of conduction to the ventricle (see Fig. 49.10). Management of blocked atrial bigeminy is the same as for isolated premature atrial contractions.

Ion Channelopathies

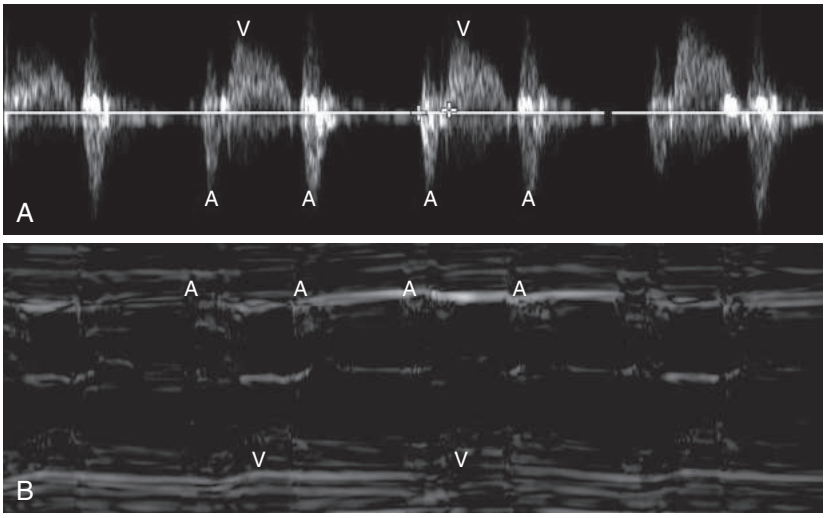
Congenital LQTS should be considered in the setting of persistent bradycardia in an otherwise asymptomatic fetus.[57] Measurement of the QTc can be performed by magnetocardiogram or fetal ECG.[50–52] Fetal treatment is not recommended; however, the development of VT requires treatment as delineated in the Fetal Tachycardia section. Management involves close observation prenatally, correction of maternal electrolyte abnormalities, and avoidance of maternal exposure to QT-prolonging medications. Postnatal evaluation includes an ECG and continued observation and management of arrhythmias associated with LQTS.[40]

Atrioventricular Block

Structural CHD accounts for 50% of fetal AV block. Fetuses with heterotaxy syndrome with left-atrial isomerism congenitally corrected transposition and AV canal defects are most at risk for the development of AV block. In a fetus, the combination of congenital AV block, complex structural CHD, and hydrops fetalis is associated with a high rate of in utero demise.[58] AV block can be diagnosed by fetal echocardiogram by evaluating the atrial and ventricular rates and the AV relationships (Fig. 49.13A and B).

Maternal anti-SSA (Ro) and anti-SSB (La) antibodies are present in the majority of fetuses that develop isolated AV block. The presumed mechanism is that the fetal cardiac structures are a target for immune complex deposition, namely the AV node and the myocardium. Ro-52-specific antibody has shown a very close association with immune-mediated congenital AV block.[59] The risk of heart block in mothers with anti-SSA/SSB antibodies is 2% to 3%, with a recurrence rate after one affected child as high as 12% to 17%.[60,61] Autopsy specimens typically reveal progressive fibrosis of the AV nodal structures in affected fetuses. The process typically spares the His–Purkinje system so that the slower "escape rhythm" tends to be relatively reliable. Damage to the ventricular myocardium and the presence of endomyocardial fibroelastosis is also found in affected fetuses.[62] The risk of transfer of maternal antibodies to the fetus, and therefore the risk for development of AV block, is highest between 16 and 26 weeks' gestation.[60] Close follow-up with a fetal echocardiogram to evaluate fetal heart rate, PR interval for prolongation, and ventricular function is recommended every other week during this critical time period for all fetuses with exposure to anti-SSA/SSB antibodies. Fetal heart rate monitoring should be performed at standard intervals by the obstetrician. In recent years, home fetal heart rate monitoring, performed by the pregnant woman twice to three times daily, has been utilized between 16 and 26 weeks gestation in order to detect evolving second-degree heart block quickly and allow for immediate admission and treatment to limit progression to third-degree AV block.[63]

Treatment of AV block depends on the cause, ventricular rate, and degree of heart failure. β-sympathomimetics such as terbutaline, salbutamol, and isoprenaline are reasonable to use in fetuses with heart rates less than 55 bpm or with higher heart rates if there is significant CHD or signs of cardiac dysfunction or hydrops. Although terbutaline may increase the fetal heart rate, improvement in survival has not been demonstrated. Terbutaline is well tolerated; however, maternal heart rates of up to 100 to 120 bpm and benign ectopy have been reported.[64] Immune-mediated AV block may benefit from dexamethasone (4 to 8 mg/day) or IVIG therapy. Dexamethasone has been shown to reduce inflammation, reverse or stabilize second-degree block, and improve hydrops or myocardial fibrosis and function.[62] Risks associated with the use of dexamethasone include



• **Fig. 49.13** (A) Fetal mitral inflow and aortic outflow spectral Doppler and (B) M-mode of the fetal heart demonstrating 2:1 atrioventricular block with an atrial rate of 120 beats per minute and ventricular rate of 60 beats per minute. *A*, Sinus atrial activity; *V*, ventricular depolarization.

fetal growth restriction, oligohydramnios, effects on the central nervous system, and maternal diabetes. A trial of dexamethasone may be considered in fetuses with second-degree AV block or first-degree AV block with signs of cardiac inflammation (valve insufficiency, ventricular dysfunction, effusion) to prevent progression from completing AV block, though its use has not been well established.[40] The treatment of mothers with hydroxychloroquine before 10 weeks' gestation has been associated with a reduced risk of anti-SSA/SSB antibody-mediated fetal cardiac disease and may be used in high-risk pregnancies.[65]

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Congenital Heart Disease

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KEY POINTS

- Congenital heart disease (CHD) is the most common birth heart defect encountered in the clinical setting, affecting 1% of live births.
- Surgical outcomes for all forms of CHD continue to improve. Early detection through fetal echocardiography, physical examination, and pulse oximetry screening allows for improved neonatal management and decreased short-term and long-term morbidity.
- The etiology of CHD remains elusive in many cases. Advances in cellular biology and genetic testing will continue to improve our understanding of its origins.
- An in-depth understanding of neonatal cardiac anatomy and physiology is necessary for proper management of infants with CHD.

General Considerations

Fetal-to-Postnatal Transition

The hemodynamic state of the fetus differs significantly from that of the newborn. In the fetus, a relatively low systemic vascular resistance exists because of the presence of the placenta, and the pulmonary vasculature maintains a high resistance. Central shunts exist for nutrient-rich blood from the placenta to be delivered to the fetal circulation both within the heart (foramen ovale) and on the arterial side of the circulation (ductus arteriosus).

The ductus venosus serves as a flow regulator between the umbilical vein and the inferior vena cava (IVC), largely bypassing the hepatic and portal venous systems. Based on studies in fetal sheep, less than one-half of the umbilical venous return enters the left lobe of the liver and reaches the ductus venosus near its insertion into the IVC, returning as relatively nutrient-rich blood.[1] The lateral position of the IVC within the right atrium results in streaming of placental blood across the foramen ovale and into the left atrium. The most desaturated blood to return to the right atrium comes from the coronary sinus, which combines with the venous return from the superior vena cava (SVC) and is directed across the tricuspid valve into the right ventricle. The nutrient-rich blood deriving from the umbilical vein, which has crossed the foramen ovale to enter the left side of the heart, predominantly supplies the heart and brain. Output from the right ventricle supplies the lungs and flows right-to-left through the ductus arteriosus to supply the remainder of the body. In the fetus, the presence of the ductus arteriosus, which is nonrestrictive, results in both ventricles being subjected to a comparable afterload. Compared with the postnatal heart, this results in an increase in right ventricular workload and some restriction to filling of the right ventricle, thus the right ventricle carries the primary workload of the fetal heart. The ventricles exist in parallel in the fetal heart and output is described as combined cardiac output.

At birth, several important transitions take place that allow the fetus to adapt to extrauterine life. First, the gradual decline in pulmonary vascular resistance (PVR) that was occurring during the last trimester of pregnancy undergoes an abrupt drop with the first breath taken by the newborn (Fig. 50.1). This decline in PVR results in a more than 20-fold increase in pulmonary blood flow and reversal of flow (left-to-right) in the ductus arteriosus before its closure.[2] Second, the shunts present in the fetus undergo closure such that blood flow transitions from parallel to flow in series through the body. The ductus venosus closes largely because of lack of flow following separation of the placenta, although some contractile elements may be present in the vessel wall.[3] The foramen ovale becomes occluded as the flap of the septum primum abuts the septum secundum following the increased pulmonary blood flow that increases filling of the left atrium. Small residual left-to-right shunts at the foramen ovale may persist and in 20% to 30% of the population a patent foramen ovale may exist throughout life (see "Atrial Septal Defects" later in the chapter). Closure of the ductus arteriosus is mediated by decreased blood flow and increased arterial oxygen tension; in contrast, patency of the ductus can usually be maintained by exogenous prostaglandin administration. The third important transition at birth is an increase in the combined ventricular output as the metabolic demands of the body increase at birth.

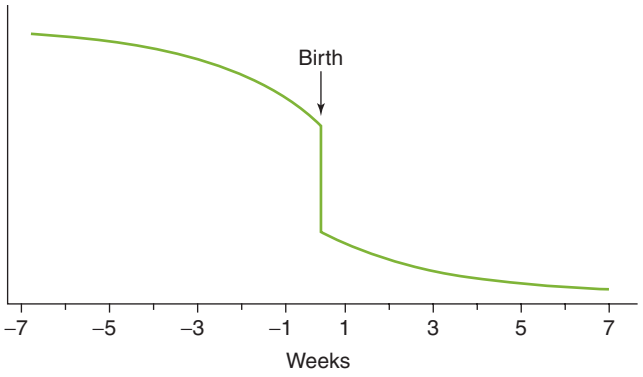
The dramatic hemodynamic changes that occur at birth continue to evolve over the next few months. There is a continued decline in PVR during the first 6 to 8 weeks after birth. In addition, the right ventricle remodels to a thinner and more compliant ventricle and the left ventricle becomes the dominant ventricle of the heart.

Nomenclature

Differing nomenclatures have evolved to define cardiac anatomy and spatial relationships. While the brief summary of nomenclature that is given here is based on the segmental approach of Anderson et al.[4] the embryologic approach of Van Praagh[5] is equally valid and used by several institutions.

The segmental approach to describing cardiac anatomy includes:

1. Cardiac position
2. Visceral sidedness
3. Systemic and pulmonary venous connections
4. Atrial sidedness and their connections



• **Fig. 50.1** Change in Pulmonary Vascular Resistance. A gradual decline in pulmonary vascular resistance is seen during the latter part of gestation followed by an abrupt decline at birth. A gradual decline occurs postnatally over the next 6 to 8 weeks.

5. Atrioventricular (AV) valves
6. Ventricular relationship
7. Ventriculoarterial connections
8. Great vessel number and position

The description of cardiac position in the chest can be separated into where the heart is located and the direction in which the apex of the heart is pointed. Normally, the heart is in the left chest with the apex pointed to the left. Dextro- (right) or meso- (midline) position of the heart can occur with decreased right lung volume, severe scoliosis, or an elevated left diaphragm. A preliminary assessment of the position of the heart in the chest can be determined by a chest x-ray. The normal leftward-pointing apex of the heart (levocardia) can vary to mesocardia (apex pointing midline) or dextrocardia (apex pointing rightward). The orientation of the apex of the heart is defined by echocardiography or cross-sectional imaging.

Visceral sidedness is often defined separately for the abdominal organs, the cardiac structures, and the lungs, although they frequently follow one another. Sidedness is referred to as solitus (normal), inversus (mirror image), or ambiguous (isomerism or indeterminate). In the latter situation, effort is made to define whether the organs that appear on both sides are right-sided (liver, right atrium, and trilobed lung) or left-sided (stomach/spleen, left atrium, bilobed lung) structures since this can have prognostic and therapeutic importance. For instance, patients with bilateral right-sidedness typically lack a spleen and require lifelong antibiotic prophylaxis for encapsulated organisms. Additionally, these patients can have malrotation of the intestines and are at risk for volvulus.

Venous connections of the superior and inferior venous systems must also be delineated. The usual connection of the SVC to the right atrium may also be accompanied by a persistent left SVC to the coronary sinus, with or without a bridging brachiocephalic vein. The IVC is derived from various embryologic vessels. Interruption of the IVC can occur due to failure of fusion of any of the embryological parts. Lower extremity blood flow is consequentially routed to the SVC through the azygous or hemiazygous systems. Various pulmonary venous connections are described below.

Atria can be solitus with the morphologic right atrium on the right (normal), inversus, mirror image, common, or indeterminate. The right atrium is typically identified by the presence of a coronary sinus, the presence of the crista terminalis (the muscular ridge separating the muscular and smooth portions of the right atrium, the large sailed-shaped appendage, and the coarse pectinate muscles of the free wall). The left atrium is characterized by its smooth walls and narrow, finger-shaped appendage. Atrial morphology can be discerned by several imaging modalities such as echocardiography, cross-sectional imaging such as magnetic resonance imaging and computed tomography and more historically angiography. When the morphologic right atrium connects to the morphologic right ventricle (and similarly on the left), the connection is *concordant*. A *discordant* connection occurs when the morphologic right atrium connects to the morphologic left ventricle as in corrected transposition of the great arteries (TGA). When both atria connect to one ventricle (as in double inlet left ventricle) or a single ventricle, the type of connection is referred to as *univentricular*. An *ambiguous* connection occurs in cases of atrial isomerism.

The AV valves generally remain committed to their respective ventricles throughout embryology. As such, the tricuspid valve, when present, connects to the morphologic right ventricle, and the mitral valve connects to the morphologic left ventricle. The tricuspid valve has three leaflets and is distinguished from the mitral valve by the septal attachments of its papillary muscles and the slight inferior position of the septal leaflet of the tricuspid valve relative to the anterior leaflet of the mitral valve. When the AV valves fail to undergo septation, a common AV valve is found, as in children with a complete AV septal defect. The position of the AV valves and their chordal attachments are used to define whether the valves are malaligned or straddling. A malaligned AV valve is not completely positioned over its respective ventricle, which is sometimes referred to as *overriding*. If the chordal attachments of an AV valve cross the septum and connect to the other ventricle, an AV valve is referred to as straddling.

The morphology of the ventricles, the associated AV valve, and the outflow portion of the ventricle can generally be used to identify the right and left ventricles. The right ventricle, besides being associated with the tricuspid valve, is more heavily trabeculated at its apex and anterior free wall than the left ventricle. The right ventricle is described as tripartite and consists of an inflow, body, and outflow. The inflow includes the tricuspid valve, the papillary muscles which are responsible for anchoring the valve leaflets, and the chordae tendineae, which are the fibrous cords anchoring the tricuspid valve to the papillary muscles. The body includes the trabeculated muscular portion of the ventricle and the outflow which extends toward the pulmonary valve in the form of a tract. Along this outflow tract is the infundibulum or conus, which is a muscular separation between the tricuspid and semilunar valves.[6] The left ventricle is more smooth-walled with finer trabeculations at its apex than the right ventricle, and in normal anatomy demonstrates fibrous continuity between the mitral and aortic valves. When the ventricular morphology is uncertain, the ventricles are said to be indeterminate. A common ventricle is defined by virtual absence of the interventricular septum.

The great vessels are largely defined by their branching pattern. The pulmonary artery bifurcates shortly after exiting the heart into the right and left pulmonary arteries that undergo subsequent branching to supply the segments of the lung. The right pulmonary artery is positioned anterior to the right upper bronchus while the left pulmonary artery is posterior to the left upper bronchus. The pulmonary arteries typically follow the situs of the lungs such that mirror image pulmonary artery branching is seen in situs inversus, bilateral branch pulmonary arteries anterior to the upper bronchus are seen in right isomerism, and branch

pulmonary arteries posterior to the upper bronchus are seen in left isomerism. The aorta is normally left sided, and courses over the left mainstem bronchus giving rise to three head and neck arterial branches. A right aortic arch crosses over the right mainstem bronchus before crossing back to the left side of the spine in the thorax and can have several different branching patterns. While the aorta is typically posterior and rightward to the main pulmonary artery, the relative position of the vessels can vary greatly. Most commonly, in d-transposition of the great arteries, the aorta is anterior and rightward to the main pulmonary artery. In situations where only a single semilunar valve is present, a truncus arteriosus (or common truncal artery) is found that gives rise to both the aorta and pulmonary artery.

The ventriculoarterial connections are said to be concordant when the right ventricle connects to the pulmonary artery and the left ventricle gives rise to the aorta. The ventriculoarterial connection is discordant when the opposite occurs. The ventriculoarterial connection can also be double, single, or common. If both great arteries arise from one ventricle, a double outlet occurs. The definition of a double outlet connection is somewhat controversial. For example, in the case of double outlet right ventricle (DORV) with normally related great vessels, some clinicians have proposed basing the definition on whether greater than 50% of the aorta overrides the right ventricle, while others define the double outlet on whether a subaortic conus exists that results in mitral–aortic discontinuity. From a patient management perspective, both situations are relevant for either placement of the ventricular septal defect (VSD) patch or the potential for development of subaortic obstruction, respectively. A single outlet occurs when severe pulmonary hypoplasia occurs such that no main pulmonary artery segment is present. A common outlet occurs in truncus arteriosus.

Clinical Evaluation of the Newborn

Even with the technologic advances in prenatal and postnatal echocardiography and genetic testing, a careful history and physical examination are needed in every newborn with suspected congenital heart disease (CHD). Birth history, including complications during pregnancy, labor, and delivery, is important to document. Often the child with cyanosis because of structural heart disease has an unremarkable birth history. A difficult labor or delivery may point toward noncardiac causes of cyanosis such as persistent fetal circulation, infection, or pneumothorax. For the child with poor systemic perfusion, a history of premature rupture of membranes or maternal fever may suggest sepsis as a cause for the diminished cardiac function. Hematologic abnormalities that may cause cardiovascular dysfunction in the neonate, such as polycythemia or anemia, may be suggested by a history of placental abruption or twin–twin transfusion.

Family history is critical to review with the biologic parents. There is a genetic basis for a growing number of congenital heart defects (see later). A sibling with CHD more than doubles the likelihood of future children having CHD.[7] A history of CHD in either of the parents also increases the chance of developing a congenital heart lesion.[8,9]

Physical examination of the newborn should initially include a general assessment looking for dysmorphic features and the degree of distress of the infant. The child with cardiac obstructive physiology may have shallow, rapid respirations with intercostal and suprasternal retractions. Cyanosis may or may not be seen depending on the degree of hemoglobin desaturation (roughly 5 g of hemoglobin must be desaturated to be clinically evident). Vital signs, including four extremity blood pressures, should be determined along with preductal and postductal oxygen saturation measurement. Palpation of the precordium may identify an overactive or displaced cardiac impulse or the sensation of a thrill caused by turbulent flow. Palpation of the abdomen for a liver edge or spleen tip can often provide an indication of volume overload or neonatal infection. Assessment of femoral and upper extremity pulses is essential. Simultaneous palpation of the right branchial and right femoral pulses allows assessment of comparable timing and intensity of the pulsations. Perfusion and capillary refill of the extremities are also important to determine.

Auscultation is often challenging in the sick neonate. However, characterizing the presence, timing, intensity, position, and radiation of murmurs that are present may provide a clue to the underlying diagnosis. In the tachypneic and tachycardic child, it is critical to listen over the head and liver for a continuous murmur that may indicate an arteriovenous malformation (AVM). The presence of a click or gallop over the precordium may indicate valvar disease or cardiac failure. Assessment of the second heart sound is particularly important. It has been suggested that the presence of physiologic splitting of the second heart sound nearly always suggests a structurally normal heart.[10]

Signs of CHF in the newborn may be subtle and include resting tachypnea (with no periodic variation), sinus tachycardia, and an enlarged liver. Tachypnea may be accompanied by nasal flaring and intercostal and subcostal retractions, particularly in situations where elevated pulmonary venous pressures are present. Grunting respirations are a particularly concerning sign in a newborn and often accompany severe heart failure and decreased systemic perfusion.

Because many infants with CHD are asymptomatic in the newborn period and have a normal physical examination and because routine prenatal ultrasound does not detect all defects, it has been recommended that pulse oximetry screening should be added to the routine newborn screening panel.[11] The screen specifically targets critical congenital heart defects or those that usually require an intervention in the first month of life and can lead to death or significant morbidity if not diagnosed in a timely manner. Since this recommendation, there have been several studies focusing on the feasibility, implementation, and impact of the screen.[12]

Laboratory Assessment of the Neonate

As mentioned previously, initial laboratory assessment should include measurement of preductal and postductal oxygen saturations in the right hand and foot, respectively, in a left aortic arch with normal branching. Values less than 93% are considered abnormal. The *oxygen challenge test* is performed by increasing the inspired oxygen concentration to 100% for at least 5 minutes. Oxygen saturation values that increase into the normal range may be useful to distinguish an admixture cyanotic heart lesion from lung disease, although this test does not discriminate with 100% accuracy. History and physical examination should be included and guide the need for further evaluation. A decrease in postductal oxygen saturations compared with preductal values suggests right-to-left shunting because of an increase in PVR. The unusual situation in which the preductal saturation reading is less than the postductal reading occurs when TGA is combined with pulmonary hypertension and a patent ductus arteriosus (PDA).

An electrocardiogram (ECG) should be obtained in the initial evaluation of the newborn with suspected CHD, although in the absence of an arrhythmia, it rarely provides a specific diagnosis. The neonatal ECG demonstrates prominent rightward forces and may have an upright T wave in the right precordial leads in the first few days after birth, and thus it may not be diagnostic of right ventricular hypertrophy. Age-dependent standards are available and should be referred to when evaluating the ECG (Table 50.1). Certain lesions may have distinctive findings on ECG such as extreme right axis deviation and Q waves in leads I and aVL (complete AV septal defect), preexcitation and right atrial enlargement (Ebstein anomaly), and Q waves in leads V1–V3 (congenitally corrected TGA).

A chest x-ray should also be obtained in every newborn that is evaluated for CHD. The chest x-ray may help to determine the heart size, shape, and border contours as well as pulmonary vascular markings. A prominent thymic shadow in the newborn may make identification of classic chest x-ray findings difficult, such as the "boot-shaped" heart in tetralogy of Fallot (TOF), the "egg on a string" in transposition, and the "snowman" appearance in supracardiac total anomalous pulmonary venous return (TAPVR), although the massively increased heart size typically found with Ebstein anomaly will not be missed. An absent thymic shadow may suggest DiGeorge syndrome (22q11 deletion), although genetic testing is still required. Increases in pulmonary vascular markings typically found in left-to-right shunt lesions may not be immediately apparent in the newborn because of the relatively high PVR and may take days or weeks to become apparent. Often, decreased pulmonary vascular markings in lesions with diminished pulmonary blood flow, such as tricuspid or pulmonary atresia, will be apparent in the newborn period. Presence of a PDA, however, will improve pulmonary blood flow in these lesions. Echocardiography is truly the mainstay in the diagnosis of CHD in the neonate. The suspicion of CHD warrants immediate consultation with a pediatric cardiologist and evaluation of the neonate by echocardiography.

Genetics and Congenital Heart Disease

About 30% of children with a chromosomal abnormality will have congenital heart disease.[13] There are an estimated 400 genes associated with CHD,[14] and the understanding of the genetic basis of CHD is progressing at a rapid pace. Blue et al. depicted the evolution of genetic testing and its impact on elucidating relevant genetic associations with CHD over the past several decades (Fig. 50.2).[15]

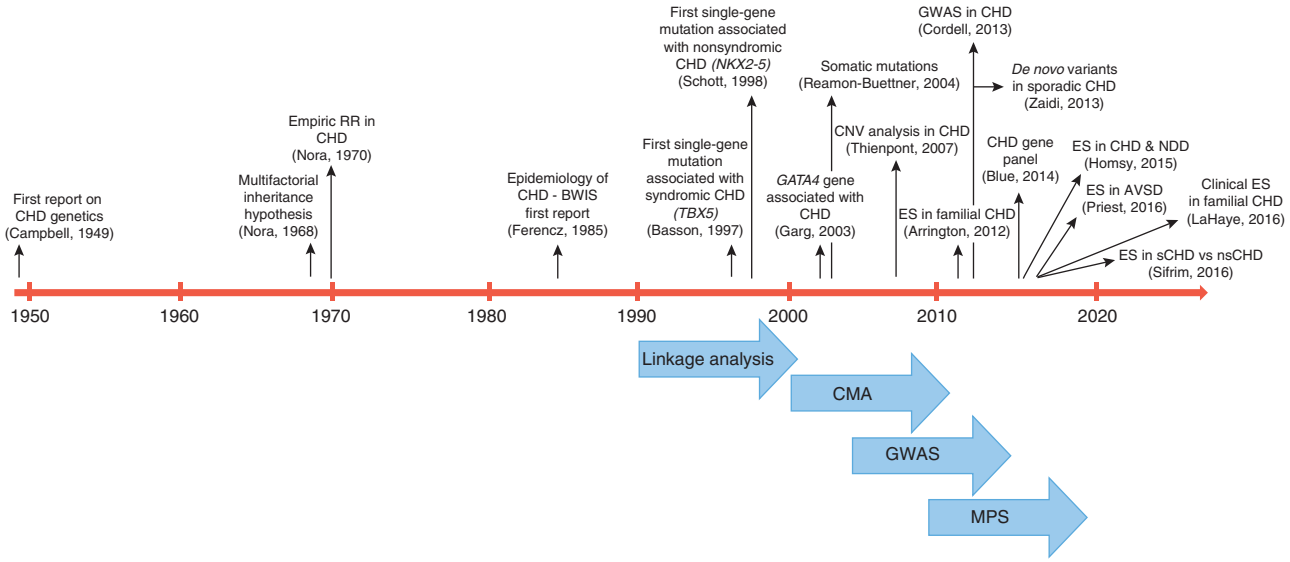
Cardiogenesis is driven by a complex interplay between several processes including but not limited to transcription factors, signaling pathways, chromatin modifiers, and protein development. Thus, the genetic pathways that drive cardiac development are multifactorial. Disruptions in genetic pathways can include point mutations, aneuploidy, and copy number variations all occurring as de novo mutations or due to Mendelian inheritance with varying penetrance. We will briefly review some of these genetic variations and associated congenital heart defects.

Point mutations are an alteration of a single nucleotide sequence in a particular gene. This generally results in one of three outcomes. The result can be inconsequential because the alteration is coding for the same amino acid. In contrast, a missense mutation can occur if the new codon corresponds to a different amino acid. Lastly, a nonsense mutation can occur resulting in a stop codon terminating the gene transcription altogether. An estimated 2% of CHDs are due to point mutations.[16] For example, HAND 1 and 2 are transcription factors that play a role in regulating ventricular looping and development. A loss of function mutation in HAND 1 can result in an arrest at the ventricular looping stage of cardiogenesis and the mutation has been identified in patients with hypoplastic left heart syndrome (HLHS), right heart hypoplasia, and double outlet right ventricle (DORV).

Copy number variants are the deletion or duplication of specific regions of DNA usually involving a significant number of base pairs. These are estimated to cause 10% to 15% of CHD.[16] An example of this type of mutation is 22q11 deletion, also known as DiGeorge syndrome or velocardiofacial syndrome

TABLE 50.1 Electrocardiogram: Age-Dependent Standards

Age Group	QRS axis	PRI	QRSD	QV6 (mm)	RV1 (mm)	SV1 (mm)	RV6 (mm)	SV6 (mm)	SV1 + RV6
<1 day	59–163	0.08–0.16	0.031–0.075	2	5–26	0–23	0–11	0–9.5	28
1–2 days	64–161	0.08–0.14	0.032–0.066	2.5	5–27	0–21	0–12	0–9.5	29
3–6 days	77–163	0.07–0.14	0.031–0.068	3	3–24	0–17	0.5–12	0–10	24.5
1–3 weeks	65–161	0.07–0.14	0.036–0.08	3	3–21	0–11	2.5–16.5	0–10	21
1–2 months	31–113	0.07–0.13	0.033–0.076	3	3–18	0–12	5–21.5	0–6.5	29
3–5 months	7–104	0.07–0.15	0.032–0.08	3	3–20	0–17	6.5–22.5	0–10	32
6–11 months	6–99	0.07–0.16	0.034–0.076	3	1.5–20	0.5–18	6–22.5	0–7	32
1–2 years	7–101	0.08–0.15	0.038–.0076	3	2.5–17	0.5–21	6–22.5	0–6.5	39
3–4 years	6–104	0.09–0.16	0.041–0.072	3.5	1–18	0.2–21	8–24.5	0–5	42
5–7 years	11–143	0.09–0.16	0.042–0.079	4.5	0.5–14	0.3–24	8.5–26.5	0–4	47
8–11 years	9–114	0.09–0.17	0.041–0.085	3	0–12	0.3–25	9–25.5	0–4	45.5
12–15 years	11–130	0.09–0.18	0.044–0.087	3	0–10	0.3–21	6.5–23	0–4	41



• **Fig. 50.2** Timeline of CHD genetic discoveries and the genetic technologies and study designs used. Genetic technologies/study designs are indicated by blue arrows and mark the approximate time when the technology was developed and used. (From Blue GM, Kirk EP, Giannoulatou E, et al. Advances in the genetics of congenital heart disease: a clinician's guide. *J Am Coll Cardiol*. 2017;69:859–870.)

which is commonly associated with conotruncal anomalies such as interrupted aortic arch (IAA), TOF, and truncus arteriosus (TA). 22q11 deletion alters the expression of TBX1, a key transcription factor in the development of the pharyngeal pouches and arches. In addition to CHD, the manifestations of the syndrome, although phenotypically heterogenous, can include parathyroid gland absence leading to hypocalcemia, thymic aplasia, neurodevelopmental delays, and characteristic facial features.[17]

Aneuploidy is the loss or gain of an entire chromosome or chromosomal segment. For example trisomy 13, 18, and 21 are all additions of chromosomes 13, 18, and 21. These defects are associated with cardiac disease 80% to 100% of the time in trisomy 13 and 18[18] and 40% to 55% of the time in trisomy 21.[19] Turner syndrome is the loss of an X chromosome. CHD can occur in up to 50% of patients with Turner, and lesions are commonly associated with the left side of the heart such as bicuspid aortic valve and coarctation of the aorta.[20] Wolf-Hirschhorn syndrome is the partial deletion of the short arm of chromosome 4 and is associated with CHD about 50% of the time. Common lesions associated with Wolf-Hirschhorn syndrome include atrial septal defects, ventricular septal defects, and pulmonary stenosis.[21]

Despite advances in the understanding of the genetic basis of CHD, the genetic basis of more than half of children with CHD is not known. It is likely that altered interactions with noncoding regions of the genome impact the degree to which genes that regulate cardiac development are expressed. There may also be gene–environment interactions, yet to be defined, which impact cardiac development.

The Genetic Work-Up of Congenital Heart Disease

A child with suspected CHD should be carefully evaluated for dysmorphic features that may indicate an associated syndrome. Similarly, newborns with suspected genetic syndromes should be screened for congenital heart disease. Fig. 50.3 demonstrates a genetic screening algorithm. Genetic testing should be performed not only in patients with suspected CHD but also in the setting of suggestive physical features, family history, or positive prenatal genetic testing. Fluorescent in situ hybridization (FISH) is the use of fluorescent probes to map specific deoxyribonucleic acid (DNA) sequences and is the test of choice for identifying 22q11, while single-gene disorders such as Noonan syndrome require whole-exome sequencing.

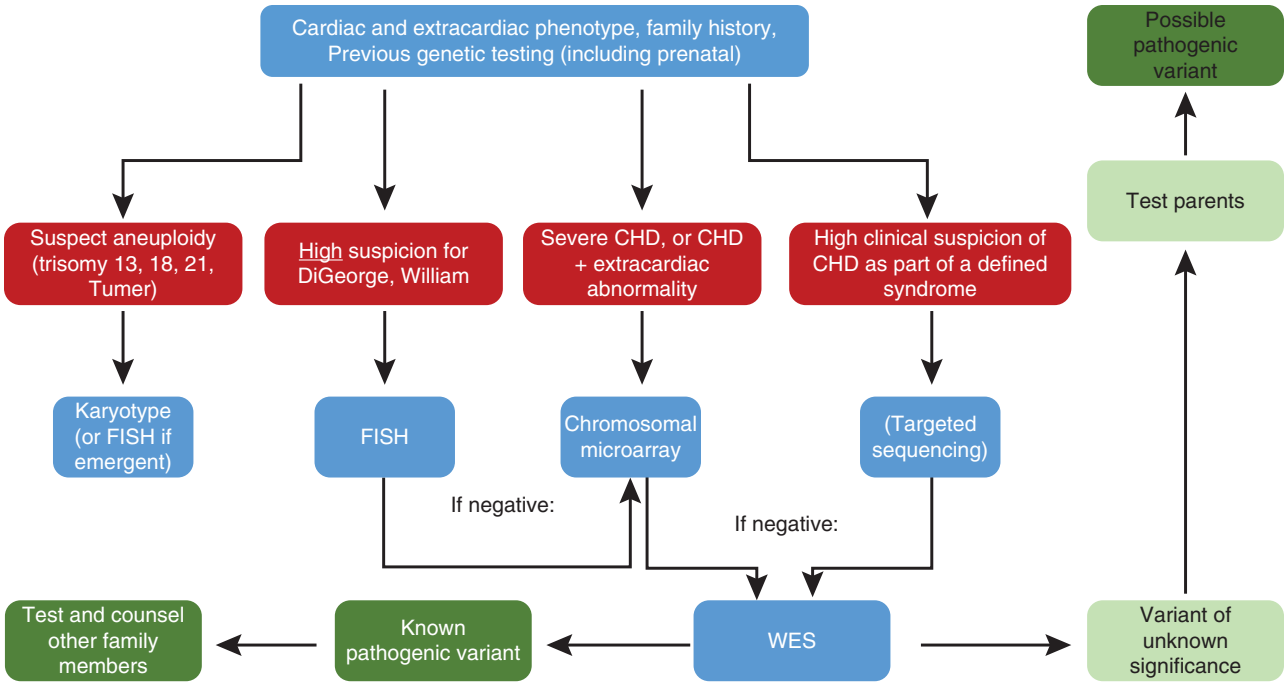
Genetic testing is not only useful for prognostic information for the individual patient but plays a significant role in pre- and postnatal family counseling and future family planning. Referral to a consulting genetics team allows for the consideration of services available to the infant, screening of additional family members, and discussion of the risks to future pregnancies.

Heart Transplantation

Although many congenital heart defects can be corrected or palliated, cardiac transplantation must be considered in instances where there is intrinsic cardiac dysfunction such as cardiomyopathy or in circumstances where surgical repair has not sufficiently corrected a child's hemodynamics. In those cases, cardiac transplantation may be the only therapeutic option.

The first heart transplant in an infant was reported in 1968.[22] Since that time, understanding of transplant immunology and medical management has made heart transplantation in infants and children an important option for inoperable patients or those with end-stage cardiac disease. The indications for pediatric heart transplant were defined by the American Heart Association as:[23]

1. Need for ongoing intravenous inotropic or mechanical circulatory support
2. Complex CHD not amenable to conventional surgical repair or palliation or for which the surgical procedure carries a higher risk of mortality than transplantation
3. Progressive deterioration of ventricular function or functional status despite optimal medical care
4. Malignant arrhythmia or survival of cardiac arrest unresponsive to medical therapy, catheter ablation, or an automatic implantable defibrillator



• **Fig. 50.3** An Algorithm for Genetic Testing in the Congenital Heart Disease Patient. *CHD*, congenital heart disease; *FISH*, fluorescence in situ hybridization; *WES*, whole exome sequencing. (From Zaidi S, Brueckner M. Genetics and genomics of congenital heart disease. *Circ Res*. 2017;120:923–940.)

5. Progressive pulmonary hypertension that could preclude future transplantation
6. Growth failure secondary to severe CHF unresponsive to conventional medical therapy
7. Unacceptably poor quality of life

Children aged 11 to 17 years account for the greatest number of transplants in the pediatric population. Fig. 50.4 demonstrates the reasons for heart transplantation among different pediatric age groups. Examples in the neonate for which cardiac transplantation has been used as primary palliation include hypoplastic left heart syndrome with significant cardiac or extracardiac comorbidities, pulmonary atresia with intact ventricular septum and presence of coronary sinusoids, complex heterotaxy or unbalanced complete AV septal defects with poor common AV valve function, and single ventricle hearts where the dominant semilunar valve is severely insufficient. When considering transplantation there is a balance between the success of palliative surgery and the availability of organs for transplantation. For example, survival of patients undergoing surgical palliation of hypoplastic left heart syndrome (HLHS) has continued to improve.[24,25] With the availability of infant donors increasing only slightly over this period of time (Table 50.2), the balance for treatment of these newborns has shifted toward surgical palliation with the Norwood or hybrid Norwood procedures as the preferred treatment option over transplant. As a result, a decreasing frequency of neonatal transplants has been seen over the last 30 years.[26,27]

Given the limited availability of organs, survival to transplant remains an obstacle in pediatric heart transplantation. Specifically in the neonate, the donor pool has remained limited as infant mortality has improved.[27,28] The use of ABO-incompatible heart transplant protocols has emerged as an option for mitigating the limited neonatal donor pool.[29,30] Evidence supporting use of these protocols has been demonstrated by comparable outcomes for ABO-incompatible and ABO-compatible heart transplantation in children. One philosophy is that the immature immune system of the neonate may slow the production of donor-specific antibodies.[31,32] This may also explain an observed survival advantage in children who receive their transplant prior to 1 month of age versus those between 1 and 12 months.[33] Other considerations may be an immunologic window where graft rejection and transplant coronary artery disease are limited.

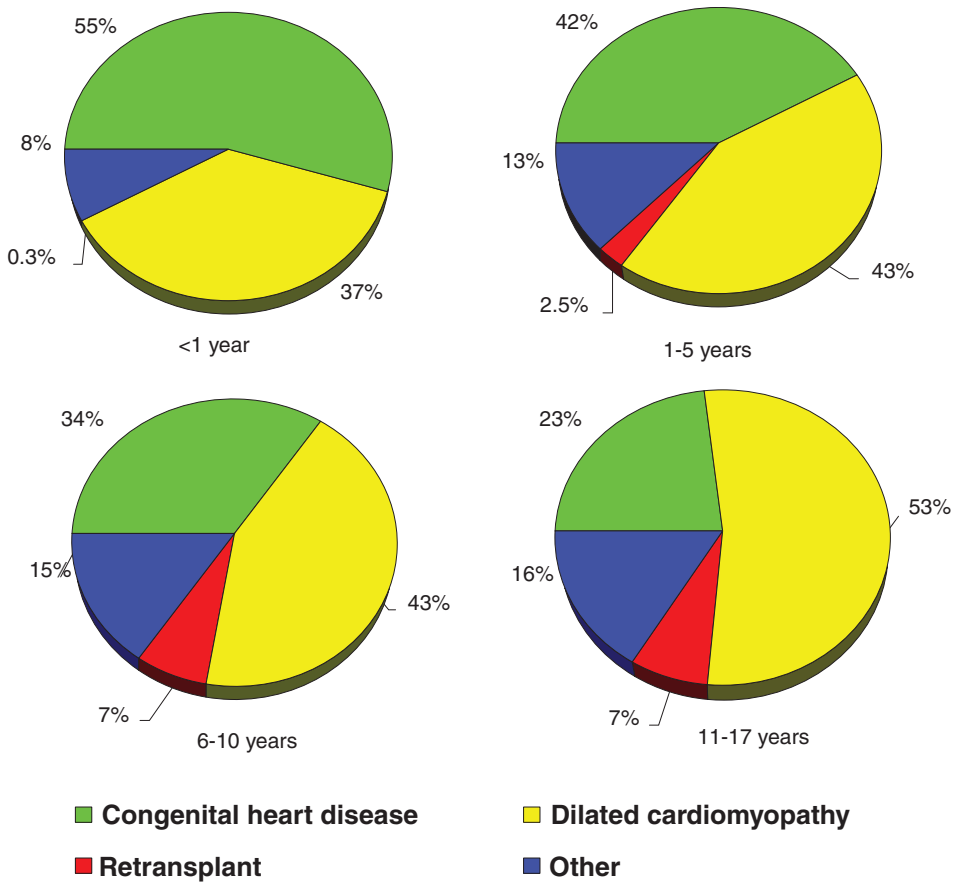
Once transplanted, the long-term survival of infants who undergo heart transplantation in general is quite good. The 10-year survival in infants continues to improve, with reported rates as high as 74%.[27]

Ventricular Assist Devices

Over the past several years, mechanical circulatory support devices have increasingly been developed for the pediatric population to serve as a bridge for postoperative cardiac recovery or heart transplantation. While the availability of devices for neonates lags far behind older children and adults, options are now available.

The mechanical circulatory support device approved for use in children is the Berlin Heart EXCOR pediatric ventricular assist device (VAD). This is a pneumatically driven device that comes in five sizes, with the smallest of the devices delivering a volume of 10 mL per stroke. One or two devices can be implanted to support one or both ventricles, respectively. Studies have looked at survival and complications in infants in the 3 to 5 kg range and a corrected gestational age of at least 37 weeks of age, in comparison with older children.[34] Early mortality was increased in association with lower body weight. In the 33 infants included in the study who were less than 5 kg, 64% died following VAD implantation.

Given the challenges of the use of current VADs in infants, other devices continue to be developed[35,36] and are either in the experimental stage or available for compassionate use. The development of these smaller devices has been supported, in part, by



• **Fig. 50.4** Reasons for Heart Transplantation in Pediatric Population Among Four Age Cohorts. (From Rossano JW, Cherikh WS, Chambers DC, et al. The International Thoracic Organ Transplant Registry of the International Society for Heart and Lung Transplantation: Twenty-first pediatric heart transplantation report-2018; Focus theme: Multiorgan Transplantation. *J Heart Lung Transplant*. 2018;37:1184–1195.)

TABLE 50.2 Number of Available Donors for Infant Heart Transplant by Year in the United States

Year	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015
Donors	89	87	100	116	105	110	106	117	120	126

Data from the Organ Procurement and Transplant Network Database (http://optn.transplant.hrsa.gov/)

the Pediatric Circulatory Support Program of the National Heart, Lung, and Blood Institute. Several devices are in various stage of testing,[35] some of which are small enough in size for use in infants.

Murmurs in the Newborn—Congenital Cardiac Lesions

Patent Ductus Arteriosus and Aortopulmonary Window

Most children are born with a *patent ductus arteriosus (PDA)* that typically closes within the first week of life. Prematurity is a risk factor for a persistent PDA. Persistent PDA in term infants occurs more commonly in females and may have a genetic component in some patients, as suggested by an animal model of inbred poodles[37] and linkage to chromosome 12.[38]

The pathophysiology of a PDA largely depends on the degree of shunting from the aorta to the pulmonary artery, which is determined by the inner diameter and length of the PDA and the relative pulmonary and systemic vascular resistances. If the PDA diameter is small, the ductus itself will provide the primary site of resistance to flow, and the shunt will be small. In the case of a larger PDA, low PVR may allow for a significant shunt that places the patient at risk for developing heart failure and, eventually, pulmonary vascular disease. The low resistance pathway through the lungs provides a route for diastolic run-off from the aorta that can lead to decreased coronary perfusion pressure, resulting in myocardial ischemia and systemic steal, resulting in end-organ dysfunction.

Clinically, patients with a small shunt will be asymptomatic. With a larger PDA shunt, the progressive decline in PVR postnatally will cause an increase in left-to-right shunt flow with signs of increasing heart failure. The murmur in a child with a PDA is generally continuous and has been described as a "machinery" murmur in the left infraclavicular region. The character of the murmur, however, varies greatly, although the continuous nature is generally present once the PVR has declined. Examination of the patient with a PDA will also include a wide pulse pressure because of decreased diastolic pressure and bounding pulses.

The diagnosis of PDA, when suspected on examination, can nearly always be confirmed by echocardiography. Even in the face of high PVR with limited left-to-right shunting, differences in the pulse waveforms between the aorta and pulmonary artery will allow left-to-right and/or right-to-left shunting to be observed by color Doppler imaging. As the degree of left-to-right shunting increases the left heart becomes volume overloaded, which also can be assessed by echocardiogram. In addition, retrograde flow will be seen in the proximal descending aorta when a large shunt is present. Cardiac catheterization is rarely needed unless device closure of the PDA is considered.

The prognosis for small PDAs is quite good, and debate exists as to whether closure of "silent" PDAs, incidentally identified by echocardiography, should be performed.[39] With the most recent recommendations from the American Heart Association suggesting that PDAs do not require subacute bacterial endocarditis prophylaxis,[40,41] there is little need to close these small vessels.

Because of the long-term concerns of pulmonary overcirculation and the development of pulmonary vascular disease, closure of hemodynamically significant PDAs is recommended particularly in the preterm infant. In the preterm infant closure is usually attempted medically with the use of prostaglandin inhibitors such as indomethacin and ibuprofen. Surgical ligation and division can readily be performed through a lateral thoracotomy. However, coil or device closure in the catheterization lab has a lower morbidity and success rates equal to surgery.[42,43] As a result, catheter closure of PDAs has become the preferred method in nonpremature infants. Success of device closure depends in part on the weight of the infant and size and shape of the PDA. With the current array of devices available, closure can be safely performed in most infants weighing more than 4 kg and can be considered in some infants weighing between 2.5 and 4 kg.[44,45] Percutaneous closure of the PDA in the extremely low birth weight population (<2 kg) is being increasingly explored with good efficacy and low adverse event rates.[46,47]

Aortopulmonary (AP) window occurs when there is direct communication between the aorta and main pulmonary artery and is a rare lesion with an incidence of 0.2%.[48] Nearly half of patients with an AP window will have an associated cardiac anomaly. These lesions nearly always result in a large degree of left-to-right shunting. Patients will show signs of CHF on examination and have physical and laboratory findings similar to those for a large PDA. Generally, all AP windows should be closed surgically when they are identified.

Ventricular Septal Defect

VSDs are the most common type of CHD (excluding bicuspid aortic valve). Generally, VSDs are classified into four types (Fig. 50.5):

1. Membranous/Perimembranous
2. Muscular (e.g., apical muscular defects)
3. Inlet (e.g., atrioventricular septal defects)
4. Outlet (e.g., subpulmonary defects)

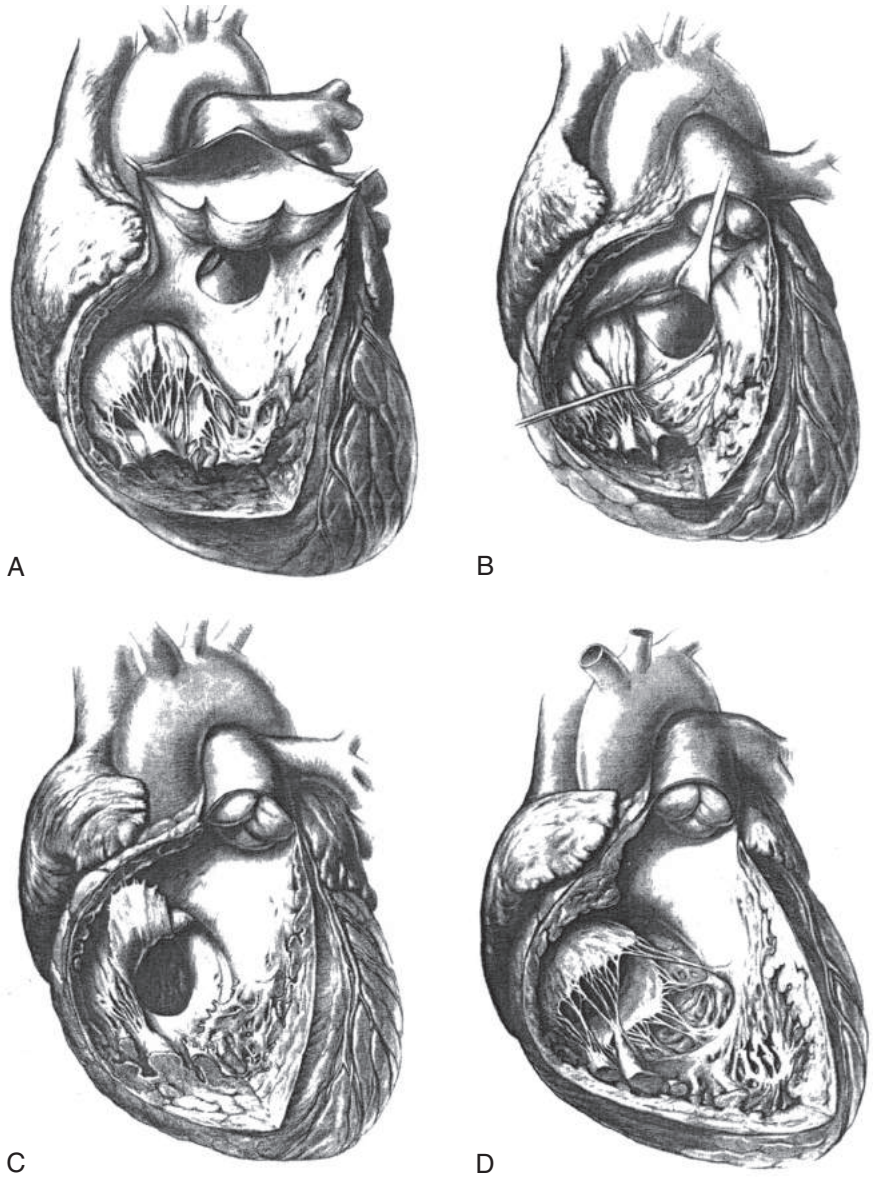
The perimembranous VSD is the most common of the four types and has variably been referred to as membranous, paramembranous, or infracristal. From the right ventricular side of the heart, these defects lie under the septal leaflet of the tricuspid valve below the crista supraventricularis and posterior to the papillary muscle of the conus. Muscular VSDs can occur in isolation or as multiples ("Swiss cheese septum") and, as the name implies, can occur anywhere in the muscular septum. Apical muscular VSDs are the most common and are sometimes difficult to accurately size by echocardiography because of the heavy trabeculations at the apex of the right ventricle. Inlet VSDs are located posterior and inferior to perimembranous defects, and although the nomenclature is controversial, this is the location of the defect in patients with complete atrioventricular septal defects. The location of the VSD in patients with outlet defects is above the crista supraventricularis and typically undermines the right aortic valve leaflet. A variety of synonyms have been used for outlet VSDs including subarterial, subaortic, supracristal, and conal VSD.

The clinical importance of any VSD is dependent upon the size of the defect and the relative pulmonary-to-systemic vascular resistance, which together determine the degree of left-to-right shunting. An additional consideration with outlet VSDs is the degree to which the right coronary cusp of the aortic valve prolapses into the defect and results in aortic insufficiency. Defects whose cross-sectional area is equal to, or greater than, the cross-sectional area of the aortic valve will not restrict flow leaving the left ventricle and entering the right ventricle. In this case, the degree of shunting will be determined by the relative resistance to flow in the pulmonary and systemic vascular beds. The normal postnatal decline in PVR will result in a progressive increase in left-to-right shunting and signs of CHF. A small percentage of children do not have the usual postnatal decline in PVR and may never develop signs of pulmonary overcirculation and heart failure despite the presence of a large VSD. This is an indication for early surgical closure of the defect.

When the VSD is small relative to the aortic valve, the defect itself will be the primary point of resistance to shunt flow. In this case, changes in PVR will have little impact on the degree of left-to-right shunting.

An important associated lesion that is critical to rule out is coarctation of the aorta. The coarctation results in a fixed elevated systemic vascular resistance that can result in significant left-to-right shunting even in the presence of a small VSD. In this case, medical therapy is often unable to control CHF symptoms, and surgery is needed.

The volume of shunted blood is most accurately quantitated at cardiac catheterization based on the step-up in oxygen saturation from the right atrium (mixed venous) to the pulmonary artery and is represented as the ratio of pulmonary-to-systemic blood flow (Qp:Qs). Generally, a Qp:Qs less than 1.5 is considered below the threshold for surgery, while a Qp:Qs greater than 2.0 is an indication for surgery. The Qp:Qs can also be estimated by echocardiography and by magnetic resonance imaging (MRI). Modern era management of isolated ventricular septal defects is rarely determined by Qp:Qs quantification and relies more heavily on clinical evaluation.



• **Fig. 50.5** Anatomic Varieties of Ventricular Septal Defect. (A) Subpulmonary defect. (B) Membranous defect. (C) Inlet (atrioventricular septal defect type) defect. (D) Apical muscular defects. (From Mavroudis C, Backer CL. *Pediatric Cardiac Surgery*. St. Louis: Mosby Year Book; 1994.)

The examination of the patient with a VSD depends on the magnitude of the shunt. Small defects that provide considerable restriction to flow often have the loudest murmur. The rapid drop in PVR immediately after birth often allows VSD murmurs to be heard in the newborn nursery, although the full extent of the murmur, and perhaps a thrill at the lower left sternal border, may not be appreciated for several weeks. The murmur may have a more ejection quality in the newborn nursery in the face of high PVR and somewhat elevated right ventricular pressure. The more typical holosystolic murmur will be more apparent as the PVR falls.

With large VSDs, little or no murmur may be heard depending on the PVR. With low PVR, signs of heart failure will likely be present including tachypnea with nasal flaring and retractions, tachycardia, diaphoresis, poor feeding, and diminished weight gain. A systolic murmur at the lower left sternal border (caused by flow across the VSD) or upper left sternal border (caused by increased flow across the right ventricular outflow tract [RVOT]) may be heard along with a diastolic inflow rumble (an absence of silence) at the apex. If PVR is high, the pulmonic component of the second heart sound may be increased although difficult to appreciate. Occasionally, large defects may allow transient right-to-left shunting to occur, particularly when the infant is crying.

In patients with an outlet VSD, the holosystolic murmur is often present, but the murmur is located higher on the left sternal border. Care should be taken to listen for the diastolic decrescendo of aortic insufficiency at the mid-left sternal border or at the apex.

The evaluation of the infant with a suspected VSD should include an ECG, chest x-ray, and echocardiogram. In infants, the ECG may not be distinctive unless an inlet VSD is present. Obtaining a chest x-ray, even in the neonate, is important in order to assess heart size and pulmonary vascular markings. It can also be an important tool in the follow-up of newborns with VSDs

when used to assess progression in left-to-right shunting as the PVR declines. Echocardiography is the gold standard for characterizing the location and size of VSDs. Associated lesions, such as coarctation of the aorta, can also be readily assessed by echocardiography. Doppler studies can estimate the degree of restriction by calculating the pressure drop at the defect. M-mode measurements can be used to determine left ventricular dimensions, which will be increased when a significant left-to-right shunt is present. As mentioned above, cardiac catheterization can accurately quantitate the degree of shunting but is rarely needed in the initial assessment of the newborn with VSD.

Up to 80% of small, muscular VSDs and 30% to 50% of perimembranous defects will close spontaneously. It is uncommon for these defects to increase in size, although the degree of left-to-right shunting can increase as PVR drops. Occasionally, inlet VSDs will undergo closure secondary to chordal attachments of the AV valves, but closure is generally present at birth if it is going to occur. Outlet VSDs virtually never close and have the associated risk of progressive aortic insufficiency because of prolapse of the right coronary cusp into the defect.

The short-term consideration in following patients with VSDs is the management of CHF if the left-to-right shunt is excessive. Medications that are used include diuretics to augment fluid shifts and less frequently afterload reduction with agents such as angiotensin-converting enzyme inhibitors. The primary goal in controlling CHF is to allow the newborn to grow adequately and hopefully allow progressive closure of the VSDs. Weight gain is a useful and objective measure to follow.

The long-term goal of therapy or intervention is to prevent the development of irreversible pulmonary vascular occlusive disease. Shunts with a Qp:Qs greater than 2.0 are at long-term risk of developing Eisenmenger disease, and closure of the defect is warranted beyond 2 to 4 years of age when further decline in defect size is unlikely.

If medical therapy fails to control heart failure and the infant exhibits failure to thrive, surgical closure of the defect in the first 6 months of life is usually necessary. Surgical closure is also indicated in the 6- to 12-month-old child with a large VSD who has not demonstrated signs and symptoms of CHF due to lack of decline in PVR. In these infants, irreversible changes in the pulmonary vasculature may occur if the pressure load is not removed from the lungs. As mentioned previously, defects with a Qp:Qs greater than 2.0 and outlet defects also require closure. The use of pulmonary artery banding is falling out of favor for palliation of patients with VSDs unless the patient's clinical condition precludes complete repair. There is growing use of hybrid procedures where the surgeon and interventional cardiologist work together to close defects in small children.[49] This combined approach has successfully been applied to the closure of muscular VSDs in infants that are positioned in a location that is difficult for the surgeon to visualize from a right atrial approach.

Atrial Septal Defects

Although atrial septal defects (ASDs) rarely produce a murmur in the neonatal period, the systolic murmur they generate later in life makes them appropriate for this section. As discussed earlier, atrial level shunting through the foramen ovale in utero allows the nutrient-rich placental blood to gain access to the left ventricle and ascending aorta. In the immediate postnatal period, careful echocardiography examination of the interatrial septum will usually identify residual left-to-right or bidirectional shunting through the foramen ovale. Measurement of the size of the shunt provides an indication of whether there will be a persistent septal defect. An opening less than 6 mm in a term infant will most likely close and is referred to as a patent foramen ovale (PFO) to distinguish it from a true ASD. ASDs that represent congenital lesions are classified as:

1. Secundum
2. Primum
3. Sinus venosus
4. Coronary sinus

Secundum defects are the most common and, when present in the neonate, allow for left-to-right or bidirectional shunting. The direction of the shunt, and the reason an ASD murmur is typically not heard in the neonate, is that right ventricular compliance remains low until the RV remodels postnatally. The reduced RV compliance increases right atrial pressure and limits the amount of left-to-right atrial level shunting. Primum ASDs are in the spectrum of atrioventricular septal defects and will be considered in the next section. Sinus venosus defects occur when the wall separating the upper or lower right pulmonary vein is deficient so that pulmonary venous return from either or both veins enters into the right atrium. This results in a left-to-right shunt. This variant of anomalous pulmonary venous return will be considered in greater detail further in the chapter. Coronary sinus defects result from an "unroofing" of the coronary sinus so that the coronary sinus enters at the left atrium–right atrium junction where the septum is deficient. Often, this lesion is associated with a persistent left SVC that enters into the coronary sinus.

In later childhood, when the compliance of the right ventricle increases and left-to-right shunting through an ASD increases, the classic physical findings of fixed splitting of the second heart sound can be auscultated. A systolic ejection murmur at the left upper sternal border and a diastolic right ventricular inflow murmur at the lower left sternal border become apparent in lesions with a Qp:Qs more than 2.0. ECG will often demonstrate an RSR pattern in the right precordial leads with evidence of right ventricular hypertrophy. Cardiomegaly with a prominent main pulmonary artery segment and increased vascular markings will be seen on chest x-ray. Echocardiogram remains the gold standard for identifying and sizing the defects.

It is uncommon for infants and children with an isolated ASD to develop heart failure. Typically, intervention for a significant ASD is performed to prevent the long-term sequelae of pulmonary vascular disease. As such, closure is rarely performed before the age of 3 to 4 years. Device closure of secundum ASDs is now routinely performed in the catheterization lab when children are greater than 15 kg, although surgical closure is still performed when lesions are large or the rim of tissue between the ASD and aorta is deficient.

Atrioventricular Septal Defects

A variety of terms have been used to describe atrioventricular septal defects including AV canal defect, endocardial cushion defect, and common AV orifice. In addition, there is a spectrum of AV septal defects:

1. *Complete*—common AV valve is present along with a significant primum ASD and inlet VSD.
2. *Intermediate*—common AV valve annulus although separate AV valves along with a primum ASD and inlet VSD.
3. *Transitional*—completely separate AV valves along with a primum ASD and inlet VSD.

4. *Partial*—separate AV valves with a primum ASD and no VSD.

These four groups have some therapeutic relevance with regard to the degree of AV valve abnormality that must be considered at surgery, although each has a cleft between the anterior bridging leaflet and the lateral leaflet of the left-sided (mitral) valve that must be addressed. In infants with a complete AV septal defect, it is important to assess whether the right and left ventricles are *balanced*, that is, equally developed so that a two-ventricle repair is possible, and the degree of AV insufficiency, which has prognostic importance in the surgical outcome. AV septal defects are common in children with Down syndrome.

In the immediate postnatal period, variations in PVR that occur, particularly in infants with Down syndrome, can result in a right-to-left shunt through a nonrestrictive primum ASD and/or inlet VSD and result in systemic desaturation. Many infants with complete AV septal defects will develop heart failure in the first 2 months of life as PVR falls. A small percentage of infants will not have a decline in PVR, making the lack of heart failure a troubling sign and an indication for early surgical repair.

While diagnosis largely rests on the echocardiogram, AV septal defects can be suspected on ECG with the presence of a superior frontal QRS axis (ranging from −30 to −120 degrees), evidence of right ventricular hypertrophy, and Q waves in leads I and aVL. Echocardiogram is used to define the type of AV septal defect and identify associated anomalies (Fig. 50.6). As mentioned above, critical assessment of relative ventricular sizes and the degree of AV valve insufficiency is necessary. Interrogation of the RVOT is also needed due to the associated pulmonic stenosis or TOF that can occur with complete AV septal defects.

Correction of complete AV septal defects is performed surgically at 4 to 6 months of age. While awaiting surgery, care must be taken not to treat minor desaturation episodes with excessive oxygen since oxygen-induced lowering of PVR can rapidly worsen heart failure and lead to further desaturation, a spiral that can be difficult to reverse. Diuretics and afterload reduction are often needed to control heart failure, and some cardiologists will start these medications in the immediate postnatal period because of the high likelihood of infants developing CHF. Complete repair (septation atria, ventricles, and AV valves) is preferred. It is very rare that palliative banding of the pulmonary artery is needed to control heart failure symptoms. As mentioned above, early repair should be considered in the infant who does not have a decline in PVR and the development of heart failure, because of the concern of early development of irreversible increases in PVR, particularly in non-Down syndrome complete AV septal defect patients.

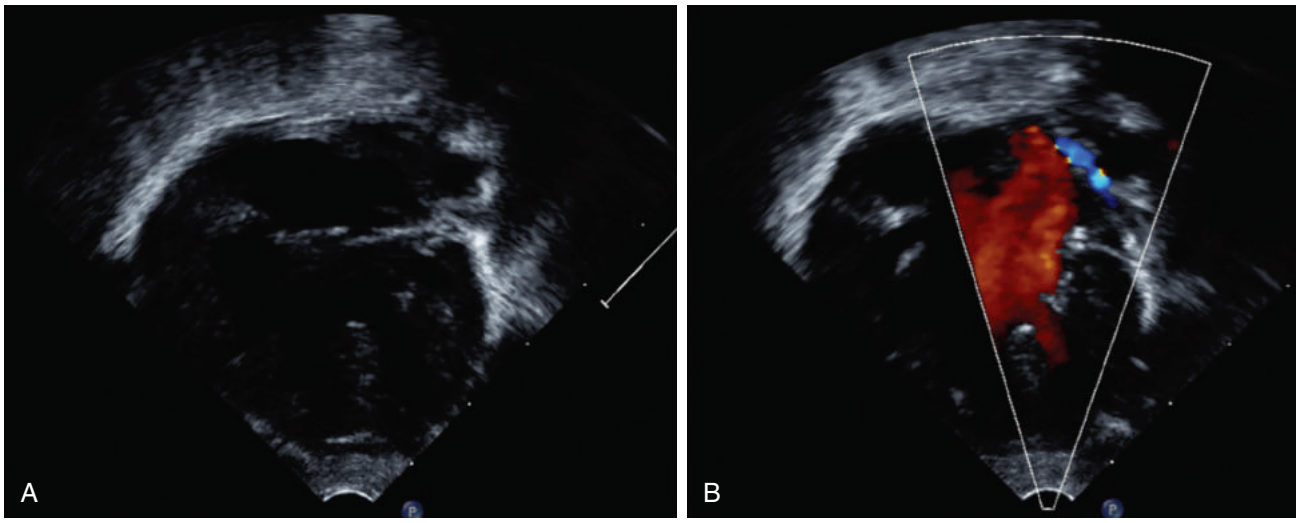
Peripheral Pulmonic Stenosis

Peripheral pulmonic stenosis (PPS) can range widely in its severity. In many instances, mild narrowing of the distal branch pulmonary arteries occurs in the neonate and produces a murmur that is heard widely throughout the chest. The murmur caused by this mild PPS is considered by some to be an "innocent murmur" of infancy and usually resolves by 2 to 4 months of age. This lesion likely reflects mild hypoplasia of the branch pulmonary arteries because of decreased in utero pulmonary blood flow and the postnatal transition where these vessels must accommodate the entire cardiac output following ductal closure.

More severe PPS is seen in cases of congenital rubella syndrome or in association with Williams or Noonan syndrome. Branch pulmonary artery stenoses can also occur with TOF. In these cases, multiple levels of obstruction may exist that require catheterization or surgical intervention.

In isolated or mild PPS, minimal right ventricular pressure overload occurs. With increasing stenosis, right ventricular pressure overload will result in right ventricular hypertrophy and, in later stages, right atrial enlargement on ECG. Echocardiogram can interrogate the proximal pulmonary arteries, but more-distal lesions require other imaging modalities such as MRI or cardiac catheterization.

Intervention to treat severe branch stenoses should be considered when right ventricular pressure is greater than 75% of systemic pressure or any clinical or laboratory evidence of right ventricular dysfunction is present. Surgical management is possible for proximal areas of stenosis, although the treatment preferred by most clinicians is balloon dilation or expandable stent placement in the cardiac catheterization laboratory. Repeated



• **Fig. 50.6** Echocardiographic View of Complete Atrioventricular Septal Defect. (A) Four chamber view showing the common atrioventricular valve that separates the atria and ventricles, which in this patient are well balanced. The large primum atrial septal defect and inlet ventricular septal defect are seen. (B) With color Doppler, inflow across the atrioventricular valve is seen.

interventions may be needed to enlarge vessels as the patient grows or to dilate other areas of stenosis that develop.

Pulmonic Stenosis

Isolated pulmonary valve stenosis is a common cause of a systolic ejection murmur in the neonatal period. While cases typically occur sporadically, pulmonic stenosis can be related to underlying genetic defects such as Noonan syndrome, Alagille syndrome, and Williams-Beuren syndrome.[50]

The degree of pulmonic stenosis determines the pathophysiology of the disease process. As the stenosis of the valve worsens, right ventricular pressure increases along with the degree of right ventricular wall stress. Right ventricular hypertrophy will develop if the stenosis is left untreated. In severe or critical pulmonic stenosis (discussed later in the section), heart failure can develop in the neonate accompanied by cyanosis due to right-to-left shunting at the atrial level.

The degree of stenosis is generally classified based on the pressure drop across the pulmonic valve with mild stenosis defined as a gradient less than 30 mmHg, moderate stenosis as a gradient of 30 to 60 mmHg, and severe stenosis as higher than 60 mmHg. While these definitions were initially based on cardiac catheterization, current follow-up of patients with pulmonic stenosis utilizes echocardiogram, which overestimates the gradient compared with direct hemodynamic measurement obtained from cardiac catheterization. Thus some use an *echo gradient* of less than 40 mmHg as mild pulmonic stenosis.

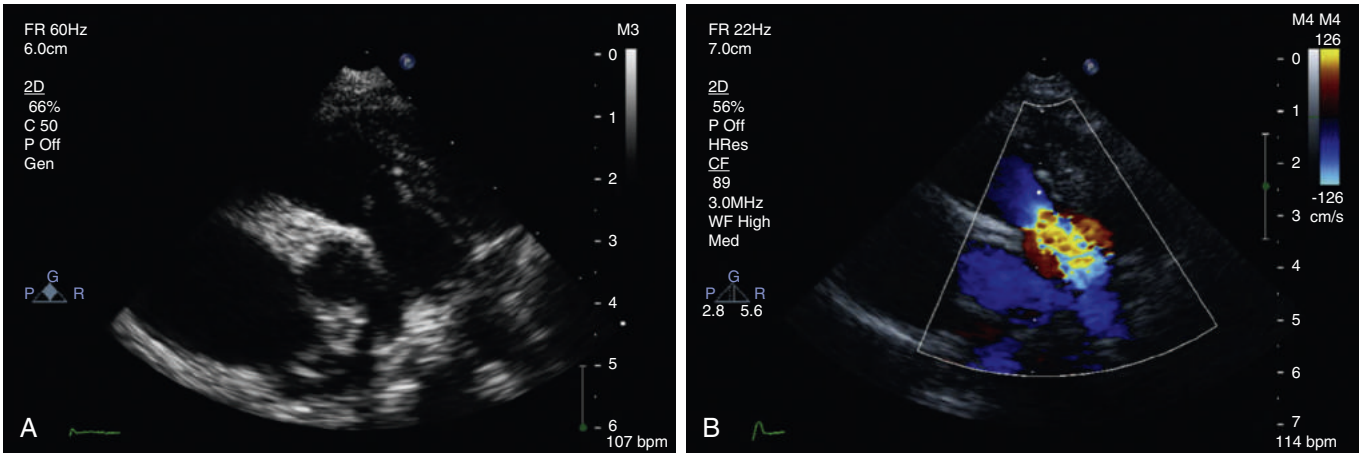
Consideration of the valve gradient is critical because of the prognostic significance of the value. In older children, mild stenosis rarely progresses.[51] In infants, however, follow-up of patients with echo gradients over 40 mmHg found that 29% developed progressive valve stenosis, with half of those showing an increase in the first 6 months of life.[52] A systolic ejection murmur of pulmonic stenosis can be heard in the neonatal period at the upper left sternal border. Typically, although the fast heart rate in the neonate may make it difficult to appreciate, a systolic ejection click just after the first heart sound (S1) can be heard in most of these infants and is an important feature to distinguish pulmonic stenosis from other lesions. Much like PPS (see earlier), the murmur of pulmonic stenosis radiates throughout the lung fields. As the gradient across the valve worsens, a thrill may be palpable at the upper left sternal border. As the degree of stenosis progresses further and becomes severe, the murmur and click will diminish and may even be absent as right ventricular dysfunction worsens. Of note is that while progressive pulmonic stenosis may be able to be estimated on the basis of the murmur, the clinical condition of the infant may not change appreciably until the degree of stenosis becomes severe.

The findings of laboratory studies in infants with pulmonic stenosis will vary depending on the degree of stenosis. ECG will demonstrate right ventricular hypertrophy in most patients with moderate stenosis, although the study may be normal when mild stenosis is present. Chest x-ray is often normal unless poststenotic dilation of the main pulmonary artery has developed. Echocardiography will be diagnostic. The valve will have varying degrees of dysplasia that is characterized by thickened, poorly mobile leaflets that dome during systole. Turbulence distal to the valve will be seen by color Doppler imaging (Fig. 50.7), while pulse wave Doppler is used to determine the degree of stenosis. As discussed previously, Doppler echo generally gives a value of stenosis that is 20% to 30% higher than the peak-to-peak pressure gradient measured as cardiac catheterization.

Treatment of isolated pulmonic stenosis, even in the neonate, can readily be performed by balloon valvuloplasty in the cardiac catheterization laboratory. The exception may be in infants with Noonan syndrome where the degree of valvar dysplasia may prevent an adequate result, although most will initially attempt a balloon valvuloplasty before progressing to surgery. Valvuloplasty should be considered in infants with moderate to severe stenosis (peak gradient of greater than 50 mmHg).[53] Mild or moderate pulmonary insufficiency, should it develop after balloon valvotomy, is usually well tolerated. Recurrent stenosis and more significant pulmonic insufficiency are found more often when valvuloplasty is needed in the neonatal period.[54]

Aortic Stenosis

Similar to stenosis of the pulmonic valve, aortic valve stenosis can produce a murmur in the neonatal period. However, aortic stenosis is more likely to be progressive than pulmonic stenosis.[55] Levels of obstruction of the left ventricular outflow tract can also occur at the subvalve and supravalve levels. Subvalvar stenosis is rarely diagnosed in the neonatal period but often progresses later in life



• **Fig. 50.7** Pulmonic Stenosis. (A) Short-axis view of pulmonic stenosis with a thickened-appearing pulmonic valve. (B) Using color Doppler imaging, turbulence in the main pulmonary artery is seen above the pulmonic valve.

as a fibromuscular ridge beneath the aortic valve. Supravalvar stenosis can present in the newborn period and is often associated with Williams syndrome.[56]

As with pulmonic stenosis, the pathophysiology and physical findings associated with aortic valve stenosis depend on the degree of obstruction. The most common aortic valve abnormality addressed in the neonatal period is critical aortic stenosis associated with decreased left ventricular function. This medical emergent situation is addressed in detail later. Mild (aortic valve gradient <30 mmHg) and moderate (valve gradient of 30 to 60 mmHg) aortic valve stenosis are not commonly encountered in the neonatal period. When they are, these milder forms of stenosis are generally associated with a bicuspid aortic valve and tend to progress with age. The rate of change of progression throughout infancy and childhood is quite variable and necessitates frequent follow-up of these patients.

The murmur in the newborn with aortic stenosis may be difficult to localize to the upper right sternal border as in older children. A thrill is usually palpable at the suprasternal notch with even mild aortic valve stenosis, with a precordial thrill felt as the degree of stenosis increases. Usually a click from the stenotic aortic valve is heard at the apex or lower left sternal border.

Electrocardiography may be of limited benefit in the newborn with mild or even moderate aortic valve stenosis, although some degree of left ventricular hypertrophy may be seen. Chest x-ray is of limited benefit in following these patients, with the focus of the diagnostic assessment being the echocardiogram. On echocardiogram, the aortic valve can be characterized. Fused leaflets that are thickened and domed are seen. Pulsed Doppler is used to determine the gradient across the valve while color Doppler imaging can define the presence of aortic insufficiency.

Initial management of moderate aortic valve stenosis is balloon valvotomy in the cardiac catheterization laboratory. The balloon is sized in an effort to limit aortic insufficiency, which is much less well tolerated than insufficiency of the pulmonic valve. If significant aortic valve insufficiency is present or develops, surgical repair or valve replacement is needed. In neonates and infants, the Ross procedure is usually the procedure of choice.[57] In this procedure, the pulmonary valve is removed intact from the patient and sewn into the aortic position, and a homograft is placed in the pulmonic position. Interestingly, the pulmonary autograft generally demonstrates good growth and excellent function in the aortic position. While the child will outgrow the pulmonary homograft, replacing it and managing progressive pulmonic stenosis are much easier than addressing the aortic valve.

Cyanosis in the Newborn

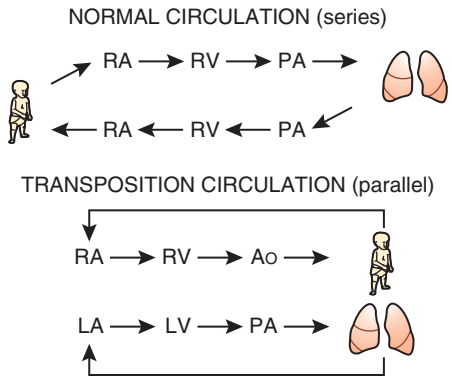
Congenital heart defects that present with cyanosis do so because of a right-to-left shunt. The shunt results in mixing of the systemic and pulmonary venous returns thereby leading to cyanosis. This mixing can occur between the atria, ventricles, or great vessels. Defects presenting predominantly with cyanosis can be further subclassified by the amount of associated pulmonary blood. If there is no restriction to pulmonary blood flow, cardiac output to the lungs will increase as the normal postnatal drop in PVR occurs. Clinically, this results in signs of congestive heart failure (CHF) such as tachypnea, poor feeding, hepatomegaly, and pulmonary edema. Much of the neonatal management is aimed at balancing the ratio of systemic to pulmonary blood flow. Defects with restriction to pulmonary blood flow typically present with cyanosis without associated symptoms of CHF. If the restriction is severe, pulmonary blood flow may be dependent upon a left-to-right shunt through the ductus arteriosus. These patients typically have some degree of right ventricular hypertension. If the ductus closes, profound cyanosis results. This is termed ductal dependent pulmonary circulation and initial management includes maintaining patency of the ductus arteriosus with prostaglandin E_1 (PGE_1).

Transposition of the Great Arteries

Transposition of the great arteries (TGA) accounts for 5% of all CHD and is the most common cyanotic cardiac defect that presents with cyanosis in the newborn period. There is a strong male predominance (60% to 70%). TGA is rarely associated with chromosomal abnormalities or extracardiac malformations. It can, however, be associated with dextrocardia and lateralization defects like heterotaxy, in particular right isomerism (asplenia).[58] It has been associated with exposure to maternal hyperglycemia as well as high maternal intake of vitamin A.[58–60]

In the most common form of TGA (D-TGA, complete transposition, or simple transposition), the aorta arises from the right ventricle anteriorly and rightward of the pulmonary artery, which arises from the left ventricle. Desaturated blood returns to the right ventricle and is recirculated to the body via the aorta, while oxygenated blood returns to the left ventricle and is recirculated to the lungs. The result is separate, parallel circulations (Fig. 50.8). Survival is dependent upon communication between the two circulations, typically in the form of bidirectional shunting at the PFO and PDA. With absent or small communications between the circulations, severe systemic acidosis and hypoxia develop after birth resulting in death.

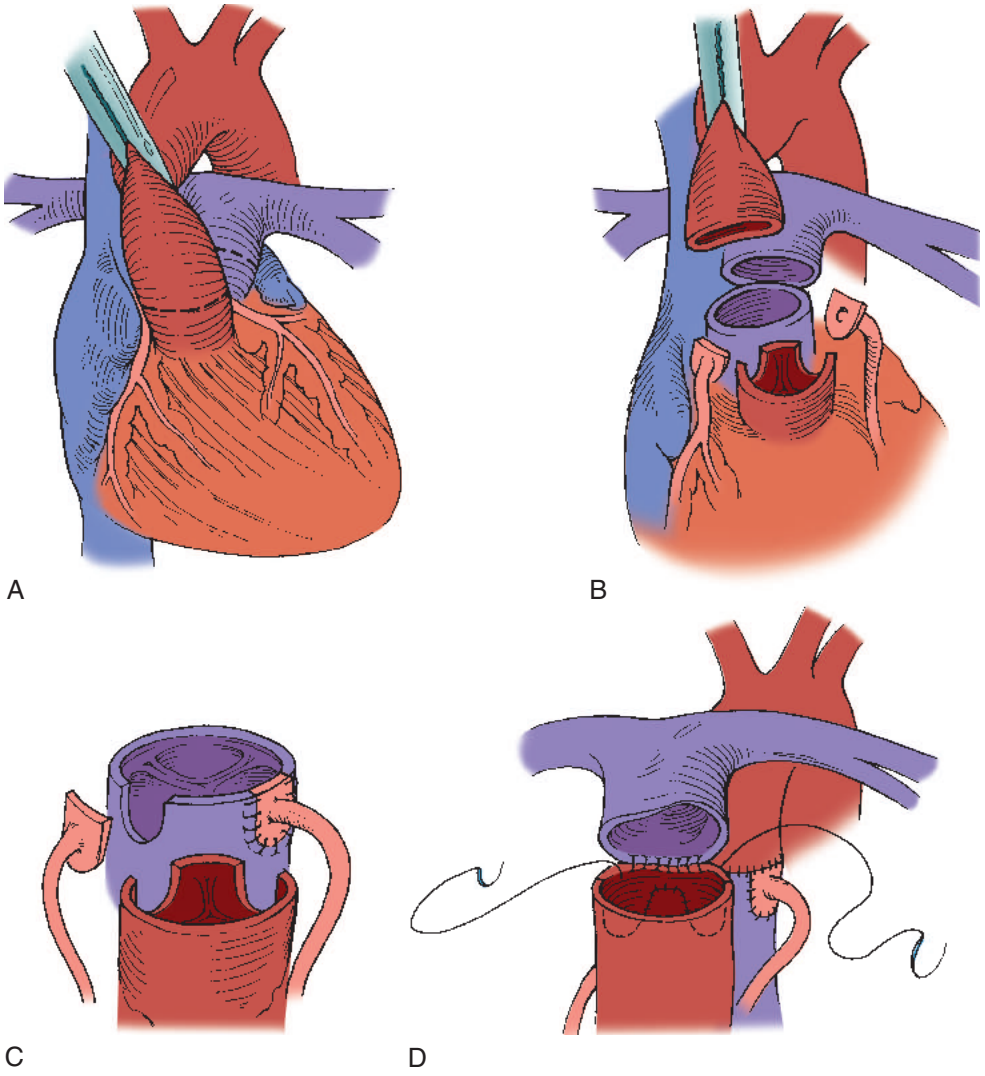
Prenatal diagnosis of DTGA has been shown to improve postnatal outcomes as it allows for prenatal planning of postnatal management.[61] DTGA is a difficult diagnosis to make likely owing to the presence of a normal 4 chamber view. This has been improving with the addition of outflow views to screening ultrasounds. However, prenatal detection rates for DTGA still remain lower than for other critical CHD.[62] In addition, consistent identification of patients with critical D-TGA who will require a balloon atrial septostomy within the first 24 hours of life has been inadequate.[63]



• **Fig. 50.8** Series and Parallel Circulations. The series circulation in the normal newborn is compared with the parallel circulation in transposition of the great arteries (TGA). In TGA, survival after birth is dependent on mixing at either the atrial, ventricular, or great vessel level. *Ao*, Aorta; *LA*, left atrium; *LV*, left ventricle; *PA*, pulmonary artery; *RA*, right atrium; *RV*, right ventricle. (From Wernovsky G. Transposition of the great arteries. In: Allen HD, Gutgesell HP, Clark EB, Driscoll DJ, eds. *Moss and Adams' Heart Disease in Infants, Children, and Adolescents: Including the Fetus and Young Adult*. 6th ed. Philadelphia: Lippincott Williams & Wilkins; 2001:1027–1084.)

In TGA physiology, cyanosis is apparent within the first few hours of life and is not responsive to oxygen supplementation. Cardiac examination is typically normal except for a loud single S2. This is because of the arrangement of the great vessels; the louder closure of the anterior aortic valve obscures the closure of the more posterior pulmonary valve. Reversed differential cyanosis, higher saturations in the lower body than in the upper body, is a phenomenon that can briefly happen during the first few hours of life as the PVR remains elevated and the duct is able to shunt bidirectionally. Chest x-ray may be normal or reveal a narrow mediastinum with slight predominance of the right ventricle resulting in an "egg on a string" appearance. Pulmonary vascular markings are normal to slightly increased. ECG is typically normal but may reveal right atrial enlargement and right ventricular hypertrophy. Echocardiogram reveals the transposed great vessels and is used to determine the size of the PFO and PDA, coronary anatomy, as well as define any associated cardiac defects.

Initial management of infants with TGA is aimed at maintaining communication between the systemic and pulmonary circulations. PGE_1 is started to maintain patency of the ductus arteriosus (dose of 0.03 to 0.1 mcg/kg/min). The lowest effective dose is used, as there are side effects to the use of PGE_1, the most common of which is apnea and may require respiratory support. In patients with a restrictive PFO, or with persistent hypoxia and acidosis despite a PDA, a balloon atrial septostomy (BAS) is performed within the first 24 to 48 hours of life to encourage mixing at the atrial level. Persistent pulmonary hypertension of the newborn can further complicate the clinical status leading to persistent hypoxia and acidosis even after balloon atrial septostomy. These children tend to have worse outcomes and may require extracorporeal membrane oxygenation (ECMO) as a bridge to surgery. In the setting of adequate mixing, pulmonary overcirculation may develop as PVR drops. Surgical treatment is the arterial switch procedure (Fig. 50.9), typically performed within the first week after birth.[64,65]



• **Fig. 50.9** Arterial Switch Operation for Dextro-Transposition of the Great Arteries. (A) The *dashed lines* depict the planned location of transection of the great vessels. (B) The coronary arteries are removed with surrounding aortic wall as "buttons." (C) The coronary buttons are translocated to the posterior neoaortic root. (D) The Lecompte maneuver brings both pulmonary arteries anterior to the neoaorta. The aortic suture line is completed, incorporating the coronary buttons. The coronary donor sites are filled in with patches of autologous pericardium, and the pulmonary anastomosis is completed. (From Wernovsky G, Jonas RA. Transposition of the great arteries. In: Chang AC, Hanley FL, Wernovsky G, Wessel DL, eds. *Pediatric Cardiac Intensive Care*. Baltimore: Williams & Wilkins; 1998:289–301.)

While TGA is usually an isolated lesion, 40% to 45% of patients have an associated VSD, 10% have a VSD with left ventricular outflow tract obstruction, and 5% have left ventricular outflow tract obstruction alone. In addition, there is a wide variety in coronary artery anatomy in patients with TGA. This can affect the surgical technique used for transfer of the coronary arteries and thus add to the complexity and risk of surgical correction.

The clinical presentation of patients with TGA and VSD depends upon the size of the VSD. Those with a restrictive VSD present like patients with TGA and intact ventricular septum. Those with a large VSD may not demonstrate any symptoms initially. As PVR drops, symptoms of pulmonary overcirculation develop. In general, presence of a VSD does not provide for adequate mixing, and balloon atrial septostomy is still warranted for patients with persistent hypoxia and inadequate atrial level mixing. Patients with restricted pulmonary blood flow (TGA with VSD and pulmonic stenosis or LVOT obstruction) appear clinically similar to patients with TOF. The timing and type of surgical repair in patients with complex TGA are variable and beyond the scope of this chapter.

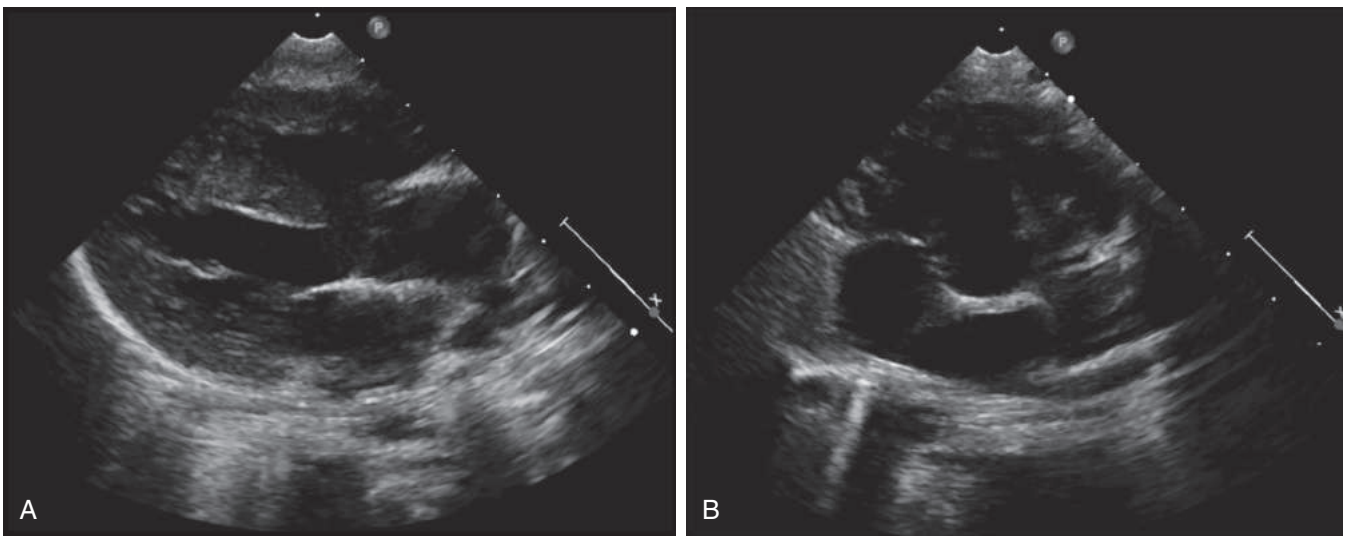
Tetralogy of Fallot

Tetralogy of Fallot (TOF) is the most common cyanotic CHD and comprises about 10% of all CHD. It consists of a VSD, overriding aorta, RVOT obstruction, and right ventricular hypertrophy (Fig. 50.10). The severity and location of the RVOT obstruction are variable and lead to a spectrum of diseases. Anterior deviation of the infundibular septum, infundibular hypertrophy, a small, thickened and frequently bicuspid pulmonary valve, and small main pulmonary artery can jointly or independently create the classic multilevel obstruction at the subvalvar, valvar, or supravalvar level. Complete obstruction of the RVOT (pulmonary atresia) with a VSD is an extreme form of TOF. TOF is associated with a right aortic arch in 25% of cases,[66] and aberrant coronary arteries in 5% to 6% of cases[67] both of which can affect surgical planning.

The etiology of TOF is heterogeneous. Maternal diabetes, phenylketonuria, and retinoic acid exposure have been associated with TOF. In addition, genetic syndromes including 22q11 deletion (20%), trisomy 21, 18, and 13, Alagille syndrome, Holt-Oram syndrome, and VACTERL/VATER association have been associated with TOF. Prenatal diagnosis of TOF can be as high as 90%.[68] It allows for identification of cases in which there will be ductal dependent pulmonary circulation and thus the need for PGE_1 at birth.

The pathophysiology and clinical presentation of TOF are directly tied to the severity of the RVOT obstruction. With mild RVOT obstruction, the predominant shunt through the VSD is left to right. Cyanosis is minimal, and symptoms of heart failure are the typical presenting symptoms. The main and branch pulmonary arteries are usually of normal size. With moderate RVOT obstruction, the shunt through the VSD is right to left or bidirectional but the prograde flow through the RVOT is adequate, resulting in mild cyanosis. The main and branch pulmonary arteries may have areas of focal stenosis or be diffusely small. These patients may have instances of hypercyanotic spells ("Tet spells"), which are periods of increased cyanosis brought on usually by agitation. With severe RVOT obstruction, there is minimal or no prograde flow across the RVOT. Pulmonary blood flow is thus dependent upon left-to-right shunt through the PDA. These patients are severely cyanotic, and PDA closure leads to cardiovascular collapse. The main and branch pulmonary arteries may be confluent and small. With pulmonary atresia (pulmonary atresia VSD or tetralogy pulmonary atresia), the pulmonary vascular bed is variable. The pulmonary arteries can be confluent, normal-sized, and fed entirely by the ductus arteriosus; confluent and small with a small ductus; or virtually absent with pulmonary blood flow supplied by multiple AP collateral vessels.

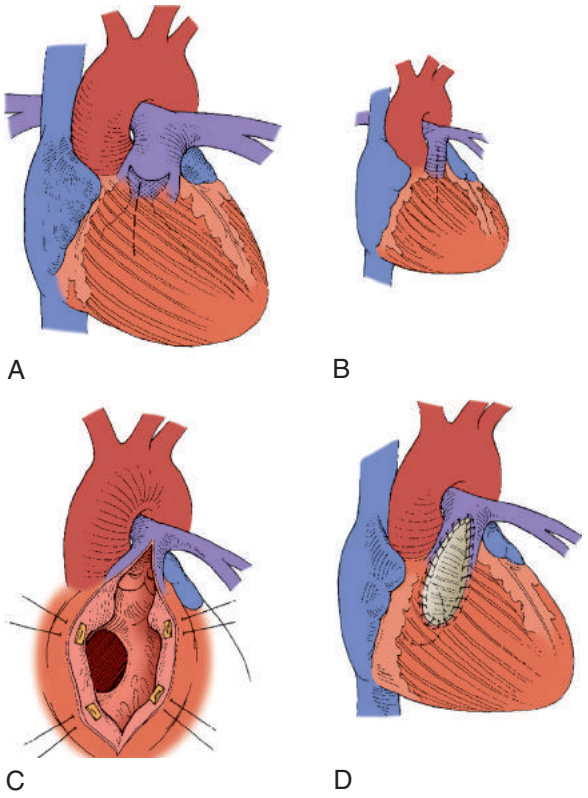
In general, physical exam findings will also vary depending on the degree of obstruction to pulmonary blood flow. A loud systolic ejection murmur can be appreciated as there is turbulence of flow across the pulmonary outflow. The murmur will be louder and shorter with increasing pulmonary obstruction. Those with pulmonary atresia and major aortopulmonary collateral arteries (MAPCAs) can have continuous murmurs heard best over the back but no pulmonary stenosis murmur. The ECG is usually consistent with right axis deviation and right ventricular hypertrophy. The CXR can have a classic "boot-shaped"



• **Fig. 50.10** Echocardiographic View of Tetralogy of Fallot. (A) Some of the anatomic features of tetralogy of Fallot are readily demonstrated on the long-axis echocardiographic view including the hypertrophy of the right ventricle, the ventricular septal defect (VSD), and the aorta overriding the VSD. (B) The short-axis view on echocardiogram shows the infundibular or right ventricular outflow tract stenosis.

appearance due to right ventricular hypertrophy. However, this is much less likely with early repair. A right-sided aortic arch can also be seen on CXR if present. Echocardiogram easily shows all components of TOF and allows for evaluation of the arch, coronary anatomy, and presence of PFO and PDA. For more complex variants including discontinuous branch pulmonary arteries and/or presence of MAPCAs, other imaging modalities may be used to further delineate anatomy prior to surgical correction.

Initial management of TOF depends upon the amount of pulmonary blood flow. Patients without significant symptoms or hypoxemia undergo a complete repair in the first 4 to 6 months after birth. Complete repair consists of VSD closure and relief of RVOT obstruction through a combination of infundibular muscle resection, pulmonary valvotomy, or RVOT patch that can extend out to the branch pulmonary arteries as needed (Fig. 50.11). Valve sparing repair, consisting of infundibular muscle resection and supravalvar patch augmentation extending into the branch pulmonary arteries if necessary, can also be performed when the pulmonary valve annulus and valve function are deemed adequate. In patients with significant cyanosis or ductal-dependent pulmonary circulation, initial management includes PGE_1. This can be followed by palliation with a systemic-to-pulmonary artery shunt in the newborn period (modified Blalock–Taussig [BT] shunt) and complete repair at a later date or a complete anatomic correction in the newborn period. The recent trend has been toward earlier complete correction in symptomatic infants.[69–72]

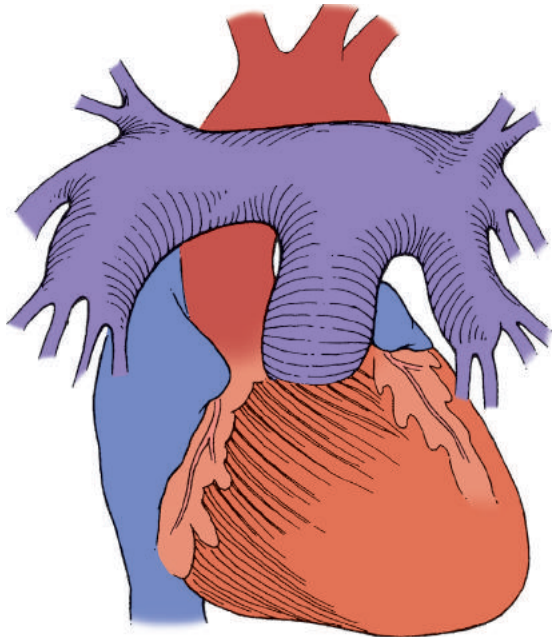


• **Fig. 50.11** Repair of Tetralogy of Fallot. (A) *Dashed line* depicts a nontransannular incision, used when the pulmonary valve and annulus are of adequate size. (B) A transannular incision *(dashed line)* is used when there is annular hypoplasia. (C) An example of transventricular exposure of the ventricular septal defect. Alternatively, the ventricular septal defect may be closed via a right atriotomy through the tricuspid valve. (D) External view of patch closure of the transannular incision. (From Spray TL, Wernovsky G. Right ventricular outflow tract obstruction. In: Chang AC, Hanley FH, Wernovsky G, Wessel DL, eds. *Pediatric Cardiac Intensive Care*. Baltimore: Williams & Wilkins; 1998:257–270.)

Tetralogy of Fallot Absent Pulmonary Valve

TOF with absent pulmonary valve (APV), or APV syndrome, is a spectrum of disorders with a rudimentary pulmonary valve. The resultant pulmonary valve stenosis and insufficiency cause dilation of the right ventricle and main and branch pulmonary arteries that is apparent in utero (Fig. 50.12). There is a higher risk of fetal heart failure, hydrops, and in utero demise with APV. This risk can be as high as 50%.[73] Most cases also have a malalignment VSD and lack a ductus arteriosus. Extrinsic compression of the airways from the dilated pulmonary arteries results in airway abnormalities as well as pulmonary hypoplasia. There is a high prevalence of 22q11 deletion among patients with APV.

Physical examination reveals a characteristic "to and fro" murmur of pulmonary stenosis and insufficiency. A prominent right ventricular heave and hepatomegaly are present secondary to the right ventricular volume overload. There is a wide range of symptoms, depending upon the degree of right ventricular volume overload and degree of airway disease. At one end of the spectrum is an asymptomatic neonate with mild cyanosis and the gradual development of heart failure symptoms during the normal postnatal decline in PVR. At the other end of the spectrum is a critically ill infant with severe cyanosis and respiratory failure caused by the combination of right-to-left shunt through the VSD and underlying airway disease. Mechanical ventilation is frequently necessary and occasionally unsuccessful secondary to airway compression by the dilated pulmonary vasculature. PGE_1 is not helpful since there is usually no ductus arteriosus. iNO and 100% oxygen can



• **Fig. 50.12** Tetralogy of Fallot With Absent Pulmonary Valve. Severe dilatation of the main and branch pulmonary arteries is seen and is frequently associated with bronchial compression and large and small airway disease. The intracardiac anatomy is usually similar to that in standard tetralogy of Fallot. (From Spray TL, Wernovsky G. Right ventricular outflow tract obstruction. In: Chang AC, Hanley FH, Wernovsky G, Wessel DL, eds. *Pediatric Cardiac Intensive Care*. Baltimore: Williams & Wilkins; 1998:257–270.)

be used to encourage forward pulmonary flow. ECMO may be needed until surgery is performed. Urgent surgery is required in these infants.

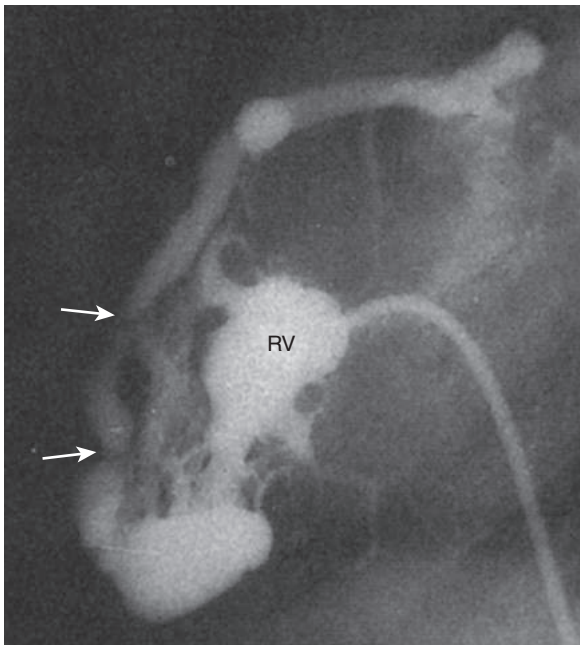
Surgery consists of VSD closure, pulmonary artery plication, relief of RVOT obstruction, and insertion of a valve or homograft in the RVOT position. This differs from the traditional approach in TOF where the pulmonary outflow is often left unguarded. In TOF with APV the goal is to limit pulmonary insufficiency with a functional valve and thus stop the dilation of right sided structures. There is significant variation in surgical technique between centers.[74,75] The surgical mortality and short-term and long-term outcomes are related primarily to the severity of pulmonary artery dilation and airway compression. More severely affected infants have a higher surgical mortality and need for prolonged postoperative ventilation. There is up to a 42% rate of death in infancy.[73] Those with less significant pulmonary artery dilation have lower postoperative mortality and long-term outcomes similar to patients with TOF.

Pulmonary Atresia With Intact Ventricular Septum

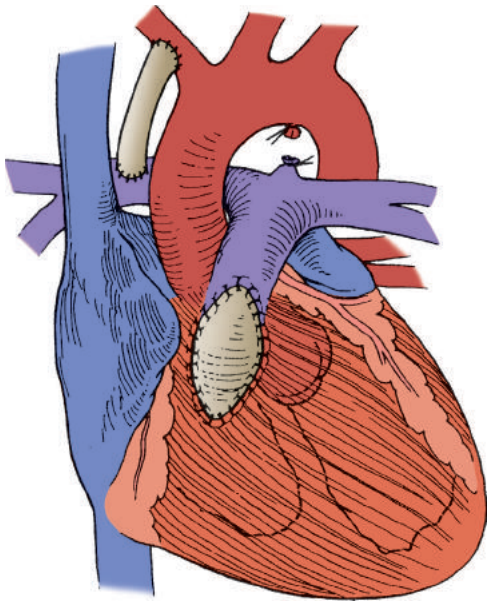
In pulmonary atresia with intact ventricular septum, the pulmonary valve leaflets are fused or fail to form. Without egress from the right ventricle, systemic venous return passes through the PFO and mixes with pulmonary venous return. Pulmonary blood flow is dependent upon the PDA, with the left ventricle providing cardiac output to both the systemic and pulmonary circulations. The tricuspid valve is usually abnormal owing to tricuspid insufficiency. Tricuspid valve and right ventricular size vary, ranging from nearly normal-sized structures to nearly atretic tricuspid valve and diminutive right ventricular chamber. The latter patients may have sinusoidal channels in the right ventricular myocardium that communicate with the coronary circulation as a way to decompress the RV. The high right ventricular pressure results in retrograde perfusion of the coronary arteries, termed right ventricle-dependent coronary circulation (Fig. 50.13).

Pulmonary atresia with intact ventricular septum presents with cyanosis. Cardiac examination reveals a single loud second heart sound. A murmur is typically not present, other than from the PDA. However, if there is significant tricuspid insufficiency, a pansystolic regurgitant murmur can be heard at the left lower sternal border. ECG typically reveals a relative lack of right-sided forces with a QRS axis between 0 and 90 degrees. The P wave is peaked from right atrial enlargement. Pulmonary vascular markings on chest x-ray are typically decreased. Heart size can go from mildly enlarged to severely enlarged depending on the size of the right sided structures. Echocardiography confirms the diagnosis and can be used to look for coronary sinusoids. Cardiac catheterization is performed in patients where coronary sinusoids are known or suspected to completely define the extent and connections of the sinusoids.

Initially, PGE_1 is used to maintain ductal patency until a more permanent source of pulmonary blood flow is provided. In patients with right ventricle–dependent coronary circulation, the outcome of surgical repair is often poor. In these cases, decompression of the right ventricle can invariably result in decreased coronary perfusion and is contraindicated. A systemic-to-pulmonary shunt followed by staged single ventricle palliation is often tried, but proceeding directly to heart transplantation may be appropriate in some cases. In patients without right ventricular coronary–dependent circulation, an egress from the right ventricle to pulmonary arteries is created by surgical valvotomy and/or RVOT augmentation or through transcatheter intervention via valve perforation and valvuloplasty (Fig. 50.14).[76,77] At times, additional pulmonary blood flow is provided by a modified BT



• **Fig. 50.13** Lateral Angiogram From a Newborn With Pulmonary Atresia and Intact Ventricular Septum. Following injection of contrast through the hypertrophied and diminutive right ventricle, there is retrograde filling of tortuous coronary arteries through sinusoidal connections to the right ventricle *(arrows)*. *RV*, Right ventricle.



• **Fig. 50.14** Newborn Surgical Palliation for Pulmonary Atresia With Intact Ventricular Septum and Normal Coronary Arteries. A right ventricular outflow tract patch is placed, the ductus is ligated and divided, and a modified Blalock-Taussig shunt is placed. (From Wernovsky G, Hanley FL. Pulmonary atresia with intact ventricular septum. In: Chang AC, Hanley FL, Wernovsky G, Wessel DL, eds. *Pediatric Cardiac Intensive Care*. Baltimore: Williams & Wilkins; 1998:265–270.)

shunt or other systemic-to-pulmonary shunt (PDA stent). The postoperative course can be complicated by low cardiac output or a circular shunt.

Tricuspid Atresia

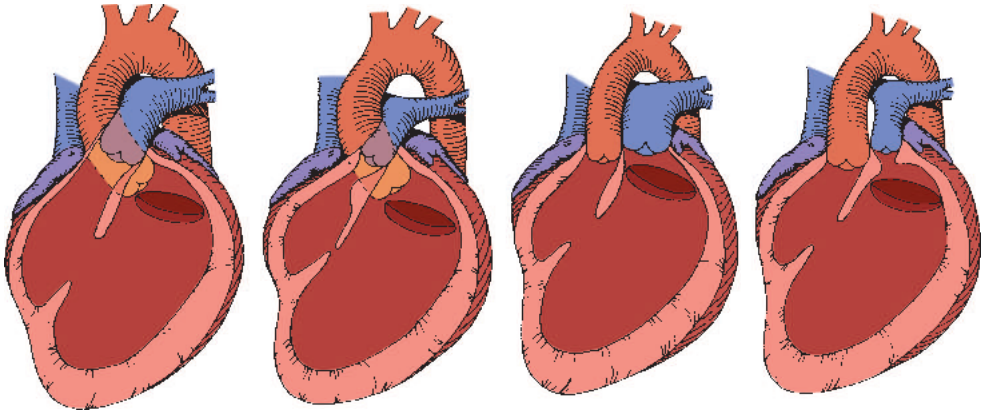
In tricuspid atresia, there is no outlet from the right atrium to the right ventricle. Systemic venous return passes from the right atrium, through a PFO or ASD, to the left atrium and then the left ventricle. The ASD is rarely restrictive. More than 90% of patients with tricuspid atresia have an associated VSD, allowing blood to pass from the left ventricle to the right ventricle and then the pulmonary arteries (Fig. 50.15). The size of the VSD determines the amount of pulmonary blood flow. The VSD is usually muscular and can become more restrictive over time. Patients with a small or absent VSD have a very hypoplastic right ventricle and pulmonary artery. The majority of pulmonary blood flow comes from the PDA. If a large VSD is present, pulmonary blood flow is prograde through the pulmonary valve, and the ductus is not necessary postnatally. Tricuspid atresia can be further classified based on the relationship of the great arteries. Most commonly, there are normally related great arteries with the above-mentioned considerations. The second most common variant of tricuspid atresia is with TGA. In this variant, pulmonary blood flow is derived from the left ventricle, and it is the systemic blood flow which must pass through the VSD and may be limited. This may lead to an associated coarctation or hypoplastic aortic arch. The third classification is with congenitally corrected TGA, which is the least common.

Clinically, cyanosis is present at birth, the extent of which is dependent upon the degree of restriction to pulmonary blood flow. A holosystolic murmur consistent with a VSD and a prominent left ventricular impulse are present on exam. ECG is nearly diagnostic of tricuspid atresia and reveals a classic left axis deviation and left ventricular hypertrophy. Chest x-ray reveals variable pulmonary vascular markings, depending on the size of the VSD and relationship of the great vessels. Echocardiogram demonstrates a fibromuscular plate in place of the tricuspid valve and a variably small right ventricle and pulmonary valve. The relationship of the great vessels can also be determined by echocardiogram. The degree of obstruction at the VSD or across the RVOT can be evaluated.

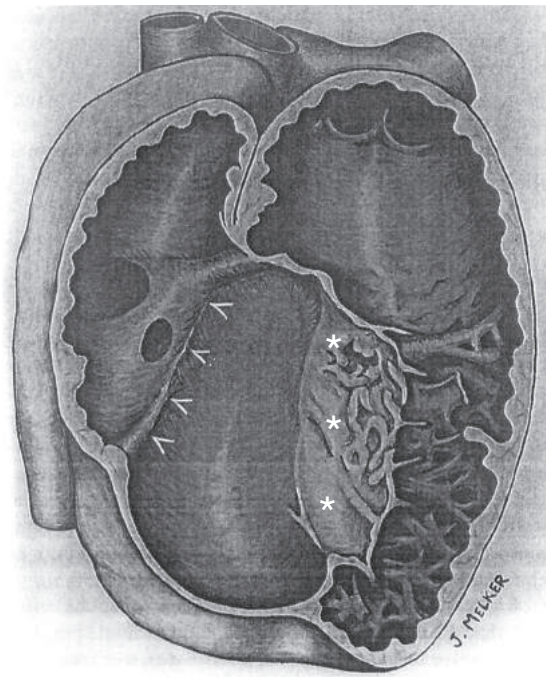
PGE_1 is used initially to maintain ductal patency. If there is restriction to flow at the atrial level, a balloon septostomy can be performed. Further management depends on the amount of pulmonary blood flow and the relationship of the great vessels. Patients with ductal-dependent pulmonary blood flow will have a systemic-to-pulmonary artery shunt placed (modified BT shunt) in the neonatal period. Those with adequate pulmonary blood flow undergo an SVC-to-pulmonary-artery anastomosis (bidirectional Glenn) at approximately 6 months of age, with completion of the Fontan procedure around 3 years of age. Patients with transposition, or more complex anatomy, undergo more extensive palliative procedures initially but continue down the pathway to Fontan palliation.

Ebstein Anomaly of the Tricuspid Valve/Tricuspid Valve Dysplasia

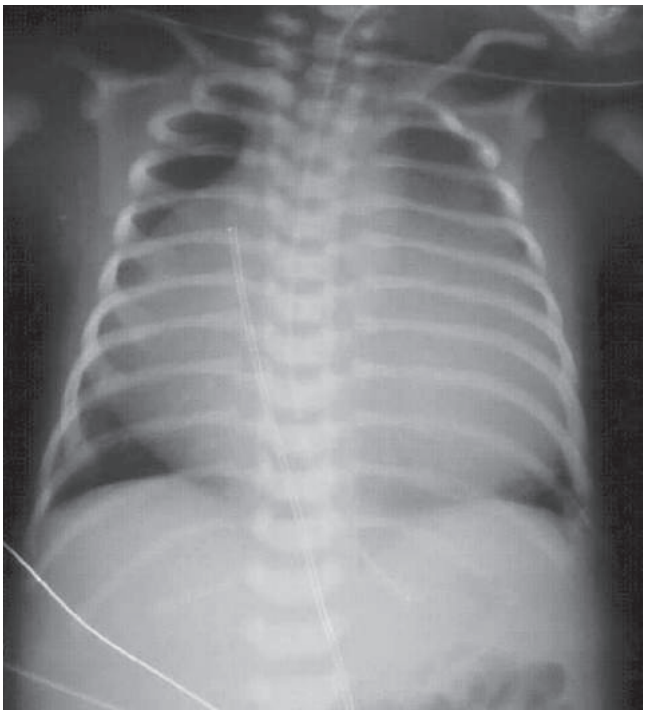
Ebstein anomaly of the tricuspid valve and the closely related tricuspid valve dysplasia are rare forms of CHD of the tricuspid valve. The presentation is variable, and there is considerable overlap between the two, more appropriately considered as a spectrum of tricuspid valve disease. For Ebstein anomaly, dysplasia of the tricuspid valve, with downward displacement of the septal and posterior leaflets, is the defining feature (Fig. 50.16). The anterior leaflet, although normally positioned in the valve annulus, frequently has abnormal chordal attachments and is large and redundant. The tricuspid valve abnormality can be accompanied by tricuspid regurgitation, right atrial dilation, abnormal right ventricular myocardium, and an increased risk of Wolff–Parkinson–White syndrome. In addition, functional and true pulmonary atresia can occur with severe Ebstein malformation. With displacement of the tricuspid valve, a significant portion of the right ventricle becomes atrialized, making it an ineffective pumping chamber. In this setting it can be difficult to differentiate functional pulmonary atresia from true pulmonary atresia. Severe Ebstein anomaly of the tricuspid valve is associated with heart failure in utero and a perinatal mortality as high as 45%.[78,79] Tricuspid valve dysplasia in its severe form can also lead to severe tricuspid regurgitation and severe dilation of the right atrium and ventricle. In this case, there may be mild displacement of the septal and posterior leaflets, but



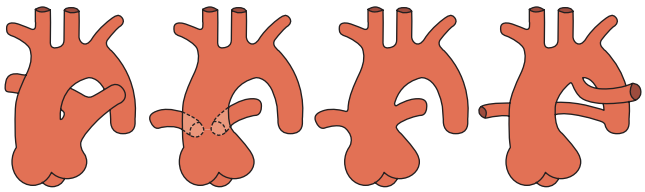
• **Fig. 50.15** Anatomic Variants in Tricuspid Atresia. (A) Normally related great vessels with a large ventricular septal defect (VSD) and normal-sized pulmonary arteries (PAs). (B) Normally related great vessels with a small VSD and PAs. (C) Transposed great arteries (left ventricle aligned with the PA, right ventricle with the aorta) with a relatively small VSD and aorta. Many patients with this variant have coarctation as well (see text). (D) Transposed great vessels with a VSD, subpulmonary obstruction, and small PAs. (From Fyler DC. Tricuspid atresia. In: Fyler DC, ed. *Nadas' Pediatric Cardiology*. Philadelphia: Hanley & Belfus; 1992:659–667.)



• **Fig 50.16** Inferior Displacement of Tricuspid Valve Leaflet Into the Right Ventricular Cavity. The *white arrowheads* indicate the normally positioned tricuspid valve annulus. The asterisks (*) indicate varying degrees of inferior or apical displacement of the septal leaflet of the tricuspid valve. The area between the true annulus and the displaced valve leaflet is considered "atrialized." (From Epstein ML. Congenital stenosis and insufficiency of the tricuspid valve. In: Allen HD, Gutgesell HP, Clark EB, Driscoll DJ, eds. Moss and Adams' Heart Disease in Infants, Children and Adolescents: Including the Fetus and Young Adult. Philadelphia: Lippincott Williams & Wilkins; 2001:810–819.)



• **Fig. 50.17** Chest Radiograph in a 1-Day-Old Newborn With Ebstein Anomaly of the Tricuspid Valve. Note the massive cardiomegaly and relative pulmonary oligemia and hypoplasia.



• **Fig. 50.18** Types of Truncus Arteriosus. The classification of truncus arteriosus is determined by the origin of the pulmonary arteries (see text for details).

it does not meet criteria for Ebstein anomaly. There is considerable overlap within the two and quite variable presentation.

The clinical presentation is variable and depends upon the degree of displacement of the tricuspid valve leaflets and severity of RVOT obstruction. In many patients, symptoms are mild and do not present until later in life. In more severe disease, cyanosis results when a right-to-left shunt occurs at the atrial level, secondary to the tricuspid regurgitation and elevated right atrial pressures. Cardiac examination reveals a holosystolic murmur at the lower left sternal border with associated gallop and clicks. Neonates with severe Ebstein present with marked cyanosis, cardiomegaly, and ductal-dependent pulmonary blood flow. Chest x-ray is characteristic in severe cases with severe cardiomegaly evident at birth (Fig. 50.17). Death can occur because of the significant heart failure and hypoxemia. Clinical improvement may occur as the PVR drops, improving the right ventricle's ability to contribute to pulmonary blood flow.

Initially, management of the severely cyanotic infant is aimed at promoting pulmonary blood flow. PGE_1 is used to maintain ductal patency. Supplemental oxygen, inhaled nitric oxide, and mild respiratory alkalosis can have marginal success in improving pulmonary blood flow by lowering PVR. After several days, attempts are made at weaning the PGE_1. If unsuccessful, surgical options are considered. Options include tricuspid valve repair or replacement, ventricle repair, or other palliative procedures such as right ventricular exclusion with a fenestrated patch and placement of a modified BT shunt.[79] While surgical outcomes have improved, the need for neonatal repair for symptomatic disease remains a risk factor for death.[80,81]

Truncus Arteriosus

In truncus arteriosus, a single arterial trunk gives rise to the pulmonary, systemic, and coronary circulation. The arterial trunk typically overrides a VSD. Truncus is classified by the origin of the pulmonary arteries (Fig. 50.18). In type I, a small pulmonary artery arises from the arterial trunk and bifurcates in the right and left branch pulmonary arteries. The right and left pulmonary arteries can also arise from separate ostia that are either close to one another (type II) or some distance apart (type III).[82] Type IV is similar to TOF with pulmonary atresia. The truncal valve has between one and six cusps. It is occasionally insufficient but rarely stenotic. Truncus usually occurs as an isolated cardiovascular defect although it has been associated with microdeletion of chromosome 22q11.[83]

As the PVR drops over the first several weeks after birth, increased shunting from the arterial trunk to the pulmonary arteries occurs, and pulmonary overcirculation develops. If left

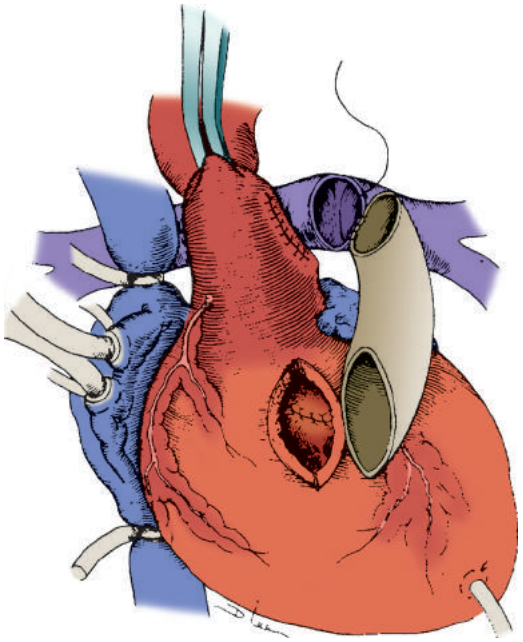
untreated, symptoms of heart failure develop. Long-term pulmonary vascular disease occurs.

The clinical presentation of truncus changes with the PVR. Initially, infants are minimally cyanotic and do not appear to be in distress. As pulmonary overcirculation occurs, symptoms of heart failure develop. Cardiac examination reveals a hyperdynamic precordium and a loud single S2. As PVR drops, the pulses become bounding, and the pulse pressure widens because of run-off into the pulmonary arteries. A low-pitched diastolic inflow rumble may be heard at the apex. In addition, a systolic ejection murmur may be present with truncal valve stenosis and a medium- to high-pitched diastolic murmur may be heard from truncal valve insufficiency. Chest x-ray typically reveals cardiomegaly with pulmonary vascular markings that increases over the first few weeks of life. ECG demonstrates right, left, or biventricular hypertrophy. Echocardiogram is used to demonstrate the anatomy and evaluate for associated cardiac defects such as right aortic arch, coronary artery anomalies, interrupted aortic arch, and secundum ASDs.

Initially, heart failure symptoms are managed medically. Surgical repair is typically performed within the first 2 months of life because of the difficulty in controlling heart failure. The usual repair involves closure of the VSD so that the arterial trunk arises from the left ventricle. A right-ventricle-to-pulmonary-artery conduit is then placed (Fig. 50.19). The right-ventricle-to-pulmonary-artery conduit needs to be replaced several times over the course of the patient's lifetime.

Total Anomalous Pulmonary Venous Return

In TAPVR, the pulmonary veins return to a systemic vein or directly to the right atrium, rather than to the left atrium. Both systemic and pulmonary venous return pass through the right atrium, right ventricle, and pulmonary artery, creating pulmonary overcirculation and right heart volume overload. An ASD is present in all cases. The right-atrium-to-left-atrium shunt supplies the systemic cardiac output. If the ASD is restrictive, then systemic output will be limited.



• **Fig. 50.19** Anatomy and Repair of Truncus Arteriosus. After the branch pulmonary arteries are removed from the common trunk (and the resulting aortic defect is closed), a ventriculotomy is performed. The ventricular septal defect is closed through this incision, and a conduit is placed from the right ventricle to the pulmonary arteries. (From Behrendt DM, Dick M. Truncus repair with a valveless conduit in neonates. *J Thorac Cardiovasc Surg*. 1995;110:1148–1150.)

Several common patterns of anomalous pulmonary venous return are seen (Fig. 50.20).[84] Most commonly, the anomalous veins drain in a supracardiac fashion, entering the left innominate vein or SVC via a vertical vein and draining into the right atrium via the SVC. Cardiac drainage occurs when the anomalous veins drain into the right atrium via the coronary sinus. Alternatively, a descending vein may pass through the diaphragmatic hiatus and enter the hepatic or portal venous system resulting in infracardiac drainage. Rarely, a mixed pattern of drainage may exist, or the anomalous veins may drain directly into the right atrium. Drainage directly into the right atrium occurs almost exclusively in patients with heterotaxy.

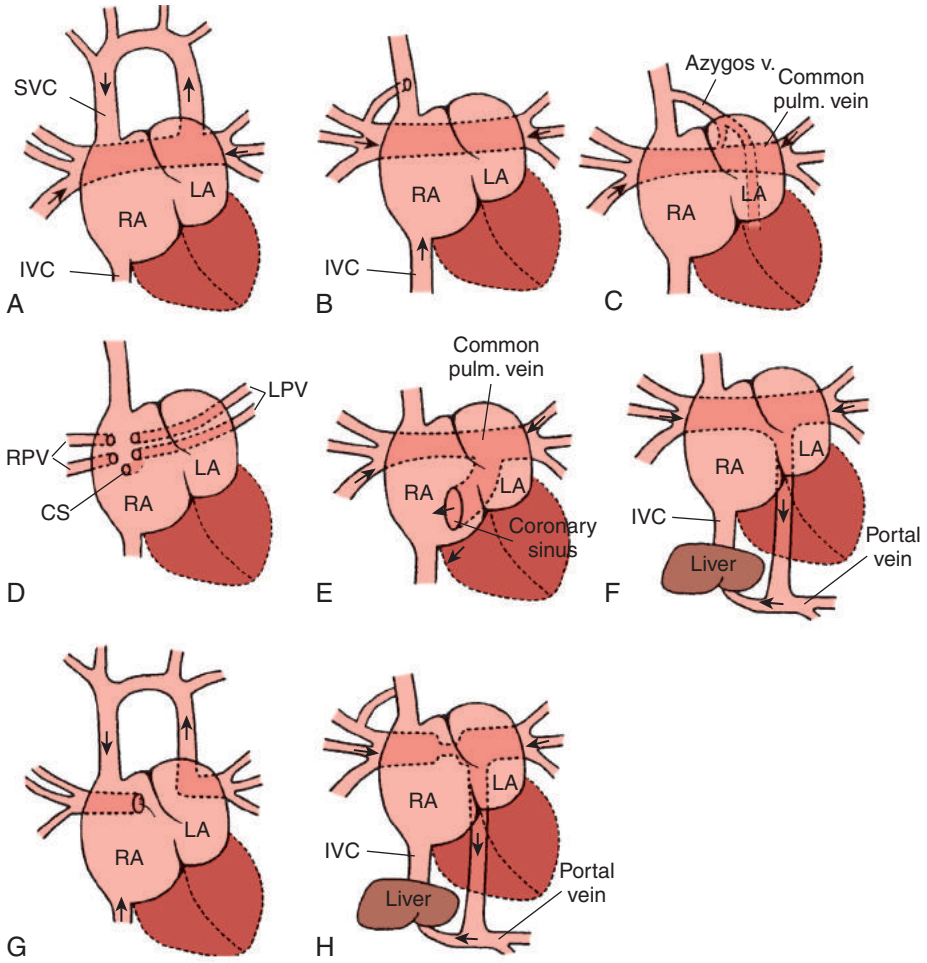
The clinical presentation of TAPVR varies, depending upon the degree of obstruction to pulmonary venous flow. Patients with unobstructed pulmonary venous flow will have minimal symptoms in the newborn period, and cyanosis is usually not apparent. Symptoms of right heart failure develop over time because of the progressive right heart volume overload. Physical examination may reveal a right ventricular heave, fixed split S2, and a soft systolic ejection murmur in the pulmonic area. Chest x-ray may demonstrate cardiomegaly due to an enlarged right atrium, right ventricle, and main pulmonary artery. Pulmonary vascular markings increase over time. ECG reveals peaked P waves and right ventricular hypertrophy. Patients with moderate restriction to pulmonary blood flow are typically cyanotic in the newborn period. There is usually adequate mixing of venous return to allow for oxygen delivery to tissues. These patients may, however, benefit from supplemental oxygen, mechanical ventilation, and sedation to decrease oxygen consumption. The presentation of patients with obstructed pulmonary blood flow will be discussed later in this chapter under obstructive lesions.

Treatment of TAPVR is surgical. In most cases, a pulmonary venous confluence is seen near the left atrium. Cardiac catheterization is used to define pulmonary venous anatomy if the pulmonary veins are not well seen by echocardiography. During surgery, the pulmonary venous confluence is anastomosed to the left atrium, the ASD is closed, and the connections to systemic veins are ligated. In the absence of pulmonary venous obstruction and in the setting of uncomplicated anatomy, surgical mortality is low and long-term results are good. Late pulmonary venous obstruction occurs in approximately 20% of cases, with relatively poor surgical and interventional cardiac catheterization results.

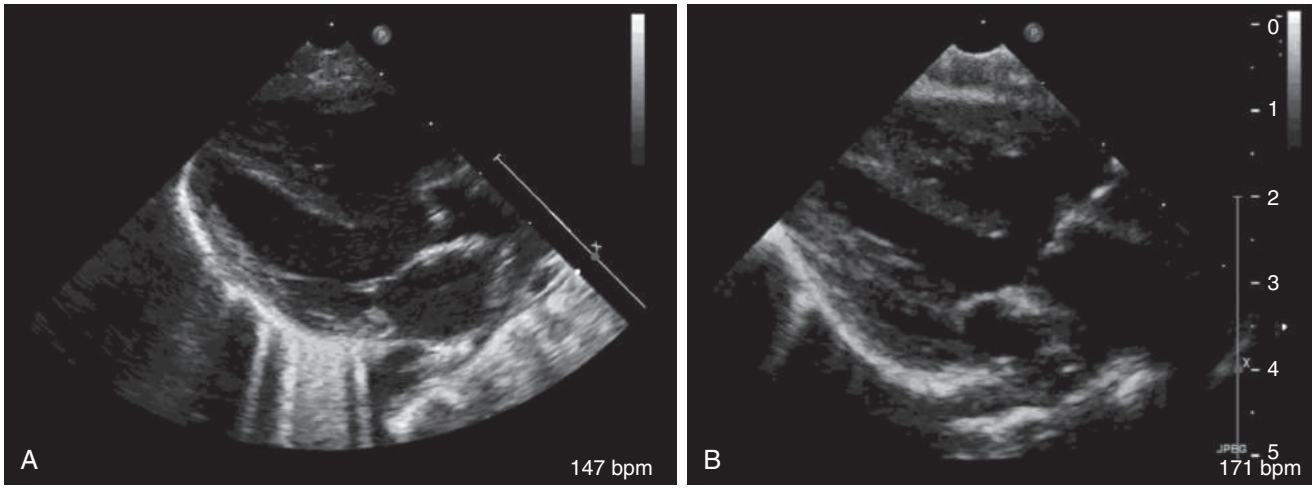
Double Outlet Right Ventricle

DORV is a relatively rare, diverse group of lesions characterized by the origination of both great vessels from the right ventricle and by the persistence of the subaortic and subpulmonary conus. There are multiple anatomic variations of this lesion that are commonly classified by the relationship of the great vessels to one another and/or by the location of the VSD to the great vessels (Fig. 50.21).[85–87] With this in mind, there are four types of DORV, each with a slightly different neonatal presentation and surgical management.

DORV of the tetralogy type typically has normally related great vessels with a subaortic VSD. The VSD directs flow into the



• **Fig. 50.20** Anatomic Varieties of Total Anomalous Pulmonary Venous Return. (A–C) Supracardiac. (D, E) Cardiac. (F) Infracardiac. (G, H) Mixed. See text for details. *CS*, Coronary sinus; *IVC*, inferior vena cava; *LA*, left atrium; *LPV*, left pulmonary vein; *Pulm*, pulmonary; *RA*, right atrium; *RPV*, right pulmonary vein; *SVC*, superior vena cava; *V*, vein. (From Eimbcke F, Enriquez G, Gomez O, Zilleruelo R. Total anomalous pulmonary venous connection. In: Moller JH, Hoffman JIE, eds. *Pediatric Cardiovascular Medicine*. Philadelphia: Churchill Livingstone; 2000:409–420.)



• **Fig. 50.21** Echocardiographic Views of Double Outlet Right Ventricle. (A) The aorta is committed to the right ventricle, and the ventricular septal defect is below the aortic valve. (B) The great vessels are transposed in this patient with the pulmonary artery overriding the ventricular septal defect.

aorta. Because the subaortic conus is pulled anteriorly, the subpulmonary conus is typically smaller, resulting in variable obstruction to pulmonary blood flow. The clinical presentation and surgical management are similar to those of TOF.

DORV of the transposition type typically has malposed great vessels with the aorta located either anterior to or rightward of the pulmonary artery. The VSD is subpulmonary and directs flow into the pulmonary artery, resulting in transposition-like physiology. This lesion is frequently associated with variable obstruction to aortic blood flow and coarctation of the aorta. Surgical repair consists of baffling the VSD to the pulmonary valve and performing an arterial switch. In the setting of coronary artery anomalies that prevent an arterial switch, the left ventricle is baffled to both the aortic and pulmonary valves, and a right-ventricle-to-pulmonary-artery conduit is placed. Arch obstruction is corrected if needed.

In DORV with a "doubly committed" VSD, the VSD is below both great vessels. The physiology and clinical presentation are variable, depending upon the size and orientation of the VSD and the degree of outflow tract obstruction. Surgical repair is directed at closing the interventricular communication without obstructing either ventricular outflow.

The least common variety of DORV is that in which the VSD is remote from both great vessels. Because of the distance between the VSD and the great vessels, a two-ventricle repair is difficult and often impossible. Pulmonary banding is frequently performed in the newborn period to limit pulmonary blood flow, delaying attempts at a biventricular repair or single ventricle palliation until several months of age. Complex procedures such as intricate intracardiac baffles, RV to PA conduit, root translocation, double root translocation, and truncal switch can be performed. If no viable pathway is achieved, single ventricle palliation is pursued.

Lesions That Present Primarily With Heart Failure

Hypoplastic Left Heart Syndrome

HLHS is an anatomically heterogeneous lesion characterized by varying degrees of underdevelopment of the left ventricle, hypoplasia or atresia of the aortic and mitral valves, and hypoplasia of the aortic arch. While accounting for only 1.4% to 3.8% of all congenital heart defects, HLHS accounts for 23% of cardiac deaths in the first week of life and 15% of cardiac deaths in the first month after birth.[88] HLHS is likely multifactorial in cause. There is a slight male predominance. While there is no clear genetic cause, familial clustering of various left heart obstructive lesions has been noted.

It is theorized that the growth of developing vascular structures is dependent upon flow. HLHS likely results from in utero obstruction of left ventricular inflow or outflow. The fetal left ventricle is predominantly filled with blood that passes through the foramen ovale. Restriction to flow or reversal of flow through the foramen ovale could then result in decreased flow to the left heart and its underdevelopment. Similarly, several studies have documented the progression of severe aortic stenosis to HLHS in utero.[89,90] The progressive left ventricular hypertrophy, dilation, and fibrosis associated with severe aortic stenosis can lead to decreased ventricular compliance, elevated left atrial pressures, and reversal of flow through the foramen ovale in utero. Prenatal cardiac intervention is still in its infancy, and much continues to be learned about fetal intervention, but successes with in utero balloon dilation of the aortic valve suggest that the progression to HLHS can be altered.[91]

Because of the underdevelopment of the left heart structures, pulmonary venous return must exit the left atrium through the foramen ovale. Pulmonary venous blood then mixes with systemic venous return in the right atrium and enters the right ventricle. Right ventricular output then passes either into the pulmonary circulation or through the ductus arteriosus into the systemic circulation; that is, the pulmonary and systemic circulations are in parallel. The ratio of systemic to pulmonary blood flow is determined by the relative resistance of the vascular beds. As the normal postnatal drop in PVR occurs, pulmonary flow increases at the expense of systemic flow. Thus, the management of HLHS is dependent upon an adequate egress from the left atrium and a balancing of the resistances of the pulmonary and systemic vascular beds.

Most cases of HLHS are now diagnosed prenatally when an abnormal four-chamber view of the heart is noted on a screening obstetric ultrasound. Ideally, delivery should occur at a tertiary care center. In the absence of prenatal diagnosis, postnatal presentation is somewhat variable and dependent upon ductal patency and the degree of restriction to flow at the atrial septum. The infant with an unrestrictive atrial septum and PDA is largely asymptomatic at birth and may be missed in the newborn period. Cyanosis is minimal and pulmonary overcirculation is mild, while PVR is high. Cardiac exam is relatively unremarkable. The second heart sound is single and loud. A third heart sound becomes apparent as heart failure develops. As PVR drops and ductal closure occurs, feeding difficulties and respiratory distress become apparent, with rapid progression to cardiovascular collapse. Physical examination following ductal restriction is significant for lethargy, pallor, and diminished or absent pulses. Chest x-ray typically reveals a relatively normal-sized heart and pulmonary edema. ECG is nonspecific but may reveal relative lack of left-sided forces.

The patient with a restrictive atrial septum presents with tachypnea and profound cyanosis shortly after birth. The elevated left atrial and pulmonary venous pressures result in pulmonary venous congestion that is apparent on chest x-ray.

The preoperative management of patients with HLHS is directed at balancing the ratio of pulmonary-to-systemic blood flow (Qp:Qs) to allow for sufficient oxygenation of blood while maintaining adequate systemic cardiac output. Prostaglandins should be started immediately postnatally to ensure ductal patency. Echocardiography is utilized to confirm cardiac anatomy and determine the degree of restriction to flow through the foramen ovale. If the atrial level shunt is restrictive with profound cyanosis and metabolic acidosis, a balloon atrial septostomy, surgical septectomy, or emergent stage I palliation should be performed (see later in the section). If the restriction was present in utero, pathologic fibrosis and arterialization of the pulmonary veins and medial hypertrophy of the pulmonary arterioles occur. Even following atrial septostomy, lung disease can persist and PVR can remain high. Oxygen and iNO may be needed to maintain saturations in an appropriate range. This subset of patients has a high mortality rate.

A small group of patients will have adequately balanced pulmonary and systemic blood flow at the time of presentation. A small degree of restriction to flow through the foramen ovale may be associated with slight cyanosis but has the beneficial effect of restricting pulmonary blood flow. In the absence of acidosis or end-organ dysfunction, this state is generally tolerated until stage I palliation is performed.

An unrestrictive ASD allows increased pulmonary blood flow as the PVR falls. Because of the parallel arrangement of the circulations, increasing pulmonary flow decreases systemic flow. In this setting, there is increasing oxygen saturation associated with inadequate oxygen delivery to the tissues. Worsening metabolic acidosis and end-organ dysfunction ensue. The ratio of pulmonary-to-systemic blood flow is balanced by manipulating the resistances of the pulmonary and systemic vascular beds. The success of these therapies is monitored by measurement of oxygen saturation, mixed venous saturation, lactate, and arterial blood gases.

PVR can be manipulated through mechanical ventilation and alteration in the amount of oxygen delivered. Mechanical ventilation with high positive end-expiratory pressure can limit pulmonary blood flow. PVR can also be increased by adding carbon dioxide or nitrogen to the ventilator circuit to reduce the fraction of inspired oxygen (FiO_2) to 0.17.[92,93] Both CO_2 and nitrogen have been shown to decrease the Qp:Qs and decrease oxygen saturation. Only hypercarbia has been shown to improve systemic oxygen delivery.[94] The goal of this therapy is to maintain normal lactate, systemic oxygen saturation at 75% to 85%, and mixed venous oxygen saturation approximately 25 percentage points lower than systemic saturations. Mechanical ventilation is typically required when manipulating inspired gas. Mechanical ventilation alone has been associated with increased infection risk, more labile preoperative hemodynamics, and increased mortality. Both elective mechanical ventilation and alteration of the inspired gas mixture may be less in favor in the current era.[95]

Vasoactive medications can be used to alter systemic vascular resistance and improve ventricular function. Use of these medications is determined by clinical presentation and echocardiographic findings. Milrinone can be used to provide some afterload reduction, if tolerated by blood pressure. The inotropic effects of milrinone are also an advantage if ventricular function is poor. In addition to decreasing pulmonary blood flow, afterload reduction has the added benefit of decreasing tricuspid valve regurgitation if it is present. Milrinone also dilates the pulmonary vascular bed, so care should be taken when it is used. While counterintuitive, when faced with an unoperated patient with high oxygen saturation and low peripheral blood pressure, the gentle addition of milrinone may improve blood pressure simply by increasing systemic blood flow.[95]

The immediate goal of surgical palliation is to provide stable unrestricted systemic and coronary blood flow and reliably restrict pulmonary blood flow. There are several strategies for stage I palliation. Traditionally, aortic arch reconstruction was performed using pulmonary artery tissue, and pulmonary blood flow was supplied using a modified BT shunt (Norwood procedure, Fig. 50.22). A recent modification of a right ventricle-to-pulmonary artery conduit has been used to supply pulmonary blood flow (Sano modification, Fig. 50.23). The Sano modification has the presumed benefit of providing pulsatile flow to the pulmonary arteries without AP diastolic run-off and coronary steal. The downside of this procedure is the need for a ventriculotomy. A hybrid procedure that combines stent placement in the ductus arteriosus by the interventional cardiologist and pulmonary artery banding by the surgeon is an approach being taken by a number of institutions and provides a relatively noninvasive stage I palliation for HLHS.[96] Each of these procedures has pros and cons and advocates and detractors.[96–98] Longer-term prospective studies are needed to determine the optimal approach to stage I palliation. In the low-risk patient, survival following the first stage nears 80% to 90%.

The remaining palliative surgeries occur outside the newborn period. Stage II palliation unloads the right ventricle and begins to separate the pulmonary and systemic circulations. The superior cavopulmonary anastomosis (bidirectional Glenn) is usually performed between 4 and 6 months of age. During this procedure, the conduit providing pulmonary flow is removed, and the SVC is anastomosed to the pulmonary artery.

Stage III palliation completely separates the pulmonary and systemic circulations. An inferior cavopulmonary anastomosis (Fontan completion) is performed by one of several techniques at around age 3.

Obstructed Total Anomalous Pulmonary Venous Return

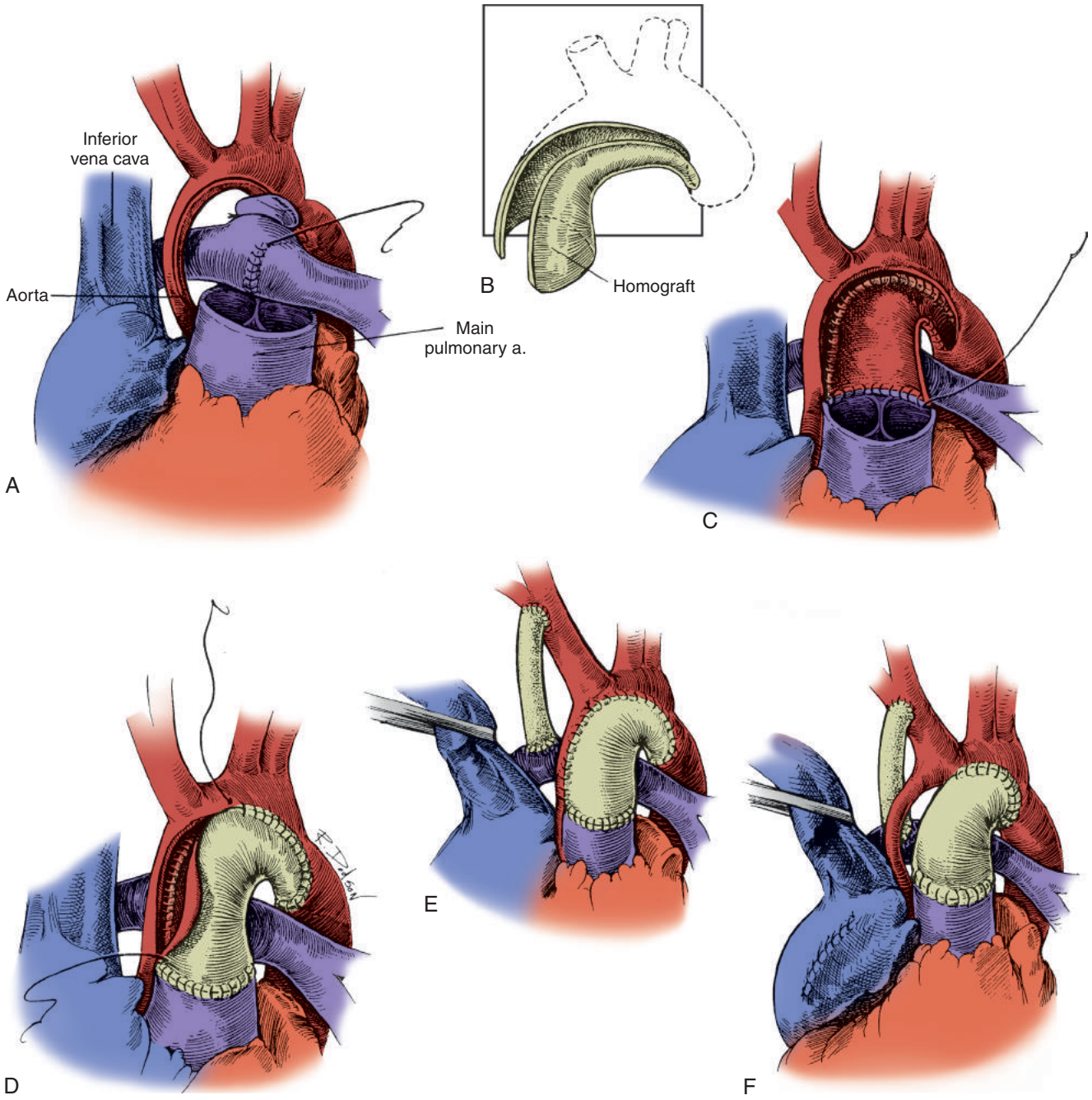
Obstructed TAPVR represents one of the few remaining neonatal surgical emergencies in pediatric cardiology. While obstruction can occur with any type of TAPVR, it is most common in infradiaphragmatic TAPVR. Physiologically, obstruction to pulmonary venous flow results in pulmonary venous hypertension that is transmitted to the pulmonary capillary bed, resulting in pulmonary edema. Pulmonary blood flow is severely limited. Newborns thus present with profound cyanosis and respiratory distress that are not responsive to medical management. Prostaglandins may help minimize the obstruction by maintaining patency of the ductus venosus, but patency of the ductus arteriosus does not improve the clinical picture as the limitation to pulmonary blood flow is not due to insufficient antegrade flow but rather obstructed outflow. Chest x-ray reveals a normal cardiac silhouette with pulmonary venous congestion that may be interpreted as interstitial pneumonia (Fig. 50.24). Echocardiography can be challenging in these patients. The pulmonary veins can be small due to limited flow, making them difficult to detect by two-dimensional imaging and color Doppler. Surgical treatment is the same as for unobstructed TAPVR. The postoperative course is frequently marked by PVR lability, right ventricular hypertension, and low cardiac output syndrome. The risk of mortality, need for prolonged ventilation, and incidence of late postoperative obstruction all increase when obstruction is present preoperatively.[99]

Cor Triatriatum

Embryologically, the pulmonary veins enter a common pulmonary vein that initially has no connection to the left atrium.[100] During normal development, the common pulmonary vein becomes incorporated into the left atrium, resulting in the usual pattern of two right and two left pulmonary veins entering the left atrium. Abnormal incorporation of the common pulmonary vein can result in cor triatriatum, a condition in which the common pulmonary vein joins the left atrium through a single opening. If the opening is small and restrictive, the clinical presentation is similar to that of obstructed TAPVR. If the opening is nonrestrictive, no symptoms are present. Surgical resection of the membrane that separates the left atrium and common pulmonary vein is an effective treatment. Significant preoperative pulmonary venous obstruction increases surgical mortality.

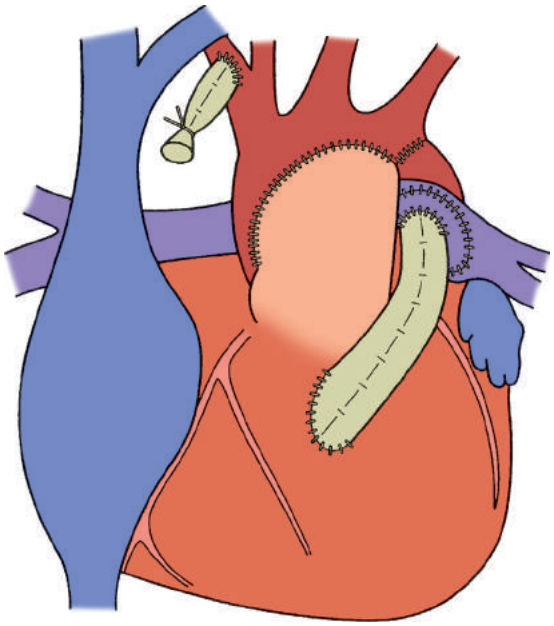
Mitral Stenosis

Congenital mitral stenosis is a rare form of CHD with several subtypes. The stenosis can occur in the supravalve region, at the valve annulus, or within the mitral valve support apparatus. Typical

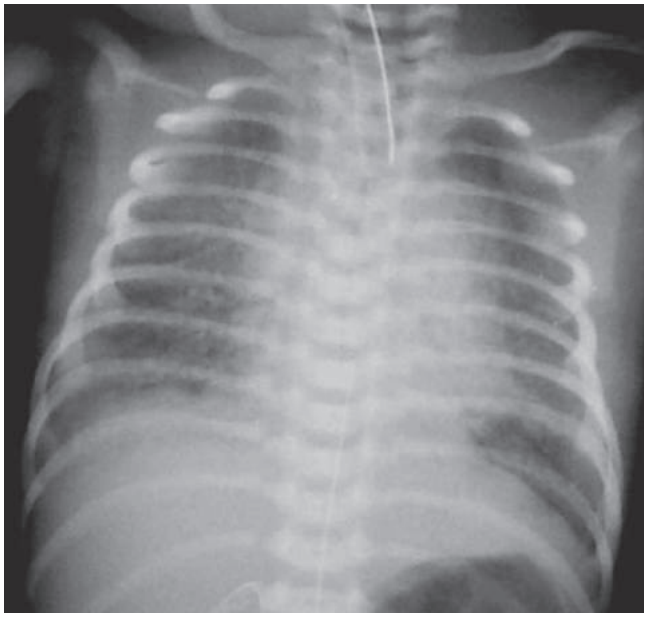


• **Fig. 50.22** Stage I Palliation for Hypoplastic Left Heart Syndrome: Classic Norwood Procedure. (A) The main pulmonary artery (*a*) is transected and the distal end oversewn. The ductus arteriosus is ligated, and an incision is made from the proximal ascending aorta around the aortic arch to the level of the ductus. (B) A pulmonary homograft is utilized to create a patch to reconstruct the neoaorta. (C, D) This homograft patch is used to connect the proximal main pulmonary artery and pulmonary (neoaortic) valve to the ascending aorta and transverse arch. (E) A modified Blalock–Taussig shunt is placed from the base of the innominate artery to the right pulmonary artery. (F) An alternate technique utilizing a circumferential tube graft from the proximal main pulmonary artery to the distal transverse aortic arch. *Not shown*: Atrial septectomy is performed to provide unobstructed egress from the pulmonary veins to the right ventricle. (From Castañeda AR, Jonas RA, Mayer JE Jr, et al. *Cardiac Surgery of the Neonate and Infant*. Philadelphia: Saunders; 1994.)

congenital mitral stenosis is characterized by thickened leaflets, short or absent chordae tendineae, obliteration of interchordal spaces, and two separate papillary muscles. Supravalvar mitral ring occurs when there is connective tissue outgrowth on the atrial surface of the mitral valve leaflets, leading to a smaller mitral valve effective orifice. The mitral valve orifice can also be stenotic secondary to a parachute mitral valve, when most or all chordae tendineae insert onto only one papillary muscle. Another form of obstruction occurs with a double orifice mitral valve, where a tongue of tissue connects the anterior and posterior mitral valve leaflets.



• **Fig. 50.23** Right Ventricle–Pulmonary Artery, or Sano, Modification of Stage I Reconstruction. The arch reconstruction is similar to that shown in Fig. 50.22. The Blalock–Taussig shunt is replaced with a Gore-Tex tube inserted from the right ventricle to the main pulmonary artery. (From Sano S, Ishino K, Kawada K, et al. Right ventricle–pulmonary artery shunt in first-stage palliation of hypoplastic left heart syndrome. *J Thorac Cardiovasc Surg*. 2003;126:504–510.)

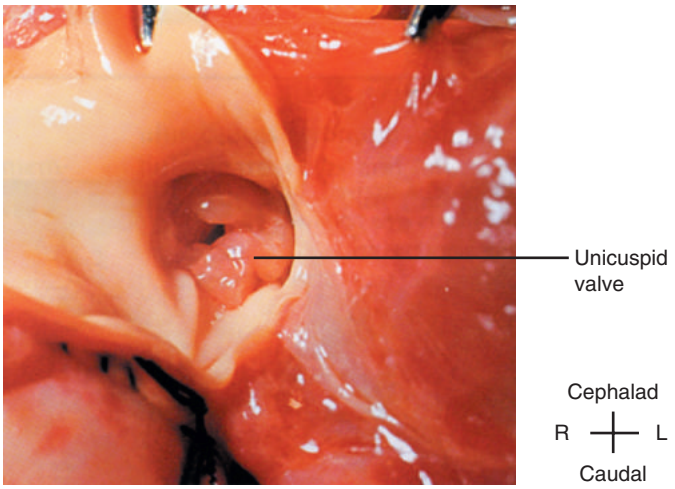


• **Fig 50.24** Chest X-ray in a 1-Day-Old With Obstructed Total Anomalous Pulmonary Venous Return.

A mitral arcade or hammock occurs when the leaflets are connected directly or by short chordae to the papillary muscles. Congenital mitral stenosis frequently occurs in conjunction with other left-sided obstructive lesions.

Symptoms from mitral stenosis usually occur in the first 2 years of life and may consist of shortness of breath, respiratory distress or wheezing, cyanosis, and pallor. Cardiac examination reveals a rumbling apical diastolic murmur, loud first heart sound, and loud split second heart sound. Opening snap of the mitral valve may be heard. Chest x-ray reveals left atrial enlargement and pulmonary venous congestion. ECG reveals right ventricular hypertrophy with normal, bifid, or spiked P waves suggesting left atrial enlargement (LAE). Echocardiogram is used to define mitral valve anatomy and localize the area of obstruction. Doppler can be used to determine valve gradient and estimate right ventricular pressure.

Treatment options for congenital mitral valve stenosis include balloon mitral valvuloplasty, surgical mitral valvuloplasty, and mitral valve replacement. There has been improvement in outcomes for mitral valve interventions in the first year of life with acceptable survival, but reintervention remains common especially in the long term.[101] Patients with additional left-sided obstructive lesions or associated defects frequently require single ventricle palliation.



• **Fig. 50.25** Congenital Aortic Stenosis. Frontal view through opened aorta demonstrates stenotic and dysmorphic aortic valve with commissural fusion. (From Litwin SB. *Color Atlas of Congenital Heart Surgery*. St. Louis: Mosby; 1996.)

Critical Aortic Stenosis

Patients with critical aortic stenosis have severe left ventricular outflow tract obstruction that limits systemic cardiac output (Fig. 50.25). The result is ductal-dependent systemic perfusion. While the obstruction can occur below the valve, at the valve, above the valve, or as a combination of these, this section focuses on outflow tract obstruction resulting from morphologic problems of the aortic valve. Severe aortic valve stenosis is defined as a Doppler-derived pressure gradient greater than 60 mmHg. Moderate stenosis is defined as gradient of 30 to 60 mmHg, while mild stenosis is a peak gradient less than 30 mmHg. In the setting of depressed left ventricular function, the Doppler echocardiogram-derived gradient may be significantly lower and underestimates the severity of the stenosis.

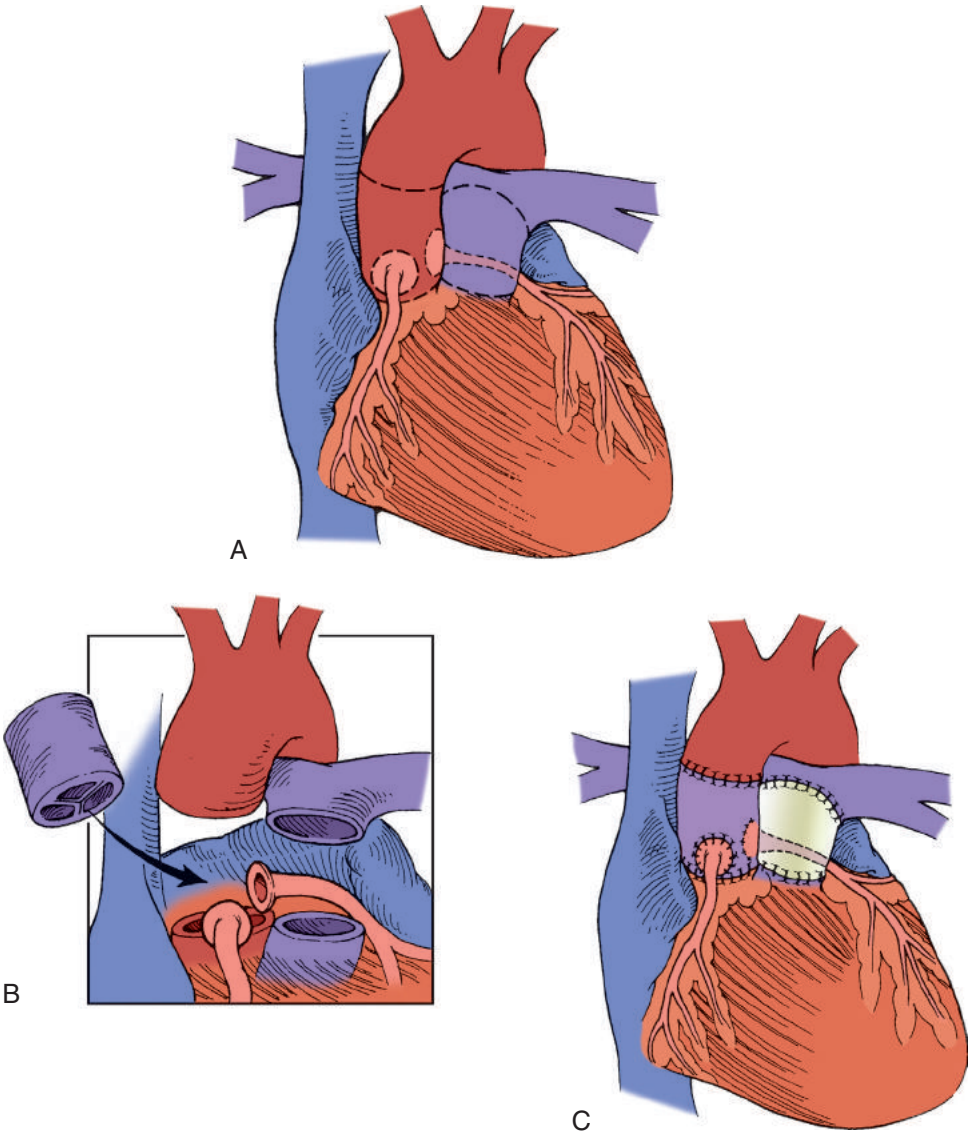
Aortic valve stenosis is detectable in utero. The long-standing pressure overload on the left ventricle causes left ventricular hypertrophy and scarring (endocardial fibroelastosis). In some cases, as discussed above, the disease progresses to HLHS. Growing experience with fetal intervention suggests that, with proper patient selection, it is possible to alter the course of critical aortic stenosis in the fetus through early intervention.[91,102,103]

Clinically, critical aortic stenosis presents in the newborn period with signs of decreased systemic perfusion: pallor, decreased pulses, and prolonged capillary refill. A harsh systolic ejection murmur is

heard on examination in the aortic area. The volume and quality of the murmur correlate with the severity of stenosis in the setting of normal left ventricular function. If left ventricular function is depressed, the murmur may be soft despite severe stenosis. ECG reveals left ventricular hypertrophy with possible T-wave abnormalities. Heart size is typically normal on chest film, although the aortic knob may be prominent due to post stenotic dilatation, and pulmonary congestion may be present. Echocardiogram is used to define the location and severity of the left ventricular outflow tract obstruction. Aortic stenosis is commonly found with other left-sided obstructive lesions, with possible underdevelopment of left heart structures. These findings may alter the treatment plan and lead to single ventricle palliation. Published models have attempted to identify echocardiographic findings that predict the suitability of a two-ventricle repair in neonates with critical aortic stenosis with variable results.[104–108]

Initial management of infants with critical aortic stenosis is directed at the treatment of cardiogenic shock. Endotracheal intubation, mechanical ventilation, secure vascular access, inotropic support, sedation, and paralysis are all frequently necessary. PGE_1 maintains ductal patency and provides systemic output. A small PFO must be present for pulmonary venous return to cross the atrial septum and enter the systemic circulation via the right ventricle and ductus arteriosus. A balloon septostomy may be necessary to decompress the left atrium.

Further management depends on left ventricular size and the presence of other left heart obstructive lesions. Patients with multiple levels of left heart obstruction, a small mitral valve, hypoplastic aortic arch, or small left ventricle may be best suited for single ventricle palliation. Options for two-ventricle palliation include balloon valvuloplasty in the cardiac catheterization lab, surgical valvotomy, or the neonatal Ross procedure (Fig. 50.26).[109]



• **Fig. 50.26** Ross Procedure: "Autograft" Aortic Valve Replacement. (A) *Dashed lines* depict surgical incisions around coronary arteries, aorta, and pulmonary artery. (B) Following removal of the coronary arteries and adjacent "buttons" and the diseased aortic valve, the patient's native pulmonary valve ("autograft") is positioned in the aortic root. (C) Completed repair with reimplanted coronary arteries and a cadaveric homograft valve inserted in the pulmonary position. (From Chang AC, Burke RP. Left ventricular outflow tract obstruction. In: Chang AC, Hanley FH, Wernovsky G, Wessel DL, eds. *Pediatric Cardiac Intensive Care*. Baltimore: Williams & Wilkins; 1998:233–256.)

Outcomes of all procedures depend, in part, on relief of obstruction, presence of aortic valve regurgitation, associated cardiac lesions, and severity of end-organ dysfunction at the time of initial presentation. The mortality of each of the interventions is relatively high. Regardless of the treatment chosen, critical aortic stenosis is a lifelong illness. Patients require close follow-up and multiple procedures throughout their lifetime.

Coarctation of the Aorta

Coarctation may occur anywhere from the transverse aortic arch to the bifurcation of the iliac arteries. Most commonly, there is a discrete narrowing distal to the left subclavian artery, across from the aortic insertion of the ductus arteriosus in a *juxtaductal* position. Conversely, there can be long segment narrowing of the transverse aortic arch, otherwise referred to as a hypoplastic aortic arch. A bicuspid aortic valve occurs in 70% of cases. Other left-sided obstructive lesions tend to occur with coarctation of the aorta. There is a 2:1 male-to-female preponderance and an association with Turner syndrome.

In neonates with a discrete juxtaductal narrowing, the PDA widens the narrowed area and provides relief from the obstruction. The net shunt through the PDA is left to right. These patients have equal oxygen saturations in the upper and lower extremities. In patients with a severe coarctation or a diffusely hypoplastic arch, descending aortic flow originates from a right-to-left shunt through the ductus arteriosus. Differential upper and lower extremity oxygen saturations occur in these patients, with the upper extremities having greater saturation than the lower extremities.

The clinical presentation of coarctation of the aorta depends upon the severity of the narrowing. Mild coarctation often does not present in infancy. Detection typically occurs when upper extremity hypertension and diminished or absent femoral pulses are noted on examination. Infants with more severe coarctation or aortic arch hypoplasia present with diminished lower extremity perfusion following ductal closure. Physical examination reveals an infant with poor perfusion and absent femoral pulses. Cardiac examination may reveal a systolic ejection click if a bicuspid aortic valve is present. A systolic ejection quality murmur is heard that radiates to the back and left infraclavicular area. Chest film demonstrates a large heart with increased pulmonary vascular markings. ECG reveals right ventricular hypertrophy. Echocardiogram is used to define the location and extent of aortic narrowing. Additional left-sided obstructive lesions are ruled out. Right ventricular hypertrophy and right ventricle hypertension are also frequently noted.

Treatment of the infant presenting with coarctation of the aorta is similar to that of infants with aortic stenosis. Intubation and mechanical ventilation are frequently necessary. Vascular access is obtained for inotropic support, and PGE_1 is started to open the ductus arteriosus. Metabolic derangements are corrected. Ideally, the infant is stabilized before surgery. Surgery is typically indicated at the time of diagnosis, even in relatively asymptomatic patients. In most cases a lateral thoracotomy is performed, the area of coarctation is excised, and an end-to-end anastomosis is performed. If long segment narrowing is present, patch material may be used to augment the arch, and a more extended end-to-end anastomosis is performed. This may require a sternotomy. Balloon angioplasty of native coarctation is not typically performed in infancy because of the risk of aneurysm, recoarctation, and vascular injury at the site of access.

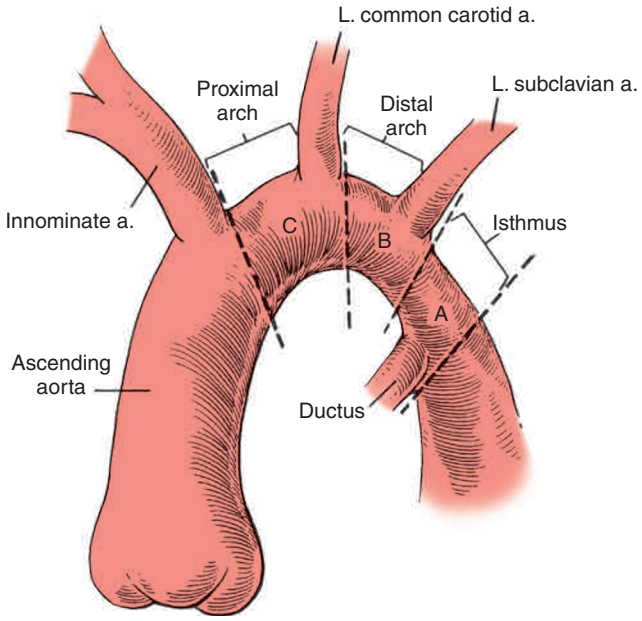
Surgical mortality is around 5%.[110,111] Re-coarctation occurs in 10% to 15% of children and can be successfully managed with balloon angioplasty on most occasions.[112] If a VSD is present, this is typically closed at the time of surgery.

Interrupted Aortic Arch

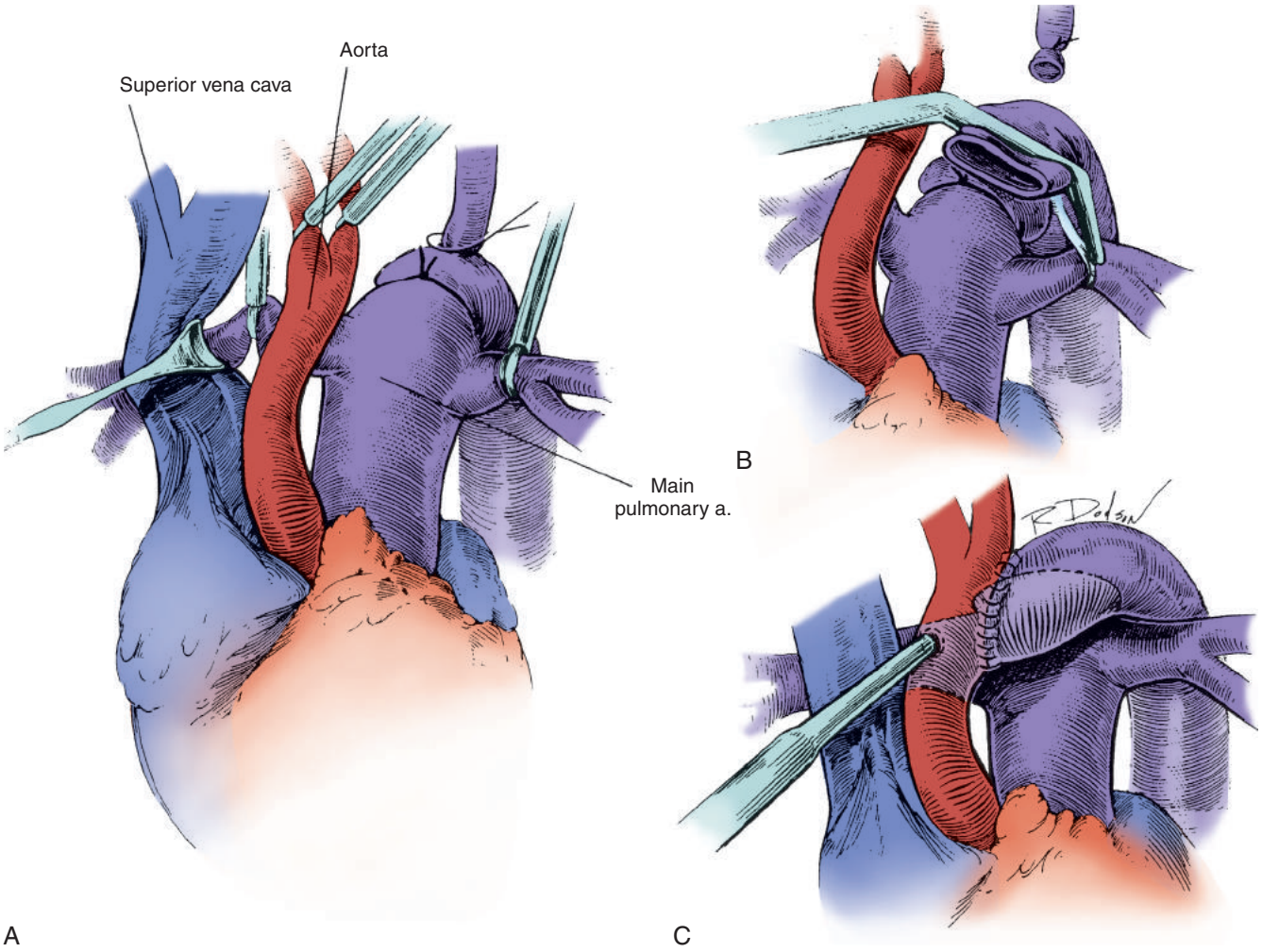
Interrupted aortic arch is a relatively rare anomaly that is defined simply as complete separation of the ascending and descending aorta. Interrupted aortic arch can be classified by the location of the interruption relative to the head and neck vessels (Fig. 50.27): type A, distal to the left subclavian artery; type B, between the left subclavian and left carotid; and type C, between the left carotid and innominate artery. All types of interruption occur in conjunction with posterior malalignment of the infundibular septum; this results in a VSD and varying degrees of left ventricular outflow tract obstruction. Aberrant arrangements of the head and neck vessels are common, with 50% of patients with type B interruption having an aberrant right subclavian artery that arises from the descending aorta. Interrupted aortic arch is associated with 22q11 deletion.

The clinical presentation of interrupted aortic arch is similar to that of other left-sided obstructive lesions. Descending aortic flow is entirely dependent upon right-to-left shunting through the PDA. Ductal closure causes cardiovascular collapse. Initial management is as described for coarctation of the aorta. PGE_1 should be started as soon as possible, as all other resuscitative efforts will have no benefit until postductal circulation is established.

Surgical repair is performed after metabolic acidosis resolves and end-organ function is improved. Continuity is established between the ascending and descending aorta via end-to-end anastomosis, homograft insertion/patch augmentation to connect the two segments, or jump grafts (Fig. 50.28). The VSD is typically closed. In



• **Fig. 50.27** Anatomic Classification of Interrupted Aortic Arch–Types A, B, and C. *Dashed lines* indicate the potential areas of discontinuity (interruption) in the aortic arch. See text for details. *a*, Artery. (From Castañeda AR, Jonas RA, Mayer JE Jr, et al. *Cardiac Surgery of the Neonate and Infant*. Philadelphia: WB Saunders; 1994.)



• **Fig. 50.28** Surgical Repair of Interrupted Aortic Arch, Type B. (A) The branch pulmonary arteries and arch vessels are snared, and a ligature is placed around the ductus arteriosus. (B) The proximal descending aorta is controlled with a clamp. To adequately mobilize the descending aorta, the left subclavian artery may need to be divided (as shown). Following resection of the ductus arteriosus, the proximal (pulmonary artery) end is oversewn. (C) The descending aorta is anastomosed directly to the ascending aorta. An alternative strategy is anastomosis of the left subclavian and left common carotid arteries combined with homograft patch augmentation of the inferior surface of the arch. Ventricular septal defect closure, when present, is also performed (not shown). *a*, Artery. (Source: Castañeda AR, Jonas RA, Mayer JE Jr, et al. *Cardiac Surgery of the Neonate and Infant*. Philadelphia: WB Saunders; 1994.)

patients with a small LVOT that is not suitable for systemic circulation, aortopulmonary amalgamation or a Ross-Konno type procedure can be performed with the goal of a biventricular repair.[113] In patients with severe left ventricular hypoplasia, a two-ventricle repair may not be possible, and a staged repair or single-ventricle palliation is performed.[114,115] Surgical mortality is less than 10% but higher in patients with additional anomalies.[115] Repeat operation because of left ventricular outflow tract obstruction and balloon angioplasty for recurrent arch obstruction are both common.

Anomalous Origin of the Left Coronary Artery From the Pulmonary Artery

When the left coronary artery arises from the pulmonary artery, inadequate oxygen delivery to the left ventricle results. Coronary artery perfusion occurs primarily during diastole. As pulmonary artery pressures drop postnatally, perfusion pressure of the left coronary artery falls, resulting in ischemia and infarction of the left ventricle. If collateral vessels connect the right and left coronary circulations, flow in the left coronary artery reverses. A left-to-right shunt occurs, resulting in coronary artery steal. Mitral valve regurgitation secondary to papillary muscle ischemia and left ventricular dilation develops.

Clinically, symptoms typically develop in the first month after birth. If adequate collateral vessels and myocardial oxygen delivery exist, the patient may present later in life with angina-like symptoms or even a murmur of collateral flow. In the infant, attacks of irritability, pallor, and diaphoresis with feeds are a common presentation. Cardiac examination reveals a displaced point of maximal impulse (PMI), gallop rhythm, and nonspecific murmur. If mitral regurgitation is present, a holosystolic, regurgitant quality murmur is heard. Chest x-ray reveals massive cardiomegaly. The ECG demonstrates a QR pattern and inverted T waves in leads I and aVL.

Leads V_5 and V_6 may also have deep Q waves, inverted T waves, and ST segment depression. Echocardiography may suggest the diagnosis but is not always reliable as the left coronary occasionally appears to arise from the aorta. Color Doppler may demonstrate retrograde flow in the left coronary with flow into the pulmonary artery. Cardiac computer tomography (CT) can be employed for further evaluation. Cardiac catheterization is diagnostic.

Symptoms of CHF are managed medically. Surgical reimplantation of the left coronary artery to the aorta restores normal coronary perfusion pressure. If the usual two-vessel coronary blood supply is reestablished, there is gradual normalization of left ventricular size and function, as viewed by echocardiography.[116,117] If myocardial damage is severe or the left coronary artery cannot be reimplanted surgically, cardiac transplantation is performed.

Systemic Arterial Malformations

Vascular anomalies are placed in two major groups: hemangiomas and malformations. Hemangiomas are tumors that demonstrate endothelial hyperplasia and undergo a period of proliferation and involution. Malformations result from abnormal vascular morphogenesis. They have normal endothelial cell turnover and grow accordingly with surrounding structures. Vascular malformations are further subcategorized by the type of vascular tissue involved (arterial, venous, and lymphatic). Because of their association with high output failure in the newborn period, two types of systemic vascular malformations will be discussed further: AVMs and arteriovenous fistulas (AVFs). An AVM results from multiple microfistulas between small arteries and veins. An AVF results from a connection between a large artery and vein.

Hemodynamically significant AVFs and AVMs present with high-output heart failure and cyanosis in the newborn period. They can even present in fetal life with development of heart failure and even hydrops if severe. An effective large left-to-right shunt occurs through the direct arterial–venous connections. Heart rate, stroke volume, plasma volume, and cardiac output are increased. The fistulous connection lowers systemic vascular resistance, promoting a right-to-left shunt through the ductus arteriosus, particularly if the normal postnatal drop in PVR has not occurred. The increased systemic venous return increases right atrial pressures and promotes right-to-left shunting through the foramen ovale.

Cardiac examination reveals a hyperdynamic precordium. A prominent second heart sound, S3, and S4 may be heard. Systolic murmurs may be present secondary to tricuspid valve regurgitation or increased flow across the pulmonary valve. Increased tricuspid valve flow may create a diastolic sound. Bruits may be heard over the vascular malformation. When a malformation is suspected, care must be taken to auscultate areas where malformations are likely such as the head, liver, and chest. Arteries proximal to the malformation are typically dilated with bounding pulses, while distal ones are small with diminished pulses. ECG is nonspecific and may demonstrate right atrial and right ventricular enlargement. Chest x-ray reveals cardiomegaly with increased pulmonary vascular markings. Echocardiogram demonstrates generalized cardiomegaly. Treatment, if necessary, requires interventional closure of the anomalous vascular connections or the surgical removal of associated anatomic abnormalities.

Cardiomyopathy

A large body of literature exists describing the diagnosis and management of structural CHD in the fetus and newborn. There is a paucity of information, however, regarding the diagnosis and management of fetal and newborn cardiomyopathy. When presented with a newborn with signs of CHF, structural heart disease should be ruled out. In the absence of structural problems, the diagnosis of cardiomyopathy should be considered. The causes of neonatal cardiomyopathy include prenatal infections (cytomegalovirus, human immunodeficiency virus, parvovirus), familial or genetic causes, maternal autoimmune disease with anti-Ro or anti-La antibodies, prenatal drug exposure, arrhythmia-induced cardiomyopathy, and twin–twin transfusion syndrome. Postnatal evaluation should include a search for the cause.

Initial management is similar to that used for other types of heart disease. Initial stabilization may require mechanical ventilation, the use of inotropes, afterload reduction, and diuresis. Long-term treatment is dependent somewhat on the cause of the cardiomyopathy, as some forms may be reversible. Cardiac transplantation should be considered if cardiac function is poor, or improvement is not noted.

Acknowledgments

Special thanks to Benjamin E. Reinking and Thomas Scholz for their original contributions to this chapter.

Suggested Readings

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Long-Term Neurologic Outcomes in Children With Congenital Heart Disease

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KEY POINTS

- Congenital heart disease (CHD) is a common birth defect with continually improving survival of neonates with complex lesions requiring heart surgery.
- Neurodevelopmental (ND) abnormalities are common in school-age children and adolescents after neonatal heart surgery.
- Magnetic resonance imaging (MRI) studies have demonstrated evidence of brain injury and delayed brain development, even before having a corrective operation, in patients with hypoplastic left heart syndrome and transposition of the great arteries.
- Fetal MRI studies suggest this delayed development begins in the third trimester.
- Aberrant fetal physiology resulting in decreased oxygen and substrate supply to the brain may contribute to these imaging and ND abnormalities in addition to other perioperative risk factors.
- Children with complex CHD have a prevalence of pervasive but subtle cognitive problems termed *the neurodevelopmental signature of complex congenital heart disease*.

Severe congenital heart disease (CHD) requiring a corrective operation occurs in 6 to 8 per 1000 live births, with up to half of the cases requiring an operation in the neonatal period to survive.[1] With improved surgical techniques, mortality for CHD has steadily declined.[2] In fact, the number of adults living with CHD has surpassed the number of children with CHD in the United States.[3]

Given the changing epidemiology of CHD, considerable effort has been devoted to evaluating both neurodevelopmental (ND) outcomes and quality of life for patients with CHD. Evidence suggests that although children with CHD may no longer have overt signs of neurologic dysfunction, they may exhibit deficits in multiple domains, including visual-spatial skills, memory, executive function, speech and language, and gross and fine motor function.[4–6] Long-term follow-up data suggest that these deficits continue into adolescence and young adulthood with potentially significant impacts on societal contribution.[7–9]

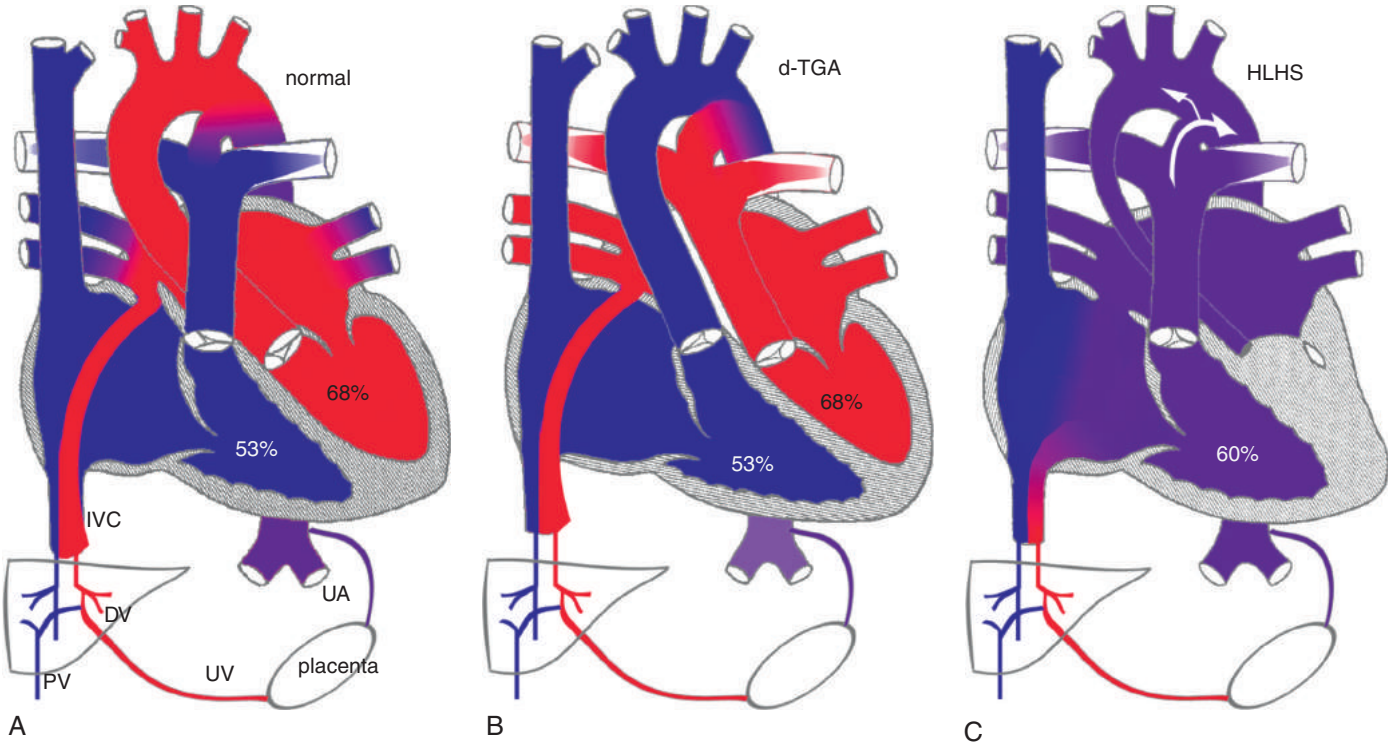
A natural assumption has been that these adverse outcomes were directly related to a brain injury sustained during neonatal cardiac surgical interventions. However, in the recent era it has become apparent that patient-specific risk factors play essential roles in determining the ultimate ND outcome and, furthermore, that the interplay between the brain and the circulation is complex, occurring at many levels throughout fetal and postnatal development. This chapter will review mechanisms influencing neurologic outcomes in CHD, including (1) the physiologic effects of congenital heart lesions on brain blood flow, (2) brain development in the context of CHD, (3) the timing, appearance, and mechanism of acquired brain injuries, and (4) current knowledge on ND outcomes in critical CHD in the short and long term.

Structural and Developmental Abnormalities of the Brain in Congenital Heart Disease

Human cardiac development is largely complete by gestational week 7.[10] In contrast, brain development extends over a much longer time period, with morphologic events (cell proliferation, migration, axon pathfinding, and target selection) occurring predominantly in the first two trimesters, followed by a prolonged period of refinement of circuits that begins in the third trimester and extends into infancy. This stage of brain development includes dramatic growth, myelination, and increasing neuronal electrical activity and depends upon receiving an adequate supply of nutrients and oxygen. Consequently, blood flow to the developing brain increases and is estimated to be a quarter of the combined ventricular output in the third trimester, demonstrating the unique heart–brain interplay critical for normal brain development.[11]

Fetal Circulation in Congenital Heart Disease: Effects on Cerebral Blood Flow

Fetal circulation is unique in many respects that can impact cerebral blood flow and development. In the normal fetus, cerebral blood flow is supplied by highly oxygenated blood from the ductus venosus preferentially streaming across the foramen ovale to the left atrium and ventricle (Fig. 51.1). In contrast, in fetuses with transposition of the great arteries (TGA), the aorta and pulmonary artery are transposed. Thus the higher oxygenated blood reaches the pulmonary vasculature as opposed to the cerebral vasculature. Similarly, in hypoplastic left heart syndrome (HLHS), inadequate left heart structures lead to a reversal of blood flow in the foramen ovale with the mixing of oxygenated and deoxygenated blood in the right ventricle and, in cases of aortic atresia, retrograde flow in the ascending aorta. The effects of these abnormal flow patterns on brain development are uncertain but may involve different mechanisms; despite that both decrease the oxygen



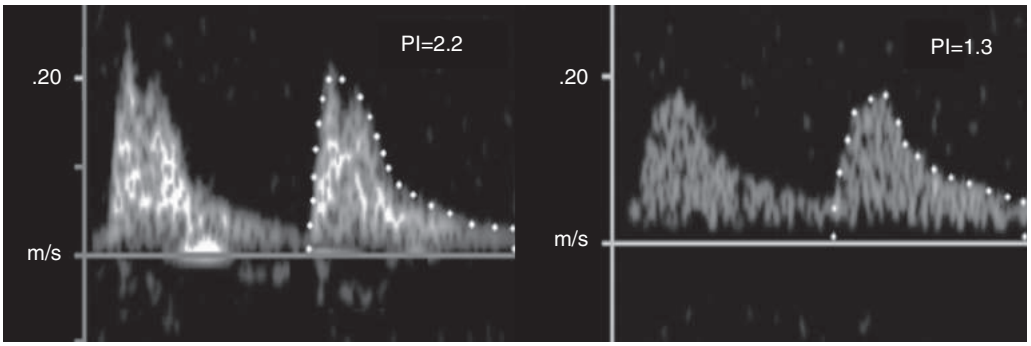
• **Fig. 51.1** Normal and Altered Fetal Circulation. (A) Normal fetal blood flow. (B) d-transposition of the great arteries (d-TGA). (C) Hypoplastic left heart syndrome (HLHS) with aortic atresia. Deoxygenated blood (*blue–purple*) flows to the placenta through the umbilical artery. Blood with higher oxygen content *(red)* returns through the umbilical vein and ductus venosus to the inferior vena cava (IVC). The higher saturated blood from the IVC is preferentially directed toward the foramen ovale into the left ventricle, ascending aorta, and head and neck vessels in the normal fetus. Desaturated blood also returns to the right side of the heart and is directed to the descending aorta through the ductus arteriosus. In the fetus with d-TGA, the aorta arises from the right ventricle such that the brain receives less oxygenated blood, while the higher saturated blood is directed to the descending aorta through the ductus arteriosus. In HLHS, reduced or absent left-ventricular ejection results in elevated left-atrial pressure, limiting or reversing flow at the foramen ovale and resulting in complete mixing of desaturated and well-saturated blood in the right atrium and ventricle. Blood flow to the head and neck may occur in a retrograde fashion from the ductus arteriosus across the aortic isthmus. *d-TGA*, d-transposition of the great arteries; *DV*, ductus venosus; *HLHS*, hypoplastic left heart syndrome; *IVC*, inferior vena cava; *PV*, portal vein; *UA*, umbilical artery; *UV*, umbilical vein. (From McQuillen PS, Goff DA, Licht DJ. Effects of congenital heart disease on brain development. Prog Pediatr Cardiol. 2010;29(2):79–85.)

content of the blood delivered to the brain. Other mechanisms may be involved, such as inadequate substrate delivery (glucose) because of decreased perfusion pressure and flow to the brain.[12] In the d-transposition of the great arteries (dextroposition [d]-TGA), the pulsatility and perfusion pressure of the cerebral circulation are normal. However, in HLHS, the hypoplastic isthmus and aortic arch may function as resistors, potentially decreasing the pulsatility and perfusion pressure to the cerebral circulation. In contrast, in d-TGA, decreased pulsatility and perfusion to the brain can result from preferential blood flow to the pulmonary vasculature because of a lower pulmonary vascular resistance than usual.

Cerebral Doppler ultrasound can assess fetal cerebral vascular resistance in the middle cerebral artery (MCA) and provide insight into fetal cerebral blood flow patterns. By calculating the MCA pulsatility index (PI, a measure of vascular resistance in the circulatory bed downstream from the point of Doppler sampling), studies have identified a pattern of "brain-sparing" in fetuses with intrauterine growth restriction and placental insufficiency as a consequence of autoregulation of fetal cerebral blood flow.[13,14] In normal pregnancies, the cerebral/umbilical PI ratio is more than 1.0, whereas, in many growth-restricted fetuses, the ratio is less than 1.0 and predicts adverse perinatal and neurologic outcomes.[15,16] This autoregulatory mechanism is thus paradoxically a harbinger of poor outcomes in the setting of fetal growth restriction.

There have been several studies examining in utero blood flow patterns in human fetuses with CHD. These have demonstrated lower MCA PI in fetuses who have lesions with the most intracardiac mixing, such as HLHS (Fig. 51.2). In fact, fetuses with HLHS have been shown to have the lowest cerebral/umbilical PI ratio among different types of CHD.[17,18] This is likely secondary to the lower oxygen content of blood delivered to the brain and abnormalities in cerebral perfusion with a hypoplastic aortic isthmus.

Cerebral blood flow characteristics have been shown to predict ND outcomes in fetuses with CHD. In a retrospective multicenter study of infants with HLHS, a lower MCA PI in utero predicted a better ND outcome at 14 months of age as assessed by the Bayley Scales of Infant Development (BSID) II.[19] These findings suggest that the autoregulatory response of cerebral vasodilation in the setting of HLHS may be sufficient and adaptive to



• **Fig. 51.2** Middle cerebral artery Doppler patterns in a normal fetus and a fetus with hypoplastic left heart syndrome (HLHS). The pulsatility index (peak systolic velocity–end diastolic velocity/mean velocity) in the HLHS fetus is lower *(right panel)*, suggesting decreased impedance in the cerebral vasculature. *PI*, Pulsatility index.

a state of chronic hypoxemia, which is in contrast to what is seen in the context of fetal growth restriction. Further large prospective studies are needed to understand the predictive utility of cerebral blood flow patterns in fetuses with CHD.

Preoperative Evidence of Delayed Brain Development by Magnetic Resonance Imaging

Structural brain malformation in neonates with CHD has been identified, even in the absence of a defined genetic syndrome. Autopsy studies have revealed multiple congenital brain anomalies in neonates with HLHS, including microcephaly, abnormal cortical mantle formation, and overt central nervous system malformations such as agenesis of the corpus callosum or holoprosencephaly.[20,21]

In addition to structural abnormalities, there is magnetic resonance imaging (MRI) evidence that brain development is delayed in CHD prior to corrective surgery. Quantitative MRI techniques such as diffusion tensor imaging (DTI) measure the direction and magnitude of water movement and thus microstructural brain development.[22] During normal brain development, the magnitude of water diffusion motion decreases (apparent diffusion coefficient [ADC]), and regional directionality increases in white matter (fractional anisotropy [FA]). Similarly, metabolic brain development can be measured with magnetic resonance spectroscopy (MRS) by measuring major metabolic compounds such as *N*-acetylaspartate (NAA), choline (Cho), creatine (Cr), and lactate. Using these techniques, researchers have discovered that newborns with CHD (d-TGA and single ventricle lesions) have findings suggesting an immature brain with abnormal DTI (4% higher ADC and 12% lower FA) and MRS (10% lower NAA/Cho ratio). Comparing these findings with those obtained in premature newborns without CHD, full-term newborns with CHD appear approximately 1 month delayed.[23] These observations have been replicated in studies assessing brain development by semiquantitative morphologic scoring, specifically the brain total maturation score (TMS). The TMS was found to be significantly lower in full-term newborns with d-TGA and HLHS compared with a normal cohort of newborns.[24] Finally, morphometry studies have demonstrated lower total and regional brain volumes in newborns with CHD compared with controls.[25] These findings help explain the abnormal somatic growth seen in the newborn with CHD. Specifically, those with d-TGA and HLHS have smaller head circumferences that are out of proportion to their weight.[26,27] These brain MRI findings in the neonate with CHD, seen before any corrective operations are performed, have led to the theory that abnormal brain development and susceptibility to acquired injury begin in utero.

Fetal Brain Magnetic Resonance Imaging Identifies Developmental Abnormalities in Congenital Heart Disease

Technical advances in fetal MRI have made it an important tool in the clinical evaluation of fetuses with suspected cerebral abnormalities. A study comparing brain volumes and MRS between normal fetuses and fetuses with CHD between 25 and 37 weeks' gestation showed definitive evidence for delayed fetal brain development.[28] During the third trimester, a progressive impairment of brain volumes was observed, particularly in those fetuses with left-sided obstruction. Additionally, larger delays in the expected increase in NAA/Cho ratio and greater impairment of growth in brain volume were noted in fetuses with aortic atresia, who have no antegrade blood flow in the aortic arch. These observations support the concept that brain development is altered during fetal life because of impaired fetal cerebral blood flow, oxygen, and substrate delivery and that compensatory mechanisms (i.e., brain-sparing effect) may not be adequate.

Recently, novel fetal cardiac MRI techniques have been utilized to understand the heart–brain interplay in CHD. These techniques enable measurements of flow and oxygen saturation in fetal blood vessels. By combining fetal brain MRI and cardiovascular magnetic resonance, researchers found a correlation between fetal cerebral oxygen consumption and brain size (estimated brain weight) among 30 fetuses with CHD in late gestation.[29] There was a direct correlation between estimated brain weight and cerebral oxygen consumption. In addition, there was a modest association between cerebral oxygen delivery and brain size. Other novels and efficient imaging techniques have been developed to understand cerebrovascular physiology while accommodating significant motion artifacts from fetal movement and maternal breathing. These studies revealed that fetuses with both left sided obstructive lesions and d-TGA have lower fetal cerebral oxygenation compared to controls.[30] The predictive value of these findings for postnatal outcomes such as brain injury and ND outcomes remains unknown. Further exploration to potentially identify targets and methods of intervention to modify ND outcome in CHD is warranted.

Trajectory of Brain Development in Congenital Heart Disease

Although much of the literature has been focused on the identification of abnormal brain development in the fetus and newborn with CHD, studies on older patients have emerged suggesting that these delays continue into adolescent years. In a study of single ventricle patients who had undergone a Fontan operation, the frequency of any structural abnormality on MRI was 11 times higher than in a normative cohort.[31] Similarly, in a brain MRI study of adolescents with d-TGA, FA was significantly reduced in several regions of the white matter compared with a normative cohort.[32] Many have postulated that the trajectory of brain development over time may be dependent on the specific cardiac lesion and whether the surgical management is corrective (i.e., d-TGA) or largely palliative (i.e., HLHS). In particular, patients with aortic atresia have been found to have the least robust microstructural brain development.[33] In a direct comparison of HLHS to d-TGA, neonates with d-TGA had a faster rate of global and regional brain growth in the perioperative time period.[34] Another study demonstrated, at 2.5 years of age, more cortical atrophy and lower brain volumes in those with HLHS compared with those with d-TGA, suggesting a lesion-specific influence on the long-term trajectory of brain development.[35]

Acquired Brain Injury With Congenital Heart Disease: Characteristics and Risk Factors

Neonates with CHD are also at risk for newly acquired brain injury. Delayed brain development itself may be a risk factor for new brain injury, particularly preoperative injury.

Focal brain injury in the term newborn can be clearly and reliably detected with conventional MRI and with greater resolution than with either ultrasound or computed tomography. The most common brain injuries observed in newborns with CHD are focal white matter injury (WMI) and small focal strokes (less than one-third to two-thirds of the arterial distribution; Fig. 51.3). These injuries are largely clinically silent and can be overlooked with routine clinical screening cranial ultrasounds.

WMI has specific imaging characteristics defined by punctate periventricular lesions seen as hyperintensity on T1-weighted MRI. Identification of this pattern of injury was unexpected because it was thought to be restricted to premature newborns with brain injury (periventricular leukomalacia). Risk factors for brain injury are summarized below and in Table 51.1. Direct comparison of focal WMI in premature newborns and term CHD newborns confirms similarities in the imaging appearance and magnitude of injury in the two groups, but topological distribution differed with more involvement of central regions in premature newborns.[36] Importantly, longitudinal studies have revealed that WMI on the moderate to severe end of the spectrum is associated with worse neurodevelopmental outcomes in late infancy and early childhood.[37,38] Thus, although these forms of early brain injury are "clinically silent," they appear to be clinically relevant.

Risk Factors for Preoperative Brain Injury

Several large prospective studies have been performed using preoperative and postoperative brain MRI to determine the frequency of acquired brain injury and associated risk factors in newborns with CHD (Table 51.2). Preoperative brain injury in the form of WMI or stroke is present in 28% to 39% of these newborns (see Table 51.2).[39–42,48] This was a surprising finding, given the focus on operative factors causing ND abnormalities in these patients. Risk factors for preoperative brain injury include hypoxemia and time to surgery,[43] preoperative base deficit, preoperative cardiac arrest, and the need for balloon atrial septostomy.[49] Other patient-specific risk factors identified include male sex and the presence of aortic atresia (lack of antegrade flow in the aorta) in those with HLHS.[46]

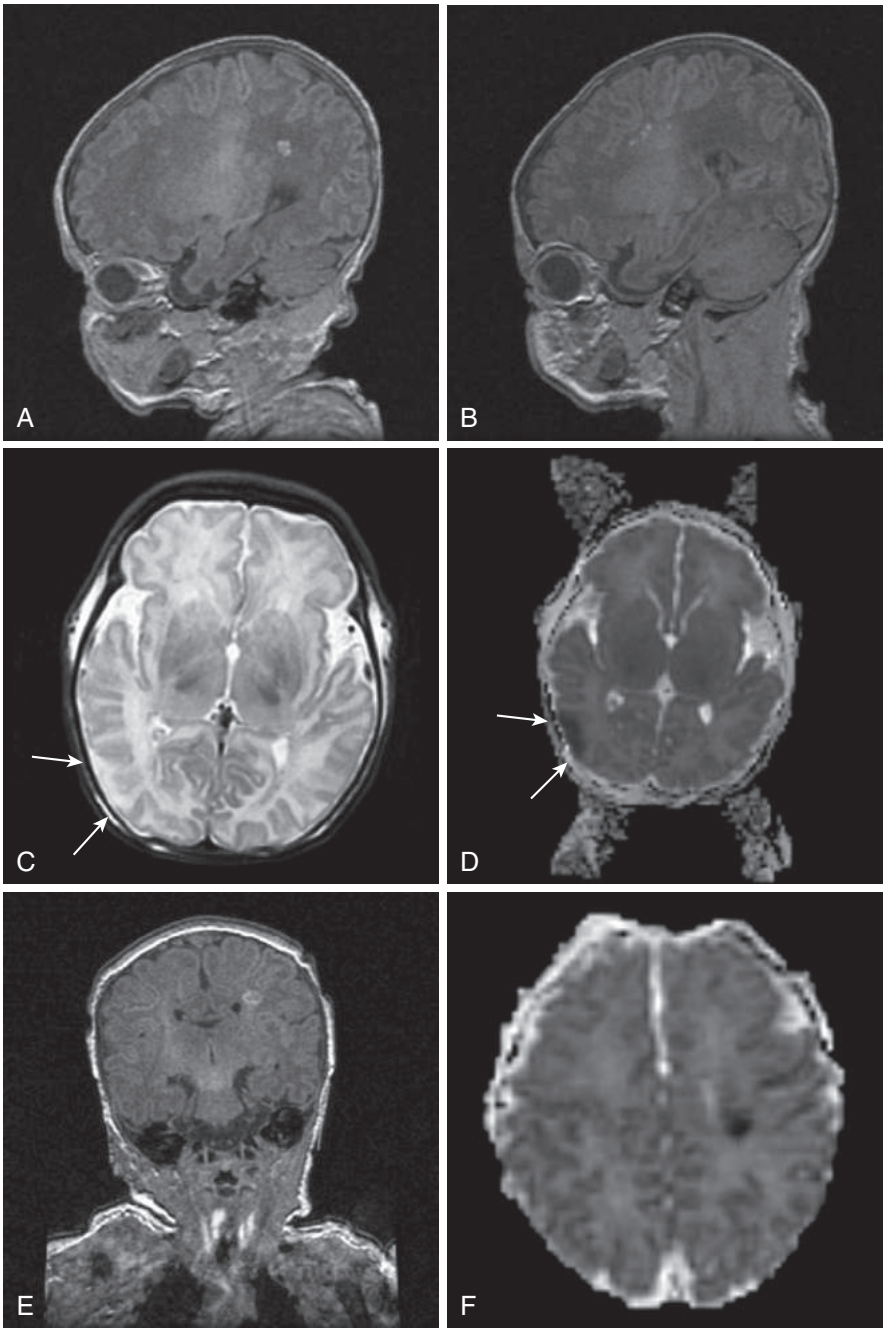
Risk Factors for Intraoperative Brain Injury

Proposed risk factors for intraoperative brain injury relate predominantly to the methods of cardiopulmonary bypass and/or hypothermic total circulatory arrest. The Boston Circulatory Arrest Trial compared two methods of vital organ support in infants undergoing open heart surgery to repair d-TGA with an arterial switch operation.[50] This operation is "corrective" since normal cardiovascular physiology is reestablished with low mortality and excellent long-term cardiac functional outcomes. Although deep hypothermic circulatory arrest, which provides the surgeon with an empty and relaxed heart, clearly allowed intricate surgeries to be performed, there was concern at the time regarding late adverse neurologic outcomes, and the "safe" duration of circulatory arrest was unknown. An alternative method (low-flow bypass) was felt to maintain some amount of brain oxygen delivery while still allowing the surgeon a relatively bloodless field. This landmark study enrolled 171 infants in a single-center, randomized clinical trial comparing deep hypothermic total circulatory arrest with low-flow cardiopulmonary bypass. All early outcome variables pointed to a benefit from low-flow bypass compared with circulatory arrest. Specifically, the circulatory arrest group had more frequent postoperative seizures, higher serum levels of brain-specific enzymes (creatine kinase), worse 1-year motor outcome (BSID—Psychomotor Development Index [PDI]), and abnormalities on a neurologic exam.

In contrast, no differences were found in cognitive development (BSID—Mental Development Index [MDI]) or MRI at 1 year of age. Importantly, these differences between the groups disappeared when the patients were assessed at older ages, but both groups remained below population norms for performance on standardized tests (detailed later in this chapter).[51] Other studies have identified circulatory arrest as a risk factor for new postoperative WMI identified on MRI.[52]

Additional variables examined included hypothermic blood pH management (alpha stat versus pH-stat), hemodilution/hematocrit (25% vs. 35%), and maintenance of regional cerebral perfusion during aortic arch reconstruction.[53,54] Very few of these studies have identified definitively improved neurologic outcomes. Patients who underwent regional cerebral perfusion tended to have worse outcomes, and this technique was associated with new postoperative injury on brain MRI.

These results suggest that although risks remain during the intraoperative period, a major burden of risk for acquired injury occurs outside of the operative period. However, the possibility that unidentified intraoperative risk factors contribute to brain injury cannot be excluded. Analysis of combined cohorts of subjects requiring neonatal cardiac surgery over a 13-year period did not identify substantial improvement in early ND outcomes despite improvements in survival.[55]



• **Fig. 51.3** Magnetic Resonance Imaging Patterns of Brain Injury in Congenital Heart Disease. (A, B) Moderate white matter injury (WMI) in a newborn with hypoplastic left heart syndrome (HLHS) is seen on sagittal T1 images in the postoperative scan. WMIs appear as small focal areas of T1 hyperintensity (brightness). (C, D) Term newborn with HLHS imaged postoperatively at day 17 of life, after a modified Norwood procedure. A small middle cerebral artery distribution infarct is seen as cortical T2 hyperintensity (*arrows* in C) and corresponding reduced diffusion (*arrows* in D) in the right parietal–occipital lobe. (E, F) Term newborn with transposition of the great arteries imaged preoperatively after a balloon atrial septostomy. A single focus of T1 hyperintensity is seen in the periatrial white matter on the coronal sequence (E). This same focus has reduced water diffusivity on the average diffusivity map (F, dark spot). This spot is larger than the typical solitary white matter lesion and may represent a small embolic stroke.

Risk Factors for Postoperative Brain Injury

Risk factors for postoperative brain injury include hypotension and hypoxemia related to low cardiac output syndrome, defined as a combination of clinical signs (tachycardia, oliguria, cold extremities, or cardiac arrest) and a greater than 30% difference in arterial–mixed venous oxygen saturation or lactic acidosis. Multiple studies have identified hypotension as a risk factor for new postoperative WMI, including low systolic blood pressure on admission, low mean blood pressure during postoperative day 1, and low diastolic blood pressure during postoperative days 1 to 2.[42,56]

TABLE 51.1 Risk Factors for Brain Injury

Preoperative	Intraoperative	Postoperative
Low arterial hemoglobin saturation	Prolonged total circulatory arrest (>40 min)	Low blood pressure
Length of time to surgery	Decreased cerebral oxygen saturation (NIRS)	Low arterial PaO_2
Catheter-based procedure (e.g., balloon atrial septostomy)	Cardiopulmonary bypass strategy (regional cerebral perfusion)	Prolonged cerebral regional oxygen saturation (NIRS <45% for >3 h)
Preoperative base deficit	Air or particulate emboli	Morphologically immature brain (total maturation score)
Preoperative cardiac arrest	Inflammation	Single ventricle physiology
Morphologically immature brain (total maturation score)		
Male sex		
Aortic atresia		

NIRS, Near-infrared spectroscopy; *PaO_2*, partial pressure of oxygen.

TABLE 51.2 Magnetic Resonance Imaging Evidence of Preoperative Brain Injury in Newborns With Congenital Heart Disease

Study	Sample	CHD	Findings	Risk Factors
Mahle et al. 2002[39]	CHD = 24	Mixed	PVL = 16% Infarct = 8% Elevated brain lactate = 53%	N/A
Miller et al. 2004[40]	CHD = 10 Control = 5	TGA	Brain injury (stroke) = 40% Elevated brain lactate = 2.7 × higher than controls	N/A
Licht et al. 2004[41]	CHD = 25	Mixed	PVL = 28%	• Decreased cerebral blood flow • Hypercarbia
McQuillen et al. 2007[42]	CHD = 62	Mixed	WMI = 18% Stroke = 21% IVH = 8%	• Low Apgar score at 5 min • BAS
Petit et al. 2009[43]	CHD = 26	TGA	PVL = 38%	• Lower preoperative oxygen saturation • Longer time to surgery
Andropoulos et al. 2010[44]	CHD = 68	Mixed	WMI = 16% Infarct = 18%	• Low total maturation score (brain immaturity)
Glass et al. 2011[45]	CHD = 127	Mixed	WMI = 24%	• Bloodstream infection in TGA subjects
Goff et al. 2013[46]	CHD = 57	HLHS	PVL = 19%	• Male sex • Aortic atresia • Low total maturation score (brain immaturity)
Peyvandi et al. 2016[47]	CHD = 153	Mixed	WMI = 24% Stroke = 20% Hypoxic–ischemic = 1%	• Postnatal diagnosis of CHD

BAS, Balloon atrial septostomy; *CHD*, congenital heart disease; *HLHS*, hypoplastic left side heart syndrome; *IVH*, intraventricular hemorrhage; *N/A*, not applicable; *PVL*, periventricular leukomalacia; *TGA*, transposition of the great arteries; *WMI*, white matter injury.

Studies utilizing cerebral near-infrared spectroscopy have also suggested that low regional cerebral oxygen saturation (<45%) for more than 3 hours was a risk factor for new ischemic injury.[57] In general, patients with single ventricle lesions carry a higher risk of postoperative brain injury, which correlates with higher postoperative hemodynamic instability, morbidity, and mortality.

Brain Immaturity as a Risk Factor for Brain Injury

The relationship between brain immaturity and brain injury has been explored in the literature although with variable results. Qualitative MRI (TMS) techniques have suggested an association between brain immaturity and the risk of preoperative and

postoperative brain injury.[44] However, quantitative MRI techniques (DTI and MRS) have demonstrated an association between brain immaturity and the risk of preoperative brain injury but not postoperative brain injury.[58] Both studies suggest that brain immaturity may be a risk factor for brain injury, with slightly different results. These differences likely relate to the method of measuring brain development. MRS and DTI exhibit changes with brain development and can be influenced by acquired brain injury based on severity, timing, and mechanism. Although overt brain injury has not been identified in the fetal period, indolent brain injury may be present influencing developmental changes and further injury in the preoperative and postoperative period.

Neurodevelopmental Outcomes

There is an increasing body of literature reporting short-term and long-term ND outcomes in patients with various types of CHD. Despite heterogeneity among these reports because of various methodologies for age at follow-up, assessment tools used, and type of cardiac lesion, these outcome data have provided us with useful knowledge on ND outcomes and provide the foundation for understanding imaging and clinical data in the fetal and neonatal period. Longitudinal studies are needed to assess the typical trajectory of brain growth and pattern of injury as these children grow older.

Immediate Neurologic Outcomes After Surgical Repair

Although the prevalence of overt neurologic dysfunction postoperatively has declined, a small percentage of infants continue to exhibit neurologic abnormalities, including clinical seizures, hypotonia, and asymmetry of tone. Combining several reports, the prevalence of postoperative subclinical seizures appears to be 4% to 11% and has been detected by continuous electroencephalogram monitoring in up to 20% of patients in the immediate postoperative period.[50,59–63] In a more recent study, a large percentage of young infants had evidence of ND abnormalities before surgery, suggesting that fetal and neonatal factors play a significant role in brain injury and development.[64] In addition, there is a higher prevalence of feeding abnormalities (swallow or suck dysfunction) in neonates undergoing cardiac surgery, which may be an early indicator of abnormal neurodevelopment later in life.[65]

Short-Term and Long-Term Neurologic Outcomes After Surgical Repair

Although there have been several reports of ND outcomes in a mixture of CHD types, it is important to recognize that outcomes can vary significantly by the cardiac lesion. Outcome studies have been carried out in two specific high-risk patient populations: those with d-TGA and those with defects requiring single ventricle palliation (i.e., HLHS).

In patients with d-TGA after an arterial switch operation, the Boston Circulatory Arrest Trial (described previously) has demonstrated that intelligence quotient (IQ) scores, although below the national average, fall within the normal range at 8 and 16 years of age.[8,9] However, d-TGA patients at 16 years of age continue to exhibit deficits in academic achievement, memory, executive function, visual-spatial skills, attention, and social cognition. A high percentage of children were judged to have behavioral problems by parents and teachers; 37% required remedial education services, and 10% had repeated a grade. Interestingly, in a multivariable regression model, socioeconomic status was a strong predictor of ND outcome in these patients at 16 years of age. These studies demonstrate that early ND testing may underestimate the ultimate burden of functional impairment in subjects with subtle but diffuse brain injuries.

Patients with single ventricle lesions, particularly HLHS, are at the highest risk of ND abnormalities based on the underlying physiology, hemodynamics, and complexity of surgical repair. These children must undergo a series of palliative surgical procedures, typically culminating in a Fontan operation. Various studies have demonstrated that children with HLHS tend to have lower IQs (typically below the general population mean) and problems with visual–motor skills, expressive language, attention, and externalizing behavior. In the largest series reported to date, children with HLHS at 12 months of age had a median MDI of 90 (range 50 to 129) and a lower PDI of 73 (range 50 to 117).[4] Risk factors for poor outcomes were mainly patient-specific and included genetic syndromes, earlier gestational age at delivery, and perioperative instability. Other studies have assessed the impact of growth and feeding issues on ND outcomes. Infants requiring device-assisted feeding and with lower weight, length, and head circumference at 3 months of age were at increased risk for ND delay at 6 and 12 months of age.[65] Long-term studies have identified persistent ND abnormalities in adolescents with Fontan physiology, including lower IQ and abnormal neuropsychological testing compared with a normative population.[31]

Genetic Susceptibility to Neurodevelopmental Abnormalities

An important point to consider is the impact of genetic comorbidities on ND outcomes in the context of CHD. Approximately one-third of children with CHD have an underlying genetic disorder such as aneuploidy or deletion syndromes (i.e., 22q11.2 deletion syndrome). Studies have shown greater impairments in cognition, IQ, motor skills, hearing, and visual skills in those children compared with children with CHD who do not have genetic comorbidity.[66,67] Several studies have been performed in children with CHD who also have 22q11.2 deletion syndrome. This microdeletion encompasses three megabases of DNA, representing 30 to 40 genes, and the syndrome is highly heterogenous with a variable phenotype. These patients have also been noted to have psychiatric disorders such as anxiety, attention deficit hyperactivity disorder, and psychosis. Interestingly, the presence of CHD does not change the prevalence of these psychiatric abnormalities.[68]

Studies have also identified patient-specific genetic risk factors that can modify ND outcomes. This has been described for apolipoprotein E (APOE) alleles in which the ApoE ε4 allele is associated with adverse outcomes in many adult conditions (e.g., Alzheimer's disease, traumatic brain injury, stroke, and subarachnoid hemorrhage). This has been explored in infants with CHD, and the ApoE ε2 allele is associated with worse outcomes.[69] Finally, in a large study of children with various forms of CHD, exome sequencing of trio samples (including the mother and father) revealed a higher burden of de novo deleterious mutations in genes with high heart and high brain expression, particularly in those that endorsed any degree of developmental impairment.[70]

Overall, more than 30% of newborns with CHD have a genetic anomaly. Ten percent of newborns have de novo single nucleotide variants (SNV) causing CHD, and this fraction increases to more than 20% in the setting of neurodevelopmental disability. Interestingly, SNVs most commonly occur in genes involving primary cilia or chromatin-modifying pathways.[71] Thus, it is likely that multiple genes and environmental factors influence ND outcomes in these patients.

Neurodevelopmental Signature of Congenital Heart Disease

Although children with complex CHD have intelligence testing results in the range of the normal population, there is a prevalence of pervasive but subtle cognitive problems that some have termed *a neurodevelopmental signature of complex congenital heart disease*. These children show behavioral and attention problems that are often not detected on standardized testing but result in poor school performance. Among a large cohort of children who were followed prospectively after undergoing cardiac surgery as infants, abnormalities on a neurologic exam at school entry were present in 28%, although less than 5% were severe. Most of the abnormalities involved fine motor coordination and tone. Cognitive difficulty and behavioral problems were identified in 30%. Certainly, survivors of CHD exhibit a "developmental profile" that changes in each stage of life and affects the quality of life and the ability to perform everyday tasks.[64] It is important to note that the American Heart Association released a statement paper recommending serial ND assessments for at-risk children with CHD.[67] This includes neonates requiring open heart surgery (before 30 days of life) and other cyanotic heart lesions such as tetralogy of Fallot that may not require a neonatal operation. In addition, serial evaluation is recommended whenever CHD is seen in combination with prematurity (<37 weeks gestation), developmental delay recognized in infancy, suspected genetic anomaly, history of extracorporeal life support, heart transplantation, the need for cardiopulmonary resuscitation, prolonged perioperative hospitalization, perioperative seizures, and abnormal neuroimaging findings.

Other populations with an increased risk of neurologic abnormalities include the rising number of premature infants with CHD. Rates of CHD are higher in premature newborns, and mortality and morbidity of CHD increase with decreasing gestational age at birth.[72,73] Both prematurity and CHD are independent risk factors for neurodevelopmental abnormalities; thus, as surgical techniques evolve and allow for intervention on premature and smaller neonates, attention must be paid to neurologic outcomes, including rates of brain injury and neurodevelopmental outcomes, to identify optimal management strategies for this unique group of patients.

Conclusion

Advances in prenatal diagnosis and care and cardiovascular surgical techniques have contributed to the overall increased survival of neonates born with CHD. Given this improvement in survival, greater emphasis is now being directed toward improving ND outcomes. More specifically, understanding the predictive value of imaging studies both in the fetus and neonate provides an opportunity for potential intervention trials to improve outcomes among this patient population. In addition, as the prenatal detection of CHD increases, informing families about potential noncardiac adverse outcomes is critical to ensure recognition and timely intervention for ND abnormalities.

Suggested Readings

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52 Central Nervous System Development

BOBBI FLEISS, HELEN STOLP, VALERIE MEZGER, AND PIERRE GRESSENS

KEY POINTS

- The brain of the preterm and term newborn infant is actively developing, with several key steps occurring during the third trimester, such as the end of neuronal migration, programmed cell death, the generation of axons and dendrites, and the first wave of synaptogenesis.
- These processes are controlled by genetic programs but are also very sensitive to environmental factors.
- Epigenetic mechanisms appear to play a central role in these processes, with long-term and potentially trans-generational consequences.

Brain development results from the accomplishment of successive genetic programs during the different ontogenic stages. It starts with the individualization of the neural plate at the beginning of the third week post-conception and is mostly complete by adolescence. However, some neuronal production persists lifelong. This late neurogenesis has been well described at the level of the olfactory bulbs and the dentate gyrus of the hippocampus, but its importance to the associative neocortex remains to be shown.

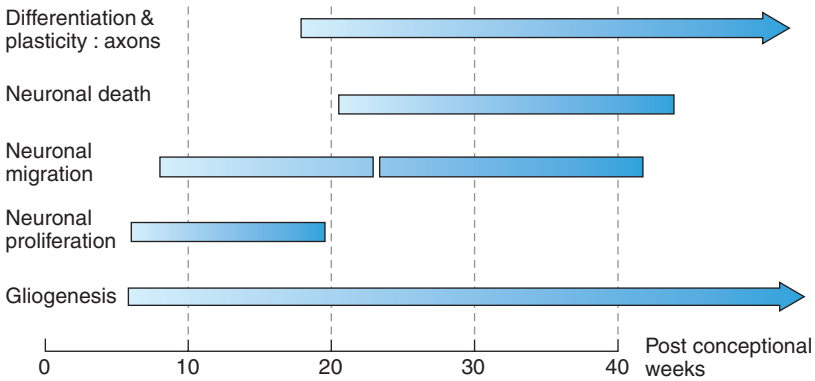
The principal stages of brain development can be summarized as follows: induction of the neuroectoderm, formation of the neural tube followed by the telencephalon, neurogenesis (production of neuronal progenitors and then of mature neurons), neuronal migration, programmed neuronal death, generation of neurites (axons and dendrites), elimination of superfluous neurites, synaptogenesis, elimination and selective stabilization of synapses, angiogenesis, gliogenesis (production of astrocytes and oligodendrocytes), and myelination (Fig. 52.1). These different stages of the development and maturation of the brain are controlled by intrinsic factors (determined genetically) and modulated by extrinsic environmental factors. This modulation by environmental factors could bring epigenetic mechanisms into play. The perturbation of the unfolding of any of these different stages of brain development leads to a deficit in brain growth and/or brain malformations. The functional consequences to the child depend on the developmental stage in question. This raises the notion of "critical periods," which we will touch on throughout this chapter; a critical period is a maturational stage in the lifespan of an organism during which the nervous system is especially sensitive to certain environmental stimuli.

Neuronal Production and Migration

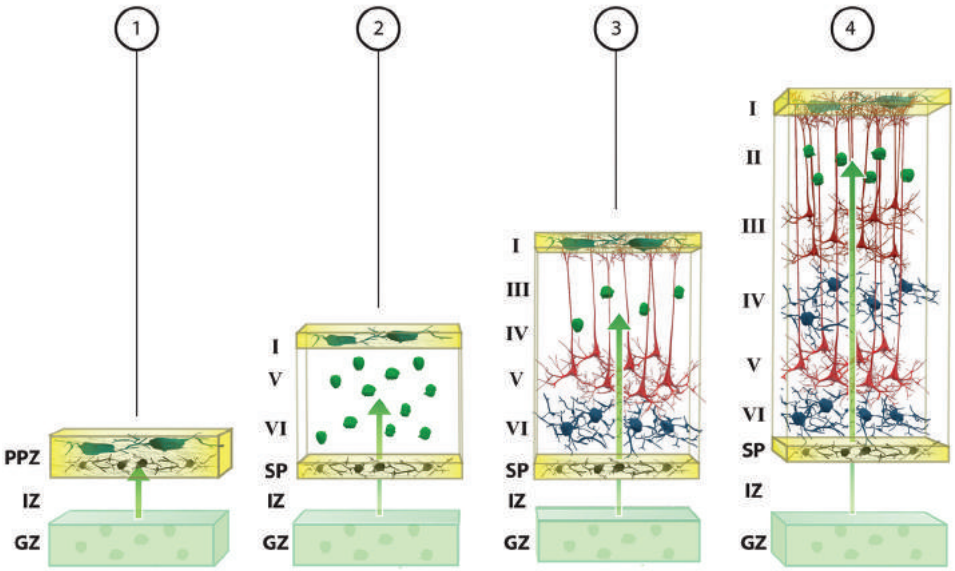
The expansion of the cortex occurs through two processes that take place in parallel: a lateral expansion process that allows the surface of the cortex to grow, and a radial expansion process that leads to an increase in its thickness. The total number of neurons in a mature human brain is estimated at between 3 and 100 billion. The cortex is initially generated from a radially oriented monolayer of proliferative neuroepithelial cells lining the walls of the lateral ventricles—the ventricular zone (VZ). Around the seventh gestational week in humans a second proliferative zone, the inner subventricular zone (SVZ), appears, derived from precursors in the VZ.[1] The cells of the inner SVZ do not adopt a radial conformation. More recently, a third proliferative zone has been identified in the developing neocortex: the outer SVZ, which appears in humans around gestational week 11. In humans, this outer SVZ displays prolonged proliferation. This difference in the behavior of neuronal precursors has been hypothesized to explain the impressive evolutionary expansion and folding observed in the surface of the neocortex.

These proliferative zones, situated on the dorsal side of the lateral ventricles, give rise to the excitatory (glutamatergic) neurons of the different cortical layers (the cortex in mammals consists of six layers), as well as potentially (as this is still controversial and seems to be species/cell subtype-dependent) a portion of inhibitory neurons (GABAergic interneurons). The other inhibitory neurons derive from another proliferative structure located on the lateral wall of the lateral ventricles, the ganglionic eminence (which is also the source of thalamic neurons) and one located in the preoptic area. Migrating neurons can adopt a radial trajectory by migrating in contact with specialized glial cells, the radial glia, which serve to guide them.[2,3]

After exiting the mitotic cycle, neurons migrate from the proliferative zones toward the future cortex.[1,4–8] The first wave of migratory neurons forms the primitive cortical plate or preplate (Fig. 52.2). The second wave of migratory neurons then splits this primitive plate into two, around gestational week 7, giving rise to a three-layered structure: layer I, which contains the Cajal-Retzius neurons, is located just below the meninges, layer VI, which contains neurons that have already completed their migration, and finally the subplate—a transient structure located below the future neocortex. Consecutive waves of migratory neurons subsequently cross the subplate and the cortical layers already in place, but stop



• **Fig. 52.1** The major ontogenic events taking place in the human neocortex.



• **Fig. 52.2** Mammalian neocortical formation and neuronal migration. *GZ*, germinative zone; *I*, cortical layer I or molecular layer; *II to VI*, cortical layers II to VI; *IZ*, intermediate zone (prospective white matter); *PPZ*, primitive plexiform zone; *SP*, subplate. Arrows and green circles indicate migrating neurons.

below layer I, thus successively forming layers V, VI, III, and II along what is known as an "inside-out" gradient.[9] Until recently, it was thought that the migration of neurons to the neocortex was complete by around gestational week 24.[10] However, recent studies suggest that GABAergic interneurons continue to be added to the neocortex practically until term.[11] In animal models of encephalopathy of prematurity and in human post mortem tissues of preterm neonates, a rarefaction of some subclasses of cortical interneurons has been demonstrated.[12–14]

Several molecules involved in controlling neuronal migration and their navigation to the appropriate destinations have been identified. These molecules can be schematically divided into four categories.[15,16]

1. Cytoskeletal molecules, which play an important role in the initiation and progression of neuronal movement (extension of the apical process and nucleokinesis). Molecules controlling initiation include Filamin-A (an actin-binding protein that is implicated in periventricular nodular heterotopias) and Arfgef2 (a molecule that plays a role in vesicular trafficking and is involved in periventricular nodular heterotopias associated with microcephaly). Among the molecules controlling progression are Doublecortin (a microtubule-associated protein—MAP—implicated in double cortex syndrome), Lis1 (a MAP implicated in type 1 lissencephaly and Miller Dieker syndrome) and Alpha-1 Tubulin (involved in the formation of tubulin heterodimers).
2. Signaling molecules that play a role in lamination, such as Reelin (a glycoprotein implicated in a human disorder combining lissencephaly with cerebellar hypoplasia).
3. Molecules modulating glycosylation that provide a stop signal to migrating neurons, such as POMPT1 (protein *O*-mannosyltransferase, associated with Walker-Warburg syndrome), POMGnT1 (protein *O*-mannose beta-1,2-N-acetylglucosaminyltransferase, implicated in muscle-eye-brain disease), and Fukutin (a glycosyltransferase implicated in Fukuyama congenital muscular dystrophy). These three human disorders display type 2 lissencephaly.
4. In addition to these three principal groups of molecules, neuronal migration can be modulated by other factors such as certain neurotransmitters (glutamate and GABA), molecules derived from peroxisomal metabolism, and certain environmental factors (inflammation, ethanol, and cocaine).

Programmed Neuronal Death

Depending on the brain region under consideration, 15% to 50% of the neurons initially produced die through a physiological process termed programmed cell death or apoptosis. Approximately 70% of the neurons that disappear seem to die between gestational weeks 28 and 41 in humans.[17]

Programmed cell death is a complex mechanism governed by a balance between cell death- and survival-inducing signals, genetic programs involved in cell death or survival, effectors of cell death, and inhibitors of these effectors.[18] Cells that have initiated programmed cell death eventually go onto to be phagocytosed by neighboring glial cells (microglia) without inducing inflammatory phenomena or scar formation. This process is facilitated by changes in the composition of the apoptotic cell membrane ("eat me signals") which are sensed by the microglia which occurs very early in the apoptotic process. Within the cell death process, the activation of caspases (proteolytic enzymes) in the form of a cascade is a key stage that culminates in DNA fragmentation and the death of the neuron. In addition, it is reported that direct interaction between healthy neurons and microglia can initiate cell death programs or even engulfment of the live cell, which is then "digested" by the microglia in a process called phagoptosis.[19]

Electrical activity appears to be a critical factor for neuronal survival. During the peak period of brain growth in rodents, the administration of substances that block electrical activity induces a serious aggravation of developmental neuronal death in various brain regions. These substances include NMDA receptor inhibitors (MK-801 or ketamine), GABA-A receptor agonists such as antiepileptics (phenytoin, phenobarbital, diazepam, clonazepam, vigabatrin, and valproic acid), and anesthetics (a combination of midazolam, nitric oxide, and isoflurane).[20]

Organization of the Central Nervous System

Subplate Neurons

Subplate neurons constitute a transient structure during brain development.[21] They are generated around gestational week 7, and the subplate itself appears around gestational week 10. This structure, located under the neocortical plate, reaches its maximum thickness between gestational weeks 22 and 36.

Subplate neurons express various neurotransmitters, neuropeptides, and growth factors. They receive synapses and form connections with cortical and subcortical structures. These neurons play an important role during brain development: (1) they produce axons that project to the internal capsule, which act as guides for neurons from layers V and VI; (2) between gestational weeks 25 and 32, they produce axons for the corpus callosum; and (3) they constitute a "waiting zone" for thalamocortical axons (with which they establish transient? synapses) before the latter invade the neocortical plate to reach layer IV. This waiting area is necessary for the adequate targeting of thalamocortical afferents.

Subplate neurons may be destroyed in preterm newborns presenting with lesions of the periventricular white matter,[22] findings which have been confirmed in animal models of periventricular white matter injury.[23,24] This damage to the subplate could participate in the thalamocortical connection anomalies demonstrated by MRI in preterm infants,[25] as well as in the associated cognitive and/or motor disorders.

Axonal and Dendritic Growth

The final morphology of the mature neuron and the formation of connections between neurons depend on complex cellular dendrite-axon interactions.[26,27] Maturing neurons develop axons and dendrites that extend as a result reorganization of the cytoskeleton, based on intrinsic and extrinsic cues. Many of the extrinsic cues include secreted chemoattractant or chemorepulsive factors, and adhesion molecules on target neurons that facilitate synapses formation. The interaction between these different ligands and their receptors leads to modifications in calcium levels at the growth cone/dendritic tip, which play a key role in motility and orientation. This ontogenic stage occurs largely, but not exclusively, during the second half of pregnancy, and extends into the postnatal period.

The formation of corticospinal projections occurs at early stages from the subplate, and then from neurons that populate the deep cortical layers (V and VI). Corticothalamic projections also appear, from both the subplate and the deep cortical layers. Corticocortical projections appear later.

A number of transcription factors (e.g., Ctip2, Satb2, Fezf2) regulate the commissural, corticothalamic, and corticospinal projections of the cortex.

Dendritic arborization progresses at slightly different rates for each neuronal subtype, though the stereotypical early branch formation is generally established in the third trimester. Even though genetic programs control the molecular mechanisms of axonal and dendritic growth and determine the initial pattern of these connections, experience and the environment then sculpt this global pattern to generate the final set of connections (through brain plasticity).

The processes underlying cortical expansion are still not fully understood,[28,29] but recent advances have strengthened a link to differences in neurite density in addition to neuronal production.[30]

Synaptogenesis

In the occipital neocortex of the monkey, five successive waves of synaptogenesis have been described.[31] Based on data obtained from the human occipital cortex,[32] the following chronology has been proposed for the human cortex: (1) a first phase starting at gestational week 6 to 8 and limited to the deep layers, such as the subplate; (2) a second phase starting at gestational week 12 to 17, with relatively few synapses produced in the cortex; (3) a third phase starting at around the middle of pregnancy and finishing at around the eighth month after birth (this phase is characterized by a rate of production of new synapses estimated at around 40,000 per second in the monkey); (4) a fourth phase that extends up to puberty and is also characterized by a high rate of synapse formation; and (5) a last phase that extends up to adulthood but is somewhat masked by the significant loss of synapses with age. Experimentally, the two first phases are not influenced by the deprivation of sensory stimuli. The third phase is partly dependent on sensory input whereas the fourth phase is strongly controlled by sensory stimuli and experience.

The concept of synaptic stabilization (by the elimination of non-stabilized synapses) was first proposed by Changeux and Edelman.[33] During brain development, there is a systematic overproduction of labile synapses in successive phases that lead to redundant connections in a fairly random manner. This stage is mainly controlled by genetic factors. Each wave of overproduction is followed by a period of stabilization of synapses that are

of functional value and the elimination of redundant or useless ones by glial cells. This period of stabilization and elimination is strongly influenced by environmental stimuli and experience. In this model, the moderate increase in the number of genes involved in synaptogenesis during the course of evolution has resulted in a richer substrate upon which the environment and experience act to generate a more complex network.

Glial Proliferation, Differentiation, and Myelination

Astrocytes

Neocortical astrocytes are of two origins.[34] At the end of neuronal migration, radial glial cells (which are both neural stem cells and play a role in guiding migrating neurons) are transformed into astrocytes, which then reside in the deep cortical layers and the white matter. In contrast, the astrocytes of the superficial cortical layers are primarily derived from glial precursors that multiply in the SVZ and then migrate into the cortex.

In the human neocortex, astrocytic proliferation begins around gestational week 24, with a peak around gestational week 26 to 28. The exact date at which the production of astrocytes ends is not known, but it could be supposed that the major part of astrocytic production is over by the end of a normal pregnancy. This peak in the production of astrocytes around gestational weeks 26 to 28 could be of particular importance for preterm newborns. Indeed, astrocytes play several important roles, including in axonal guidance, the stimulation of neuronal growth, synapse formation, the transfer of metabolites between blood vessels and neurons, the establishment of the pattern of certain brain structures, the production of extracellular matrix components, the production of trophic factors, neuronal survival, myelination, and maintenance of the blood-brain barrier. For example, experimentally blocking astrocyte production temporarily in the neocortex of rodents induces an increase in programmed cell death in neurons and long-term changes in neocortical synaptic equipment.[35] In addition, recent studies show that astrocytes can acquire different phenotypes which depend upon the physiological or pathological conditions they are exposed to.[36] These different phenotypes can have beneficial or toxic effects on neighboring cells.

Oligodendrocytes and Myelination

Oligodendrocytes can be divided into four types depending on their degree of maturation[37]:

1. Oligodendrocyte precursors that arise from the SVZ are bipolar and mitotically active; their differentiation into preoligodendrocytes occurs during their migration into the future/putative white matter.
2. Preoligodendrocytes that are multipolar cells that retain their proliferative capacity. This second cell type is predominant in the periventricular white matter during the second half of pregnancy.
3. Immature oligodendrocytes that are multipolar cells that appear during the third trimester and wrap axons in preparation for their myelination.
4. The last stage is their differentiation into myelinating and extremely multipolar mature oligodendrocytes.

The oligodendrocyte precursors and preoligodendrocytes, the predominant varieties in the brain of preterm newborns, are extremely vulnerable to injury caused by inflammatory mediators including oxidative stress, the excitotoxic cascade, and hypoxic-ischemic insults.[38] This death of preoligodendrocytes has been implicated in cystic periventricular leukomalacia. This type of severe lesion has progressively decreased in incidence and has been replaced by more diffuse lesions.[39] In this diffuse white matter lesion in preterm infants, the primary phenomenon is a blockade of oligodendrocyte maturation,[12,40,41] in which the hypoxia inducible factor (HIF)[42] and the Wnt[43] pathways play a role.

Myelination occurs during a prolonged period, persisting into childhood. The chronology and the degree of myelination vary according to the brain structure studied. There is no detectable myelination in the prosencephalon before the seventh month of pregnancy. Myelination in the telencephalon is most intense during the third trimester and postnatally and is mostly complete by the age of 2 to 3 years. The olfactory and auditory pathways and the sensorimotor cortex are the first to be myelinated, while projection and association pathways (in particular the prefrontal cortex) are the last.[44]

It is also worth noting that preoligodendrocytes are currently indistinguishable from another glial population—the polydendrocytes, or NG2 glia. Polydendrocytes give rise to oligodendrocytes, but these proliferative, lineage plastic, motile cells also play independent roles in reposes to injury and form unidirectional synapses with neurons.[45,46] However, very little is known about the function of these cells due to the current lack of specific tools with which to study them, an issue that should be a research priority for understanding brain development and health.

Microglia

Microglia constitute 5% to 15% of the total number of brain cells. Microglia are derived from a pool of embryonic myeloid precursors that arise from the yolk sac and infiltrate the developing brain, distinct from tissue resident macrophage.[47] They have distinct maturational stages, are self-renewing, and can live for up to 4 years in the human brain.[48] During the first trimester of pregnancy in humans, microglia have an amoeboid morphology that evolves progressively toward an intermediate and then a mature phenotype with a small cell body and long processes. Around mid-pregnancy, microglial populations are principally detected in white matter fascicles such as the internal and external capsules, the corpus callosum, and axonal fascicle crossings.[49] Experimental data support the hypothesis that these microglia play an important role in the origin of injury to these developing white matter tracts in preterm infants.[12,50,51]

Microglia play a suite of key roles in brain development,[52] including regulating synaptic and neurite pruning and thus connectivity, regulating programmed cell death including the formation of cortical layers, and the initiation of oligodendrocyte maturation impacting myelin production. Like astrocytes, microglia can acquire various phenotypes in response to injury or insult. Depending on brain regions and pathological conditions the microglia can be directly deleterious or can attempt to repair the brain. These diverse roles for microglia mean that during brain development they can directly injure the brain via their inflammatory responses, and by not being capable of participating in brain building due to "distraction" by inflammatory activation. This is particularly relevant for preterm born infants, as the systemic

inflammation commonly seen in these premature babies spreads to the brain and leads to the immune activation of microglia. This "double jeopardy" for the developing brain is an important contributor to the subsequent onset of cognitive and behavioral deficits seen in many formerly very premature babies.[53,54]

The Environment and Epigenetics

The different stages of brain development are finely controlled by diverse genetic programs. Nevertheless, experience, the environment, or stimulation can modulate, adapt, or refine the initial pattern to allow the brain to adjust to its environment. These adaptive processes are the expression of the great plasticity of the developing brain and allow the acquisition of new skills throughout childhood and adolescence. This is true even regarding developmental stages that were initially thought to be almost completely controlled by genetic programming, such as the proliferation of neuronal precursors. Experimental studies have shown that a maternal factor (vasoactive intestinal peptide or VIP) can change the proliferative capacity of these precursors by up to 20%.[55] However, the environment can also have deleterious effects on brain maturation. Preterm newborns are exposed to numerous stimuli that exist only sparingly or not at all with a fetus of the same age, such as excessive or repeated sensory stimulation, painful stimuli, stress, several neuroactive drugs, as well as the withdrawal of maternal and placental factors. Any environmental factor or drug that affects the brain is capable of altering a few or several stages of brain development.

The major mechanisms by which these environmental factors (positive or deleterious) act on brain maturation depend on the epigenetic events that control the organization and compaction of the genome, and thus the expression of genes. Chromosomes in eukaryotes result from a complex between DNA and a protein sheath called "chromatin," whose basic unit is the nucleosome, around which the DNA is wrapped. Each nucleosome is composed of an octamer of histones (H2A, H2B, H3, and H4). The compaction of DNA—and thus the accessibility of genetic information to the transcriptional machinery—is regulated on the one hand by covalent modifications of the DNA (including DNA methylation), and on the other by post-translational modifications that decorate the N-terminal regions (tails) of histones (acetylation, phosphorylation, and methylation). The combination of these two types of events governs the opening or closing of chromatin, and the transcription or repression of the gene in question.

The methylation of DNA at CpG islands located in transcriptional regulatory regions of genes represses their transcription by blocking the binding of transcription factors to the DNA.[56] DNA methyltransferases (DNMTs) catalyze the methylation of DNA by using S-adenosyl-methionine (SAM) as a methyl group donor. In addition, the information deposited by the methylation of the DNA is "read" and "translated" by proteins that recognize methylated DNA: the methyl-CpG-binding proteins (MBPs). These MBPs recruit histone-deacetylases, HDACs (and thus constitute the link between DNA methylation and the post translational modifications of histones), as well as other chromatin remodelers, and contribute to the establishment of transcriptionally inactive chromatin. The crucial role of histone acetylation in brain development is illustrated, for example, by the role of the histone-acetyl-transferase CBP, whose mutation in the heterozygous state is characteristic of Rubinstein-Taybi syndrome in humans, and leads to anomalies of cortical gyration, a reduction of white matter, and regional hypoplasia in association with intellectual disability.[57]

The level of DNA methylation is much higher in the brain than in other tissues and is essential for brain development in mouse models.[56] DNA methylation especially affects neuronal differentiation and survival and the neurogenesis/gliogenesis "switch." In humans, several syndromes with intellectual disability have a developmental origin linked to the DNA methylation pathway. This is the case with Rett syndrome, which is associated with mutations of the MBP MeCP2, and ICF syndrome (combined immunodeficiency, instability of the pericentromeric heterochromatin, and facial dysmorphia), caused by mutations in Dnmt3b.[57,58] The recent discovery that DNA methylation occurs in a non-CPG context, mostly in brain neurons after birth, has greatly modified our understanding of the role of this epigenetic mark in pathophysiological brain development, in particular for RETT syndrome.[59] There are numerous imprinted genes (i.e., those expressed uniquely from the paternal or maternal allele), which are affected by epigenetic regulation, expressed in the brain. The deregulation of their expression is often associated with intellectual disability. This is the case, for example, with the neurodevelopmental syndromes, Prader-Willi and Angelman syndromes.[60] Interestingly, fetal stress modifies the pattern of DNA methylation of key genes involved in neurodevelopment and the integrity of the adult brain (such as BDNF, Reelin, or GAD67).[57,61] Thus, women suffering from depression or anxiety-related disorders during the third trimester of pregnancy give birth to newborns in whom the promoter region of the glucocorticoid receptor gene is hypermethylated. Additionally, neuronal plasticity as well as learning and memory rely on the establishment of epigenetic events, and neuronal activity modifies patterns of DNA methylation.[62]

In summary, one could speak of "neuro-epigenetics" and all that this entails in terms of future therapeutic possibilities, since an in-depth knowledge of epigenetic events and their reversibility opens the door to the search for compounds that could remodel the epigenome of the brain and reprogram neural cells. In this context, diets poor in methionine (the precursor of SAM) and treatment with HDAC inhibitors (such as valproic acid) have been widely prescribed for psychiatric disorders such as schizophrenia or bipolar disorder.[57]

Suggested Readings

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Congenital Malformations of the Central Nervous System

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KEY POINTS

- Brain malformations are a significant source of morbidity and death in neonates.
- Advances in imaging and genetics now allow more specific diagnoses.
- Early and specific diagnosis allows more precise prognostication, including improved monitoring for complications and treatment decisions.
- Early diagnosis and management of patients with brain malformations require multidisciplinary team management.

Prosencephalic Cleavage and Related Events

Normal Prosencephalic Development

The prosencephalon refers to the future forebrain, which includes the telencephalon and the diencephalon; these structures give rise to the cerebral hemispheres, thalamus, and hypothalamus. The prosencephalon develops after the closure of the anterior neuropore, through processes that induce the bifurcation of the rostral extent of the fluid-filled neural tube (Fig. 53.1) to form the right and left forebrain structures.[1] During the fifth and sixth weeks of development, the structure of the forebrain is defined by cleavage along three major planes. As the anterior neuropore is closing, the first major event is the formation of the optic vesicles and nasal placodes, separated along the *horizontal* plane. When the embryo has reached a length of about 5 mm, both anterior and posterior neuropores have closed, isolating the developing ventricular system from the amniotic fluid. The retinal and lens placodes also develop at this time. In the hindbrain, the cerebellum begins to form, along with somatic and visceral efferent nuclei, the common afferent tract, and the ganglia for most of the cranial nerves. At about day 32 of gestation, when the embryo is 5 to 7 mm long, the forebrain divides in the *sagittal* plane to give rise to the paired structures. Specific areas, including the hypothalamic, amygdala, hippocampal, and olfactory regions, are identifiable at this time. The third major event in forebrain development occurs shortly thereafter when the forebrain divides in the *coronal* plane. This event separates the telencephalon from the diencephalon, defining the epithalamus, subthalamus, and hypothalamus. During the remainder of the second and third months of gestation, multiple midline structures form, including the corpus callosum, anterior and hippocampal commissures, optic nerves, optic chiasm, and hypothalamus.

Disorders of Structures Derived From the Prosencephalon

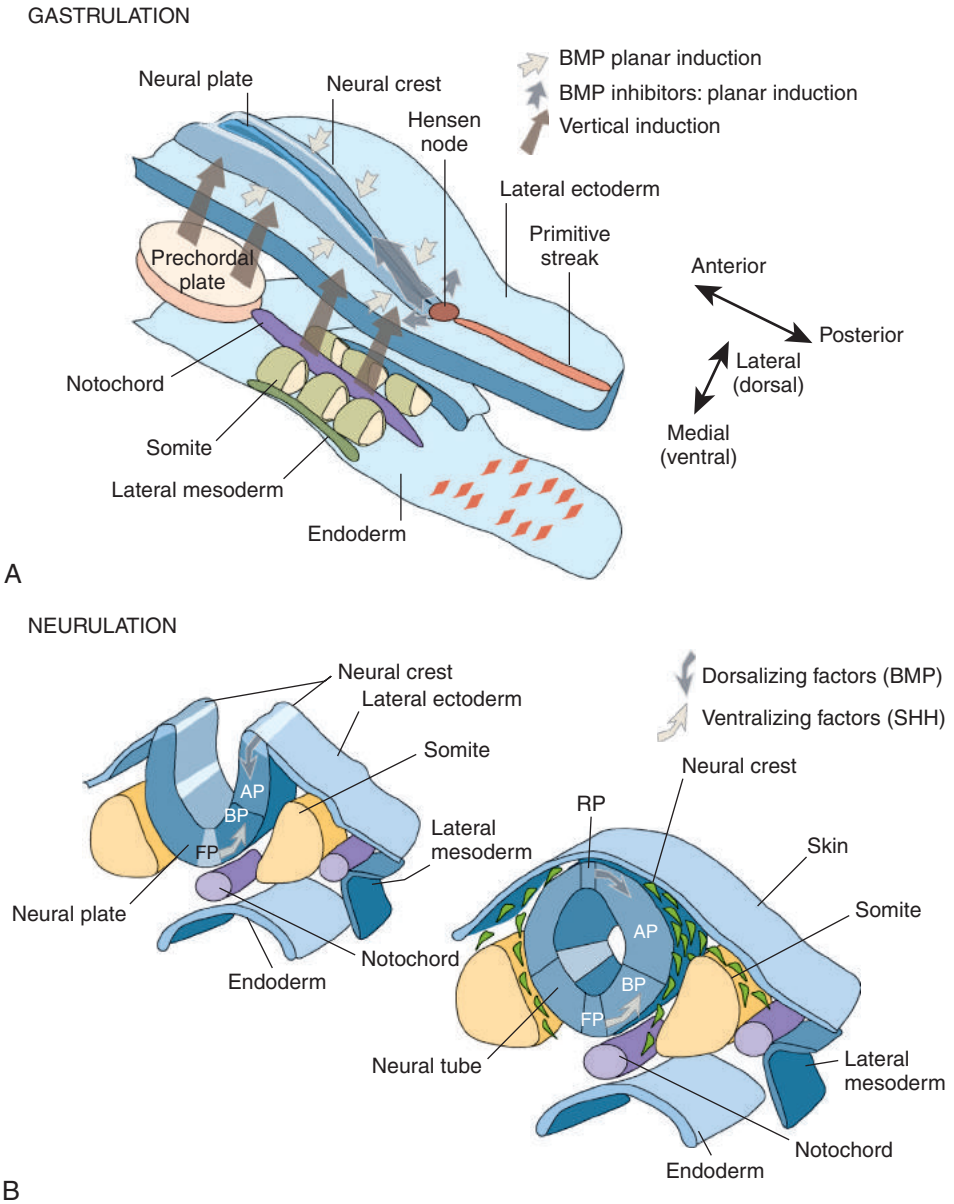
Disorders of development of the prosencephalon include the severe malformations: atelencephaly, aprosencephaly, and holoprosencephaly (HPE). Milder defects include agenesis of the corpus callosum (ACC), septo-optic dysplasia (SOD), and isolated absent cavum septi pellucidi (CSP).

Aprosencephaly and Atelencephaly

Aprosencephaly and atelencephaly are two rare and very severe cerebral malformations. In aprosencephaly, neither telencephalic nor diencephalic structures develop. In atelencephaly, there remains a rudimentary prosencephalon. Craniofacial abnormalities associated with this condition are secondary to deformation brought on by the absence of the telencephalon, rather than true malformation.[2] These disorders may result from possible autosomal recessive inheritance versus an abnormality of chromosome 13.[3] Aprosencephaly/atelencephaly has also been reported in a family with pathogenic variants in the *SIX3* gene, which has also been associated with HPE.[4] Typically, these are diagnosed prenatally by ultrasound and fetal magnetic resonance imaging (MRI).[5] Although considered "lethal" malformations, it is important for the neonatologist to be prepared for infants with severe malformations to live longer than expected and even go home from the hospital.

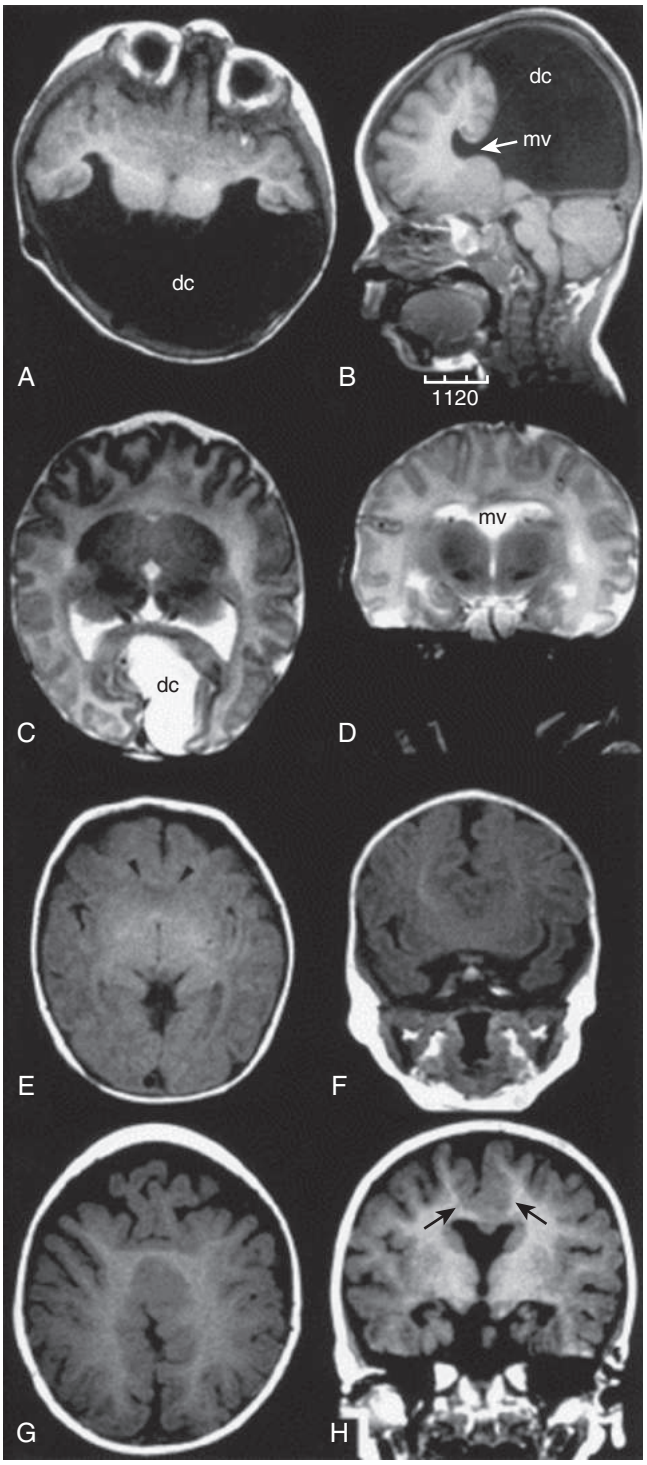
Holoprosencephaly

HPE represents a spectrum of defects in forebrain development and is the most common brain malformation,[6,7] although most affected fetuses are miscarried early in gestation. HPE represents a variable degree of incomplete separation of the prosencephalon along one or more of its three major planes during the third and fourth weeks of gestation (discussed earlier). The DeMyer classification scheme groups the degree of separation as "alobar," "semilobar," and "lobar" as well as a milder middle interhemispheric



• **Fig. 53.1** Formation of the Neural Tube. (A) During gastrulation, at the neural plate stage, dorsoventral polarity and early anteroposterior regionalization are defined by a process of vertical induction by fibroblast growth factor 8 and other factors *(long gray arrows)* derived from mesendoderm (notochord and prechordal plate). Planar induction occurs via bone morphogenetic proteins *(BMPs)* and BMP inhibitors that are derived from lateral ectoderm *(short light gray arrows)* and the Hensen node (*short dark gray arrows*) respectively. (B) The process of neurulation proceeds with the approximation of the neural folds toward the dorsal midline. Before the closure of the neural tube, neural crest cells delaminate and migrate from the neural folds. Dorsalizing factors (BMPs; *dark gray arrow*) derived from the dorsal midline roof plate *(RP)* and ventralizing factors (sonic hedgehog [SHH]; *light gray arrow*) from the floor plate *(FP)* establish dorsal-ventral gradients of these key signaling molecules that induce the formation of the alar plate *(AP)* and the basal plate *(BP)* from the lateral wall of the neural tube. (Adapted from Vieira C, Pombero A, García-Lopez R, et al. Molecular mechanisms controlling brain development: an overview of neuroepithelial secondary organizers. Int J Dev Biol. 2010;54:7–20; Courtesy Dr. Salvador Martinez, Institute of Neuroscience, Universidad Miguel Hernandez, San Juan de Alicante, Spain.)

variant subtype, also referred to as *syntelencephaly*[8]; however, HPE can be difficult to classify in a given patient. In alobar HPE (Fig. 53.2), a single anterior ventricle is contained within a holosphere with complete lack of separation of the prosencephalon. Alobar defects may be classified by fetal imaging findings. Depending on the severity of the defect, other midline structures such as olfactory bulbs/tracts, the corpus callosum, the anterior commissure, and the optic nerves may be affected. In addition, midline deep structures such as the basal ganglia, hypothalamus, and thalamic structures are fused, and vascular malformations may also be present.[9] The milder semilobar and lobar forms have distinct hemispheres and the presence of at least a portion of



• **Fig. 53.2** The Spectrum of Holoprosencephaly as Demonstrated by Magnetic Resonance Imaging. (A, B) Magnetic resonance imaging *(MRI)* of the brain in a patient with alobar holoprosencephaly. The T1-weighted axial image (A) reveals a lack of separation of the two hemispheres and deep gray nuclei. A large dorsal cyst *(dc)* is observed posteriorly. The T1-weighted sagittal image (B) reveals a midline monoventricle *(mv)* that communicates posteriorly with the dorsal cyst *(dc)*. (C, D) MRI of a patient with semilobar holoprosencephaly. The T2-weighted axial image (C) indicates separation of the hemispheres posteriorly but not anteriorly. Anterior horns of the lateral ventricles are absent, whereas the posterior horns are well formed and separated. Note the formation of a posterior dorsal cyst (dc). There is also an incomplete separation of the basal ganglia. The T2-weighted coronal image (D) reveals a lack of interhemispheric fissure and a monoventricle *(mv)*. (E, F) MRI of the brain in a patient with lobar holoprosencephaly. The T1-weighted axial image (E) reveals that the two hemispheres are fairly well separated as manifested by the presence of an interhemispheric fissure both anteriorly and posteriorly. Note that the frontal horns of the lateral ventricles are only rudimentary *(black arrowheads)*. The T1-weighted coronal image (F) documents incomplete separation of the inferior frontal lobes near the midline. (G, H) MRI of the brain in a patient with the middle interhemispheric variant of holoprosencephaly. The T1-weighted axial (G) and coronal (H) images demonstrate the continuity of gray matter in the posterior frontal lobes across the midline *(black arrows)*. (From Hahn JS, Plawner LL. Evaluation and management of children with holoprosencephaly. *Pediatr Neurol*. 2004;31:79–88.)

the corpus callosum. In "semilobar" HPE, the frontal lobes are more than 50% fused, and the thalami and hypothalamus may also be fused. "Lobar" HPE is associated with the fissure along almost the entire midline, separation, or near separation, of the thalami and absence of the CSP. A middle hemispheric variant has been described in which the posterior, frontal, and parietal lobes fail to separate, with an absence of the body of the corpus callosum.[10,11] Typically, patients with the middle interhemispheric variant are not identified in the neonatal period unless the variant was diagnosed prenatally. Finally, a septo-preoptic type has been suggested, where the nonseparation is restricted to the septal and preoptic areas.[12]

Diagnosis

With the advent of prenatal ultrasonography, patients with HPE are usually identified before birth. Alobar HPE may be identified as early as 10 weeks' gestation and can be reliably identified by 14 weeks. False-positive findings by ultrasonography are common,[13] so fetal MRI can be very useful for clarifying the diagnosis and severity of the malformation, particularly if pregnancy

termination is being considered. Subtle findings such as the absence of CSP may suggest the milder forms of HPE, although absent CSP can be isolated or associated with ACC.[9] Given the high rate of chromosome abnormalities seen in HPE (25% to 50%), amniocentesis for chromosome array and possibly DNA sequencing is indicated.[12,14]

Clinical Features

Up to 80% of children with HPE have a craniofacial anomaly.[12] The findings may range from cyclopia (a single central eye) with a nose-like structure (proboscis) above the eye, to cebocephaly (a flattened single nostril situated centrally between the eyes), to median cleft lip. Mild cases may have a single central incisor or hypotelorism. The concept of "the face predicts the brain," refers to the fact that more severe facial malformations are often associated with more severe brain malformations; for example, patients with alobar HPE can have normal facies, while those with lobar or semilobar HPE can have severe facial malformations.[8,15,16]

Newborns with HPE present with low tone and microcephaly. However, the microcephaly may be obscured by a co-occurring hydrocephalus due to the blockage of cerebrospinal fluid (CSF) flow through the fused thalami[17]; these cases are often associated with a large dorsal cyst.[18] Ventriculoperitoneal shunts can relieve symptoms of increased intracranial pressure.[19] Over time, patients may develop spasticity and dystonia. Oromotor dysfunction is frequent, and many children with HPE require tube feedings.

As with other midline brain defects, endocrinologic abnormalities are very common. Diabetes insipidus occurs in up to 70% of patients, with hypothyroidism, hypoadrenocorticism, and growth hormone deficiency being less common.[20] These endocrine abnormalities can develop over time, requiring periodic monitoring. Hypothalamic dysfunction may also cause irregularities of sleep, temperature regulation, appetite, and thirst.

Approximately 40% of children with HPE have epilepsy, but one-third of these will have intractable epilepsy.[16,19] Seizures may also be provoked by endocrinologic abnormalities such as hypernatremia or hypoglycemia. Given the range of possible medical issues, a multidisciplinary approach to the care of these children is necessary, including continued surveillance for future complications such as hydrocephalus, seizures, and pituitary insufficiency.

Prognosis is related to the severity of the defect, the involvement of other organ systems, and the genetic cause.[9] Fetal or neonatal death is typical for most individuals with chromosome abnormalities.[21] In contrast, more than 50% of cytogenetically normal patients with all types of HPE are alive at 12 months.[7,22] Survival into late adolescence and adulthood has been reported.[16,19]

Epidemiology and Etiology

The live birth prevalence of HPE is 1 in 10,000 to 1 in 20,000.[21,23–25] The prevalence is higher in miscarried embryos and fetuses, representing as many as 1 per 250 pregnancies.[26]

The cause of HPE is multifactorial, with both genetic and environmental factors appearing to contribute to the variable spectrum of presentations.[27,28] Up to 45% of HPE is caused by chromosomal abnormalities detectable by standard karyotyping.[9] The most common chromosome abnormalities are trisomies 13 and 18.[29] Chromosome microarrays can identify smaller copy number variants in 10% to 20% of individuals with HPE with normal karyotypes.[30] Pathogenic variants in at least 14 genes have been associated with HPE, accounting for up to 25% of syndromic forms (e.g., Smith–Lemli–Opitz, Meckel, Rubenstein–Taybi, Kallman, and Pallister–Hall syndromes).[28] Six genes have been well established to cause nonsyndromic HPE: *SHH, ZIC2, SIX3, TGIF, GLI2*, and *PTCH1*. *SHH* accounts for up to 40% of familial cases of HPE.[26,31]

Genotype-Phenotype Variability

HPE is characterized by extreme intrafamilial variability. The same loss-of-function pathogenic variant can be found in asymptomatic or mildly affected family members as in family members who are severely affected. Incomplete penetrance can result in "microforms," including hypotelorism, midface hypoplasia, or a single central incisor.[32] One explanation for these observations comes from studies suggesting that the mode of inheritance for HPE may be multigenic,[33] though examples of digenic, "double hit," cases are quite rare.[34] This suggests a certain complexity to genetic modifiers of known pathogenic variants. Furthermore, the severity of expression of the HPE phenotype throughout a given family may be influenced by the additive contributions from multiple genetic factors as well as environmental or teratogenic effects.

Still, some genotype-phenotype correlations have emerged. Patients with *ZIC2* pathogenic variants can have a characteristic facial appearance (bitemporal narrowing, upslanting palpebral fissures, large ears, and a short nose with anteverted nares). *ZIC2* pathogenic variants also appear to be the most common de novo pathogenic variant and have a high penetrance.[35]

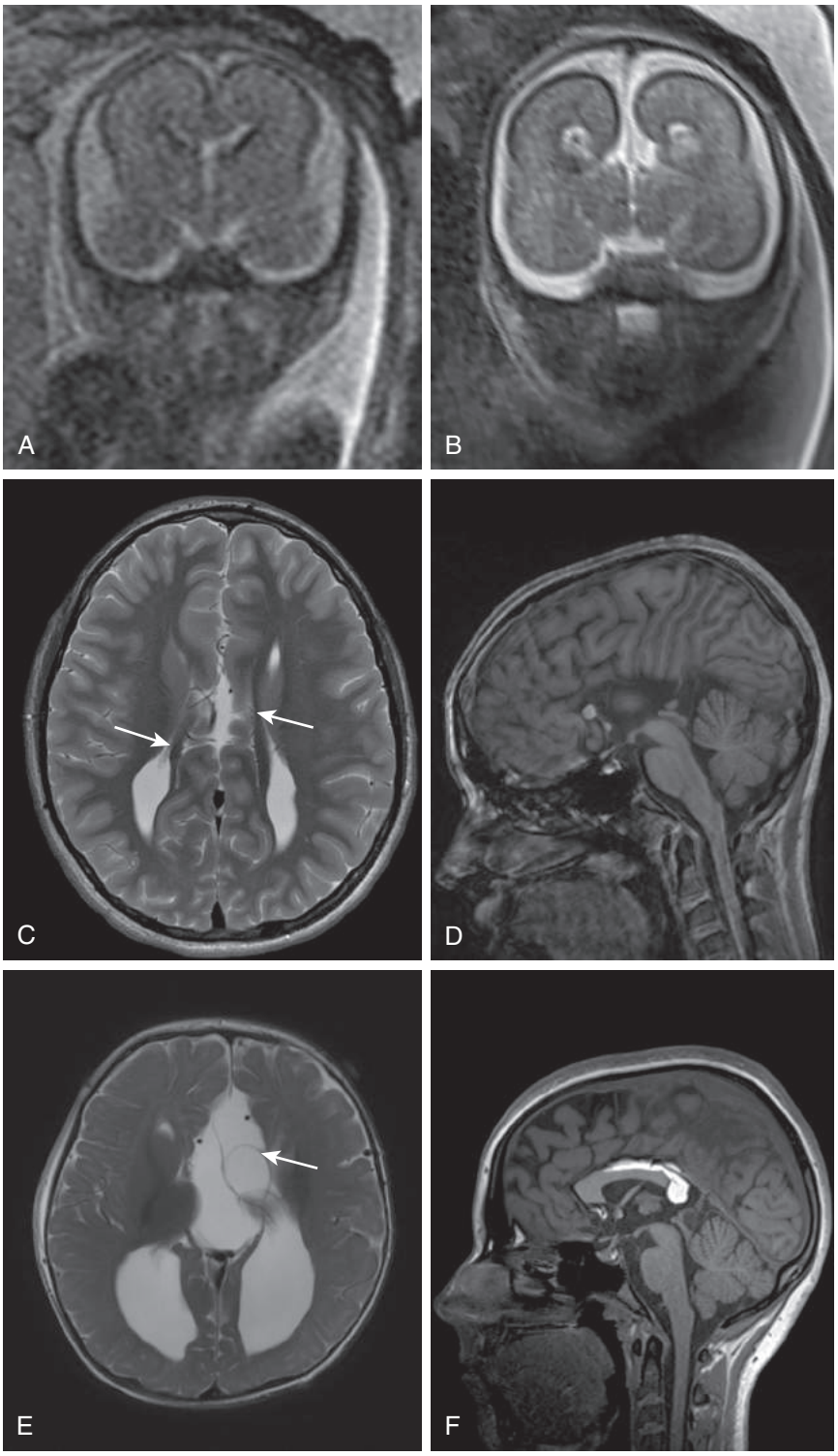
Environmental Factors

Environmental factors (e.g., ethanol, vitamin A toxicity) can produce cyclopia during the early phase of gastrulation. Maternal diabetes mellitus increases the risk of HPE to approximately 1% to 2% of all pregnancies. Cholesterol-lowering agents have been associated with HPE,[36] presumably because of their effects on *SHH* signaling. Smith–Lemli–Opitz syndrome is caused by a defect in the terminal step of cholesterol biosynthesis, and HPE manifests itself in approximately 5% of affected individuals. Additionally, ingested plant alkaloids have caused epidemics of cyclopia in sheep by processes that inhibit cholesterol biosynthesis. Other environmental associations (cytomegalovirus [CMV] infection, medications, assisted reproductive technologies) have been suggested by case reports or animal studies.[32]

Agenesis of the Corpus Callosum

The corpus callosum consists of approximately 190 million axons and is formed by a complex, multistep process that involves midline patterning, cellular proliferation, migration, and axonal growth.[37] Initial formation of the corpus callosum proceeds over 11 weeks or so and is mediated by multiple gene networks.[38] Development continues through adolescence as these connections are refined.[39] Genetic as well as environmental factors affect callosal formation.

ACC results in abnormal gyration of the medial portion of each hemisphere, eversion of the cingulate gyri, and sulcation perpendicular to the long axis of the hemisphere.[40] The external angles of the lateral ventricle are oriented parallel and upward, and the fornices are widely separated. If present, a useful distinguishing feature is Probst bundles (Fig. 53.3), which are fiber bundles



• **Fig. 53.3** Findings Associated With Agenesis of the Corpus Callosum as Demonstrated by Magnetic Resonance Imaging. (A) Coronal fetal magnetic resonance imaging *(MRI)* demonstrating the presence of the corpus callosum. Note that the medial cortex curls toward the midline. (B) Coronal fetal MRI demonstrating agenesis of the corpus callosum *(ACC)*. Note that the medial cortex curls away from the midline. (C) Postnatal axial T2-weighted image of ACC demonstrating colpocephaly and classic Probst bundles *(arrows)*. (D) T1-weighted sagittal section demonstrating near complete ACC (rostrum remains). (E) Postnatal axial T2-weighted image demonstrating a multiloculated cystic structure *(arrow)* that can be seen with ACC. (F) T1-weighted sagittal image demonstrating a lipoma *(bright white area)* that can be seen with ACC.

that run parallel to the ventricle in an anterior-to-posterior direction. ACC can be partial, more often involving loss of posterior segments.[41,42]

ACC often occurs in association with other structural abnormalities. These may be minor, such as cysts or lipomas (see Fig. 53.3), or more severe, such as heterotopia, polymicrogyria (PMG), microcephaly, abnormal sulcation, commissural and white matter abnormalities, and malformations of the posterior fossa.[38,43]

Epidemiology and Etiology

The prevalence of ACC ranges from 0.5 in 10,000 in the general population to 600 in 10,000 in children with neurodevelopmental disabilities.[39] ACC can also be associated with prenatal infections, vascular accidents, and teratogen effects.[39] Infection results in a thinner corpus callosum with a typical rostral-to-caudal extent and is a rare cause of isolated ACC. Ethanol exposure has the strongest association with ACC, which was reported in 7% of children with fetal alcohol syndrome in one series.[44]

ACC is a feature of hundreds of different disorders, and all modes of inheritance have been observed. Glass et al. (2008) found that callosal anomalies were associated with a chromosomal abnormality 17% of the time, commonly aneuploidy (chromosomes 13, 18, and 21).[45] ACC may be associated with either inherited or de novo copy number variants, some overlapping with those found in patients with autism.[46] ACC is also a primary feature of several important disorders, such as Mowat–Wilson, Aicardi, acrocallosal syndromes, *L1CAM*-related spastic paraplegia, and X-linked lissencephaly with ACC and ambiguous genitalia. Given the number of genetic syndromes associated with ACC, some authors have suggested a subdivision of groups based on the associated conditions (craniofacial, metabolic, ocular, ciliopathies), which aids in the diagnostic evaluation of these patients.[39]

Prenatally, fetal MRI at 20 to 22 weeks' gestation maximizes the identification of additional cerebral findings when pregnancy decision-making is still an option. Earlier MRI scans yield more false-negative results and miss structural changes. Additional diagnostic options include genetic (chromosomal microarray, cell-free fetal DNA) and infectious (serologic tests, polymerase chain reaction testing on amniotic fluid) disease testing. Given the challenges with accurate prenatal diagnosis, newborns should be evaluated by brain MRI shortly after birth and by subspecialty consultations (neurology, ophthalmology, genetics, developmental pediatrics, audiology). Laboratory testing (chromosome microarray, DNA sequencing, metabolic testing) should be considered, depending on the examination and MRI findings. Surveillance for neurodevelopmental issues, visual impairment, and pituitary insufficiency is recommended.[39]

Prognosis

The outcomes of ACC are variable. A systematic literature review revealed that up to 75% of patients with MRI-confirmed isolated ACC were developing typically at early school age, while 11% had severe disability.[47] Associated microcephaly, epilepsy, cerebral palsy, and cerebral dysgenesis are correlated with a higher risk of the abnormal neurodevelopmental outcome. Neuropsychological studies have shown specific impairment in abstract reasoning, problem-solving, and category fluency.[48,49] Difficulty with higher-level language such as comprehension of syntax and linguistic pragmatics is also evident.[50] These findings underscore the need for continued evaluation for learning and language difficulties in these patients.

Septo-Optic Dysplasia

Classic SOD is the triad of the absence of the septum pellucidum, optic nerve hypoplasia, and pituitary dysfunction. The diagnosis of SOD is made when two or more features of the triad are present[51]; 30% of patients have all three features.[52] SOD is a clinically and etiologically diverse group of disorders and overlaps with isolated optic nerve hypoplasia.[53] This diversity makes it difficult to provide counseling about prognosis and associated medical issues.

Diagnosis and Prognosis

Prenatally, SOD may be suspected in fetuses with absent CSP or ACC, although most fetuses with isolated absent CSP do not have SOD, posing a challenge for counseling. Postnatally, SOD may be suspected in a patient with growth failure, visual abnormalities, or genital abnormalities secondary to pituitary dysfunction. Early diagnosis is important to reduce the risk of adrenal crisis and hypoglycemia and requires brain MRI, ophthalmologic evaluation, and laboratory testing for pituitary insufficiency.

Children with SOD may be blind or have a visual impairment, presenting with roving gaze, nystagmus, or strabismus. Neurodevelopmental prognosis is controversial and may be correlated with the presence of other brain abnormalities. In early studies, cerebral palsy was found in 57% of children with SOD, intellectual disability was found in 71%, epilepsy was found in 37%, and behavioral problems were found in 20%.[54,55] A later neurodevelopmental study of seven children with unilateral or bilateral optic nerve hypoplasia with the absence of CSP found normal cognitive development, intact neurologic status, normal language development, and age-appropriate behavior in six of the seven children.[56] Conversely, a descriptive series of three children with SOD plus cortical dysplasia showed that all three had abnormal development and neurologic examination findings.[57] Specialists in development, neurology, endocrinology, and ophthalmology should follow these children. Hypopituitarism is seen in up to 80% of patients, with growth hormone deficiency being the most common. Lifelong monitoring is needed as pituitary insufficiency can become clinically apparent over time.[51]

Epidemiology and Etiology

The prevalence of SOD is 1 in 10,000 births, with increased risk in younger mothers.[58] Pathogenic variants in several genes are associated with SOD (e.g., *HESX1, SOX2, SOX1, OTX2*)[51,59]; however, a genetic origin is not identified in most patients, suggesting environmental or complex genetic causes. The disease is sporadic in most patients, and SOD can exhibit dominant and recessive modes of inheritance. Some instances of SOD have been associated with prenatal drug and alcohol exposure or a vascular pathogenesis[60–62]; however, causation remains unproven. The recurrence risk is less than 1% in the absence of consanguinity.

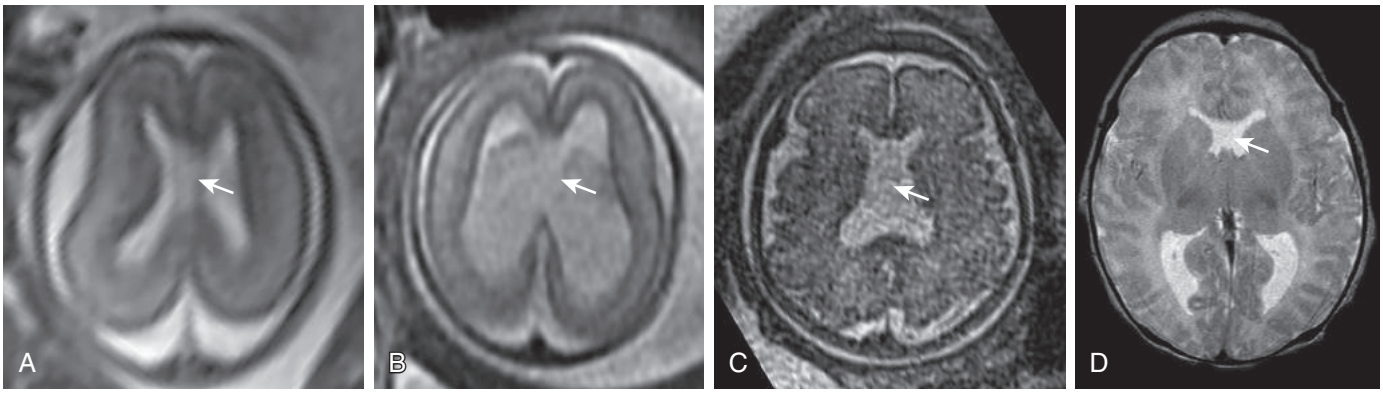
Absent Cavum Septi Pellucidi

The presence of the CSP is a normal finding on ultrasonography and MRI of fetuses and premature infants. This normal finding represents a fluid-filled space between the lamellae of the septi pellucidi, which typically fuse as the fetal brain matures. The absence of the CSP in fetuses and premature infants may be associated with neuroanatomic anomalies.[63] It may be an isolated finding (Fig. 53.4), but MRI is recommended for further evaluation.[64] In addition, a larger than expected CSP width should prompt a detailed ultrasound evaluation as this anomaly can be seen in fetuses with aneuploidy.[65]

Cortical Defects in Size and Organization

Defects in proliferation and neuronal survival include microcephaly and macrocephaly as well as cortical dysplasia. Migration defects result in disruption of the layered cortical structure, which can be seen grossly (as with lissencephaly) or microscopically (focal dysplasia). We will also discuss cortical defects such as heterotopia, PMG, cobblestone cortex, schizencephaly, and other destructive lesions. We will not discuss disorders of neuronal crest migration (e.g., neuroblastoma, Hirschsprung disease).

Projection neurons originate from neural progenitor cells that reside in the periventricular zone and subventricular zones. These progenitors undergo symmetric and asymmetric cell divisions to generate more progenitors as well as post-mitotic neurons, which



• **Fig. 53.4** Fetal and Postnatal Magnetic Resonance Imaging With Absent Cavum Septi Pellucidi. (A) Fetal magnetic resonance imaging (MRI) demonstrating expected cavum septi pellucidi (CSP) at 21 weeks' gestation *(arrow)*. (B–D) MRI demonstrating absent CSP *(arrow)* at 20 weeks' gestation (B), 34 weeks' gestation (C), and birth (D). The fetus in (B) also has mild ventriculomegaly.

subsequently differentiate into projection neurons.[66] The cerebral cortex develops in an inside-out pattern, with early-born neurons forming the deepest layers and later-born neurons populating the superficial layers. This process occurs during weeks 7 to 11 of gestation. Intrinsic and extrinsic signals direct appropriate arrest of migration, positioning of the final cell layers, and terminal differentiation of projection neurons into a variety of subtypes. Disruption of any of these processes can result in the malformations we discuss.[67] Most interneurons are born in the median ganglionic eminences and migrate long distances to populate the brain, a process termed tangential migration. Non-neuronal cells (astrocytes and oligodendrocytes) arise from the same neural progenitors that generate projection neurons, but form later.[68]

Disorders of cortical development may be genetic or caused by in utero insults (hypoxia, ischemia, metabolic derangements, toxins, and infection). Diagnosis is based on clinical presentation, imaging, pathology, and genetic testing. Malformations can manifest clinically as epilepsy or intellectual disability and may be part of syndromic disorders with other organ involvement. Management includes both medical and surgical interventions for seizure control, therapy support for motor and cognitive disabilities, and appropriate surveillance and involvement of specialists if needed. The framework of cerebral cortex development has been used to classify cortical malformations and is being updated on the basis of expanding genetic knowledge.[69]

Cortical Defects in Proliferation and Neuronal Survival

Microcephaly

Disorders caused by abnormal proliferation include microcephaly (small brain) and megalencephaly (large brain). Microcephaly is defined as an occipitofrontal circumference greater than two standard deviations (SDs) below the mean and can be either primary (evident at birth) or secondary (normal to small occipitofrontal circumference at birth with progression to greater than two SDs below the mean after birth). The causes for either can be genetic or acquired. Microcephaly may be associated with other extracranial malformations (syndromic microcephaly) or may be an isolated finding. Von der Hagen et al.[70] described a retrospective cohort of 680 children with microcephaly and found that around 60% had an identifiable etiology.[70] Of these patients, roughly half had genetic causes of various types (aneuploidies, de novo copy number variants, autosomal dominant, autosomal recessive, and X-linked). The remaining cases were secondary to injury around or after birth (which can include birth complications, infection, maternal disease, teratogen exposure, infarction, and craniosynostosis). This leaves 40% of cases without a clear etiology.

Imaging may suggest a normal brain organization, but oligogyria, gray matter disruption, and hypomyelination may be microscopic and not easily identified on imaging. Patients with microcephaly can also have other cortical malformations, suggesting an overlap in mechanisms. It is not surprising that genetic defects that cause primary microcephaly affect cell cycle progression, cell proliferation, mitotic spindle formation, and DNA repair—processes that a crucial to generating the appropriate number of neurons.[71,72] Secondary microcephaly becomes apparent only after birth and is associated with defects in genes regulating protein synthesis and a number of syndromes, including Rett and Angelman.[73] The later onset of this subgroup of microcephaly may afford opportunities for early diagnosis and treatment.

Primary microcephaly can be detected in utero by ultrasonography, and when severe, it is associated with a high risk of abnormal neurodevelopmental outcomes. Additional prenatal evaluation may include fetal MRI, infectious evaluation, and genetic testing. Recently, the Zika virus has been implicated as the causative agent of microcephaly in infants whose mothers were exposed to the virus during pregnancy.[74,75] Diagnosis of secondary microcephaly occurs via routine head measurements after birth, and then further evaluation can include brain imaging (ultrasonography or MRI), infectious disease evaluation, and subspecialty consultation (e.g., neurology, genetics, ophthalmology) as indicated.

Prognosis is related to the severity of microcephaly,[76] as well as the presence of other brain abnormalities, somatic malformations, and genetic diagnosis. Developmental disabilities and imaging abnormalities are seen in approximately 80% of children with severe microcephaly, which is defined as a head circumference greater than three SDs below the mean, while the risk is much lower in children with a head circumference greater than two SDs below the mean. Comorbid conditions include intellectual disability, epilepsy, cerebral palsy, and ophthalmologic disorders.

Macrocephaly and Megalencephaly

Macrocephaly refers to a large head size (greater than two SDs above the mean) that can be due to a variety of causes, such as hydrocephalus, thick skull, or megalencephaly (large brain size). *Megalencephaly* is rare in neonates since it is usually due to increasing brain size over time and is often associated with developmental delay and epilepsy.[77] Increasing head circumference in infancy needs to be followed closely, and imaging should be considered to evaluate the infant for causes of increased intracranial pressure. In the absence of other malformations or neurodevelopmental issues, accelerated head growth with increased extra-axial fluid during the first year or two after birth is generally thought to be benign and may be due to an imbalance in CSF production versus CSF absorption by immature subarachnoid granulations.[78,79]

Macrocephaly can be seen in a diverse group of conditions characterized by a large brain, most commonly manifesting itself as an isolated finding in familial and sporadic cases. At birth, the head circumference is greater than the 90th percentile. Macrocephaly can also be seen in fragile X syndrome and Klinefelter syndrome and in neuroendocrine disorders (Beckwith–Wiedemann syndrome, cerebral gigantism, and achondroplasia).[80,81] The prognosis depends on the cause.

The causes of megalencephaly can be divided into metabolic and anatomic categories. Metabolic causes include organic aciduria (e.g., glutaric aciduria), lysosomal storage diseases, and leukoencephalopathies, while anatomic megalencephaly can be due to pathogenic variants in genes affecting the pathways involved in cell growth such as *PI3K/AKT/mTOR, Ras/MAPK*, and *SHH*.[77] Megalencephaly can present syndromically as in *megalencephaly, polymicrogyria, polydactyly, and hydrocephalus (MPPH) syndrome* and *megalencephaly–capillary malformation (MCAP) syndrome*.[82,83] Pathogenic variants in *AKT3, PIK3R2*, and *PIK3CA* have been identified.[84–86] Interestingly, pathogenic variants in the PI3K/AKT/mTOR pathway seem to drive less extensive overgrowth conditions such as hemimegalencephaly and focal cortical dysplasias.[87] *PIK3CA* is also associated with other overgrowth disorders, including MCAP, congenital lipomatous overgrowth, vascular malformations, epidermal nevi, skeletal/spinal abnormalities (CLOVES) syndrome, and fibroadipose hyperplasia.[88]

Megalencephalic brains are most often dysplastic showing ectopic tissues (periventricular heterotopias), atypical cells on pathology, white matter abnormalities, and altered gyral patterns (often coarse). Clinically, they are associated with intellectual disability and intractable epilepsy, with onset usually in infancy.[67,72] If the dysplastic overgrowth is limited to a single hemisphere (as in hemimegalencephaly), hemispherectomy may be considered as the neonatal period to treat severe epilepsy.[89] Given the growing molecular understanding of brain overgrowth syndromes, novel medical therapies, including mechanistic target of rapamycin (mTOR) inhibitors, offer an alternative to nonsurgical candidates.[90]

Megalencephaly may also be associated with neurocutaneous syndromes such as sebaceous nevus syndrome, hypomelanosis of Ito, facial lipoma, and tuberous sclerosis.[91–95] The prognosis and recurrence risk for macrocephaly and megalencephaly depend on the underlying cause.

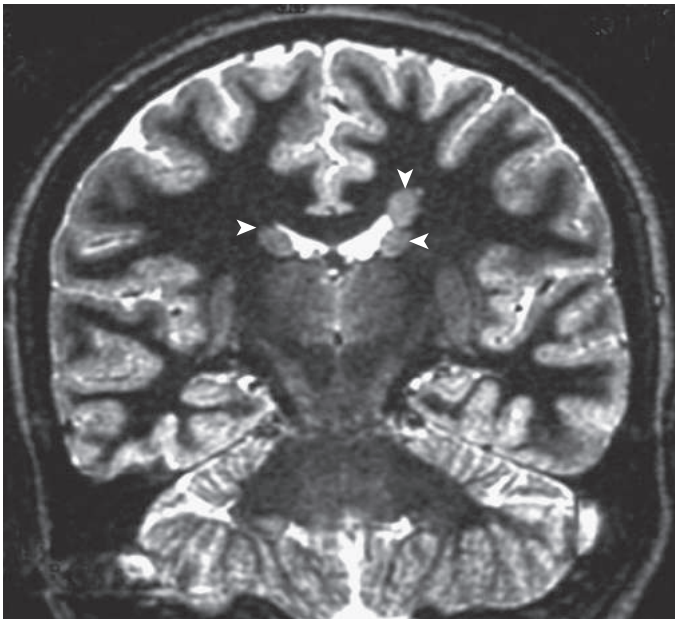
Cortical Defects in Migration

In addition to appropriate proliferation, cortical development requires appropriate neuronal migration. Disruption in the initiation, continuation, or arrest of neuronal migration results in several organizational abnormalities, including heterotopia, lissencephaly, and cobblestone cortex. These abnormalities are often accompanied by other malformations, such as hypoplasia or ACC, pointing to a complex interplay between the mechanisms that determine neuronal migration and axon path finding. Structural imaging has identified many defects in radial migration of projection neurons, while defects in tangential migration of interneurons are not typically identified.

Heterotopia

Heterotopia can be periventricular (suggesting disruption of initiation of migration), subcortical (as in subcortical band heterotopia), or transmantle (as seen with injury). Periventricular heterotopia (PVH) is characterized by masses of gray matter adjacent to the walls of the lateral ventricles (Fig. 53.5). Patients with PVH often present with focal seizures. Bilateral PVH may be associated with other malformations such as hypoplasia of the corpus callosum. *FLNA* pathogenic variants cause bilateral contiguous PVH. There is a clinical predominance of cases in females as hemizygous FLNA mutations are usually lethal in males. *FLNA* is a key component of the cytoskeleton, a cellular structure needed for motility, offering a mechanistic explanation for this malformation.[94] Among cases of familial PVH, 80% have *FLNA* pathogenic variants. Twenty percent of sporadic cases have *FLNA* pathogenic variants, indicating the importance of environmental influences, or unidentified genetic causes.[96] Given associated cardiac problems with *FLNA*-related PVH, echocardiogram and cardiac surveillance are recommended. Neurodevelopmental outcomes are varied and may be associated with learning disabilities.[97] Pathogenic variants in *ARFGEF2* have also been identified in patients with PVH.[98]

Subcortical band heterotopia is characterized by a band of heterotopic gray matter within the white matter (Fig. 53.6). Somatic pathogenic variants in *DCX* and *LIS1* have been

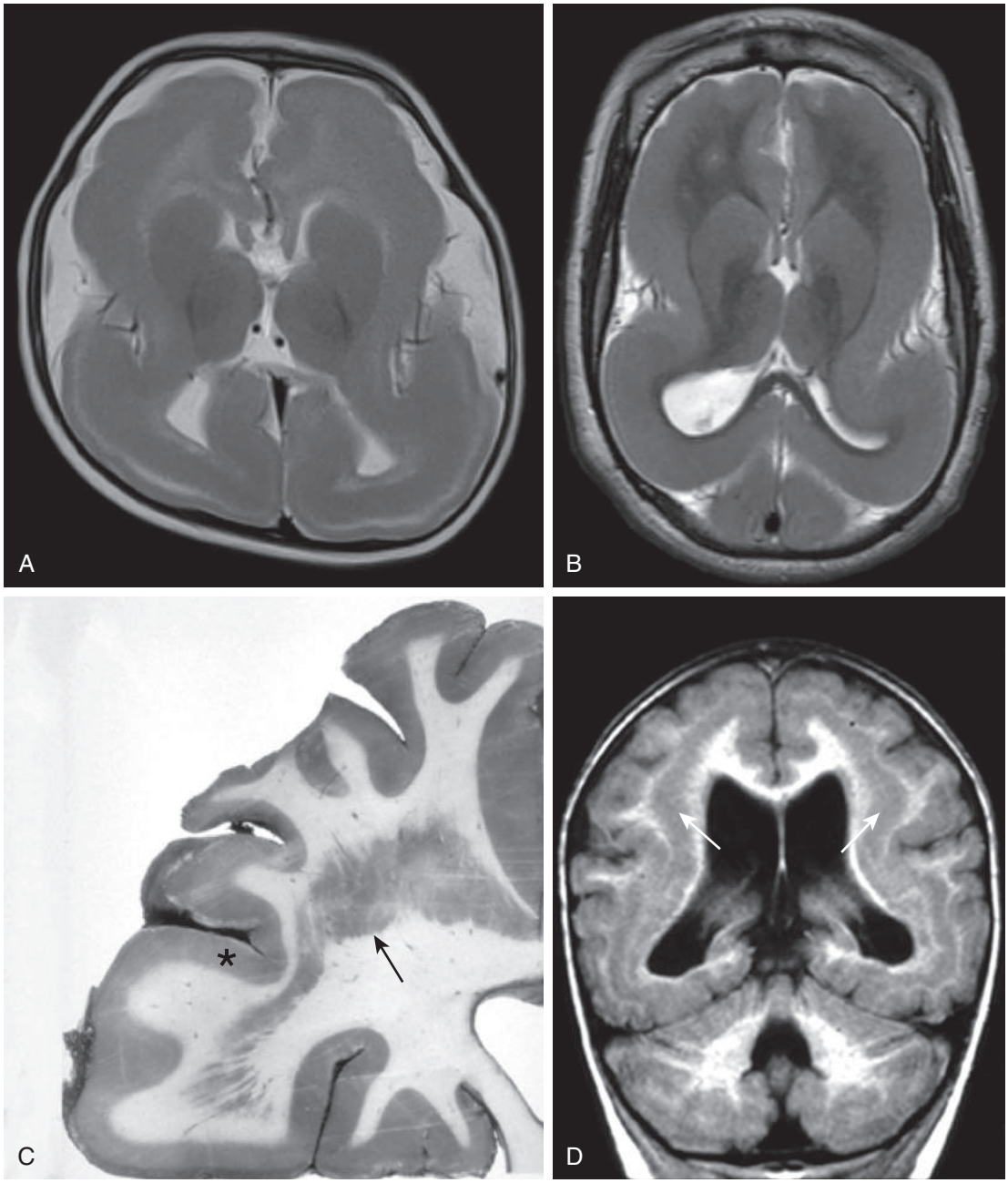


• **Fig. 53.5** Magnetic Resonance Image Scan Demonstrating Periventricular Nodular Heterotopia. Periventricular nodular masses *(arrowheads)* are visualized adjacent to the lateral ventricles in this T2-weighted coronal image. (Courtesy Dr. Martin Salinsky, Department of Neurology, Oregon Health and Science University, Portland, OR.)

associated with subcortical band heterotopia.[73] Patients typically present with developmental delay, intellectual disability, and/or epilepsy but have less severe disability than those with lissencephaly, and 25% have normal or near normal intelligence. Neurologic deficits roughly correlate with the thickness and extent of the subcortical band.[99]

Lissencephaly

The lissencephalies are characterized by reduced or absent gyri, giving the cortical surface a smooth or nearly smooth appearance.[100] Patients with lissencephaly have intellectual disability, hypotonia, epilepsy, and feeding difficulties.[94] The classification



• **Fig. 53.6** Classic Lissencephaly/Subcortical Band Heterotopia Spectrum. (A) Axial T2-weighted magnetic resonance image (MRI) of the brain in a child with *LIS1* pathogenic variant. (B) Axial T2-weighted MRI of the brain in a child with a *TUBA1A* pathogenic variant. (C) Histologic specimen demonstrating the presence of a broad band of heterotopic gray matter *(arrow)* that is situated within the cerebral white matter and distinct from the cerebral cortex *(asterisk)* in a female patient with a *DCX* pathogenic variant. (D) T1-weighted axial MRI demonstrating the circumferential nature of the subcortical band heterotopia *(arrows)* in another female patient with a *DCX* pathogenic variant. (Courtesy Dr. Joseph G. Gleeson, Department of Neurology, School of Medicine, University of California, San Diego, CA; M. Elizabeth Ross, Department of Neurology, University of Minnesota, Minneapolis, MN; Christopher A. Walsh, Department of Neurology, University of Minnesota; and Christopher A. Walsh, Department of Neurology, Harvard Medical School, Boston, MA.)

of the lissencephalies reflects the rapidly evolving molecular basis of these disorders (see Fig. 53.6).[101,102] Most patients are included in the classic lissencephaly/subcortical band heterotopia spectrum (previously type 1), which is associated with deletions or missense pathogenic variants in *LIS1*.[103] Other associated genes include *DCX*, *YWHAE*, and several tubulin genes.[104] These gene products have roles in neurogenesis, cell motility, and microtubule polymerization.[105,106]

Lissencephaly is not only seen in isolation. Pathogenic variants in the *ARX* gene, which encodes a transcription factor, result in X-linked lissencephaly with abnormal genitalia and, in the CNS, abnormal basal ganglia, immature white matter, and ACC.[107] Lissencephaly can also be seen with cerebellar hypoplasia.[96,100–102,108] The most common causes of this phenotype are dominant pathogenic variants in tubulin genes, which can often be distinguished by characteristic basal ganglia, brainstem, and cerebellar dysplasia.[109] Less frequently, lissencephaly with a moderately thick cortex, severe global cerebellar hypoplasia, and a malformed hippocampus is caused by recessive pathogenic variants in the *RELN* gene, which encodes a large extracellular matrix protein involved in signaling via the apolipoprotein E and very low-density lipoprotein receptors.[108,110] Pathogenic variants in very low density lipoprotein receptor (VLDLR) cause a less severe phenotype with mildly thickened and simplified cortical gyri and cerebellar hypoplasia.[111]

Clinical Features

Lissencephaly may be diagnosed in utero or present as hypotonia, developmental delay, or seizures during infancy. Spasticity may develop later in the first year. Isolated lissencephaly carries a poor long-term prognosis dominated by cognitive disability, spastic quadriparesis, and epilepsy. Craniofacial anomalies can be subtle in patients with *LIS1* pathogenic variants or more pronounced as in Miller–Dieker syndrome caused by deletion of *LIS1* and *YWHAE*.[112] Miller–Dieker syndrome is characterized by severe lissencephaly, a high forehead with bitemporal hallowing, short nose, upturned nares, downturned vermillion border, and small jaw.[113] Head circumference is typically normal at birth, but progressive microcephaly occurs during the first year. Neonatal seizures may occur, and severe myoclonic epilepsy typically develops in the latter half of the first year (e.g., infantile spasms or Lennox–Gastaut syndrome).[114]

Cobblestone Malformation Syndromes

Cobblestone cortex (previously known as *lissencephaly type II*) is characterized by a nodular appearance of the cortex, which is due to disruption of the normal arrest of cells at the pial surface.[67] On MRI, T2/fluid-attenuated inversion recovery signal is often markedly increased in cortical white matter. Cobblestone cortex is seen in the congenital muscular dystrophies: Walker–Warburg syndrome, muscle–eye–brain disease, and Fukuyama congenital muscular dystrophy.[115] These autosomal recessive disorders belong to a class of glycosylation-deficient muscular dystrophies,[116] the dystroglycanopathies, that are related to pathogenic variants in enzymes that catalyze the posttranslational O-glycosylation of a small number of mammalian glycoproteins.[117,118]

Walker–Warburg syndrome is the most severe congenital muscular dystrophy associated with cobblestone cortex.[119] Macrocephaly is present at birth or develops in the first year and is due to hydrocephalus. Kinked brainstem, cerebellar hypoplasia, and elevated creatine kinase level are strongly suggestive of the diagnosis. Ocular anomalies include retinal detachment, optic nerve hypoplasia, microphthalmia, and coloboma; muscular weakness is typically severe.[120] Survival beyond the first year is uncommon. The brain malformations and neurodevelopmental outcomes in patients with muscle–eye–brain disease are less severe, but patients still have substantial disability and most experience seizures. Individuals with Fukuyama congenital muscular dystrophy can have variable cortical and eye findings. Hypoplasia and cysts can be seen in the cerebellum while the cortex can show PMG. Moderate cognitive disability can be seen, and some patients may walk independently.[115]

Pathogenic variants in the laminin subunit β_1 (encoded by *LAMB1*), which localizes to the pial basement membrane, also result in cobblestone cortex and other brain conditions, but these malformations do not exhibit the ocular or muscular abnormalities seen in the syndromes mentioned above.[121] Similarly, G protein-coupled receptor 56 (*GPR56*) pathogenic variants can cause a recessive syndrome with cobblestone-like cortex, abnormal white matter signal, and cerebellar cysts as well as bilateral frontoparietal PMG.[122,123] Neuroimaging shows bilateral white matter changes, small brainstem, and small dysmorphic cerebellum, which is unique for the PMG syndromes.[124,125]

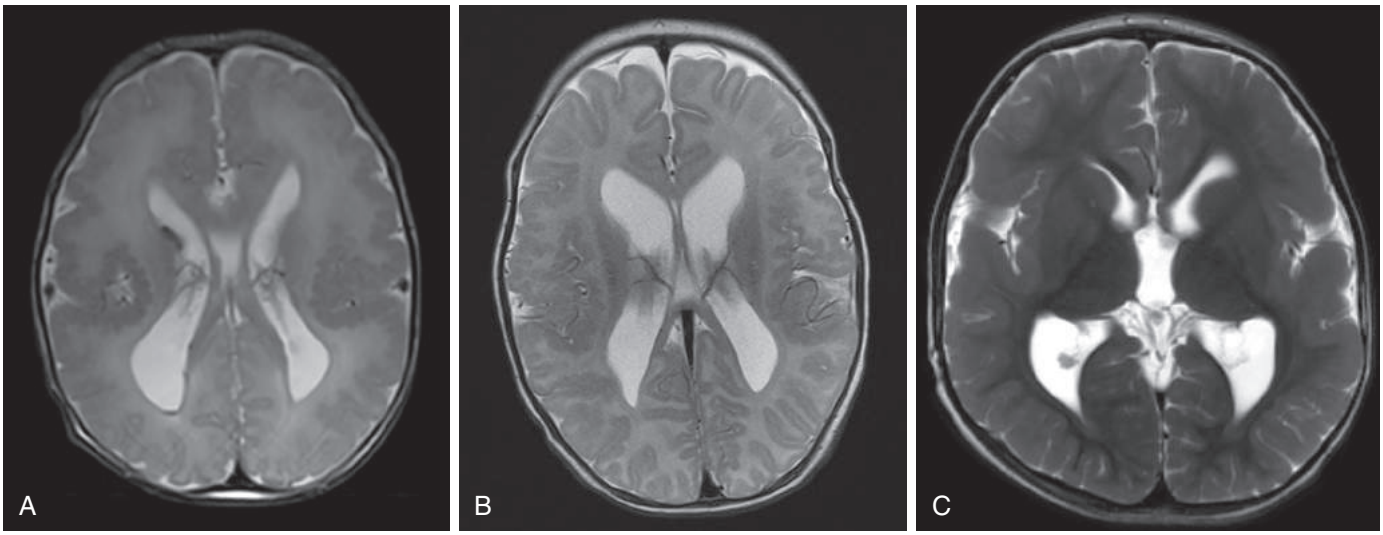
Polymicrogyria

PMG was originally defined by histopathologic findings. With the advent of brain MRI, the term is now commonly used to refer to a variety of appearances with an irregular gray–white border and many very small gyri or a thickened cortical ribbon (Fig. 53.7). PMG or PMG-like appearances can be caused by pathogenic variants in a variety of genes, as well as by infection (CMV, toxoplasmosis), ischemic/vascular anomalies, and metabolic disorders. A variety of other malformations, such as schizencephaly, can be seen in association with PMG. Recently, some ischemic etiologies have been found to be genetic in origin (*OCLN* or *COL4A1* pathogenic variants, 22q11 deletions, and Sturge–Weber syndrome).[126]

Historically, two major types of PMG have been proposed: layered and unlayered. *Layered PMG* has four rather than six distinct cortical layers. This "classic" form is localized adjacent to regions of encephalomalacia and is thought to be frequently caused by injury. *Unlayered PMG* is cortex that lacks distinct cortical layers and is associated with other migrational disturbances, such as subcortical nodular heterotopia, lissencephaly, and schizencephaly. At times, multiple types of PMG may be present in the same patient,[127] and the term has been used to refer to a variety of appearances on structural MRI without correlation to pathologic findings (see Fig. 53.7). PMG is usually sporadic, but it can be seen in association with several syndromes, especially if it is bilateral, and has been seen in multiple modes of inheritance in rare familial cases.[108,128]

Clinical Features

The clinical presentation of PMG relates to the extent of cortical dysplasia and the underlying cause and is difficult to predict on the basis of imaging findings alone. Bilateral PMG or involvement of more than half of a single hemisphere carries a high risk of moderate-to-severe developmental disability and significant motor dysfunction.[129] Hemiparesis or quadriparesis is often seen. Refractory epilepsy with complex focal seizures or multiple generalized seizure types may be delayed in onset beyond the neonatal period.



• **Fig. 53.7** Spectrum of Findings With Polymicrogyria. T2-weighted axial images (A–C) showing the varied magnetic resonance findings with polymicrogyria: (A) frontal and perisylvian polymicrogyria; (B) predominantly perisylvian; (C) globally thickened cortex.

Bilateral perisylvian PMG is the most commonly observed pattern. Patients with bilateral perisylvian PMG are likely to have epilepsy and intellectual disability.[130–132] In bilateral frontoparietal PMG, patients have global developmental delay, dysconjugate gaze/esotropia, and bilateral pyramidal and cerebellar motor signs.[124]

Tubulinopathy-Related Dysgyria

Pathogenic variants in α- and β-tubulin genes have been implicated in a range of cortical malformations.[133] So-called 'tubulinopathies' are usually due to de novo dominant pathogenic variants, with rare examples caused by germline mosaicism.[109] A more subtle malformation, called *tubulinopathy-related dysgyria*, shows a subtle irregular cortical gyral pattern with basal ganglia and brainstem dysplasia, associated with *TUBA1A, TUBB2B*, and *TUBB3* pathogenic variants that occur de novo or are inherited from a mosaic parent.[134] Patients can present with motor, cognitive, and language delays that can be mild, abnormal eye movements, behavioral issues, and seizures.

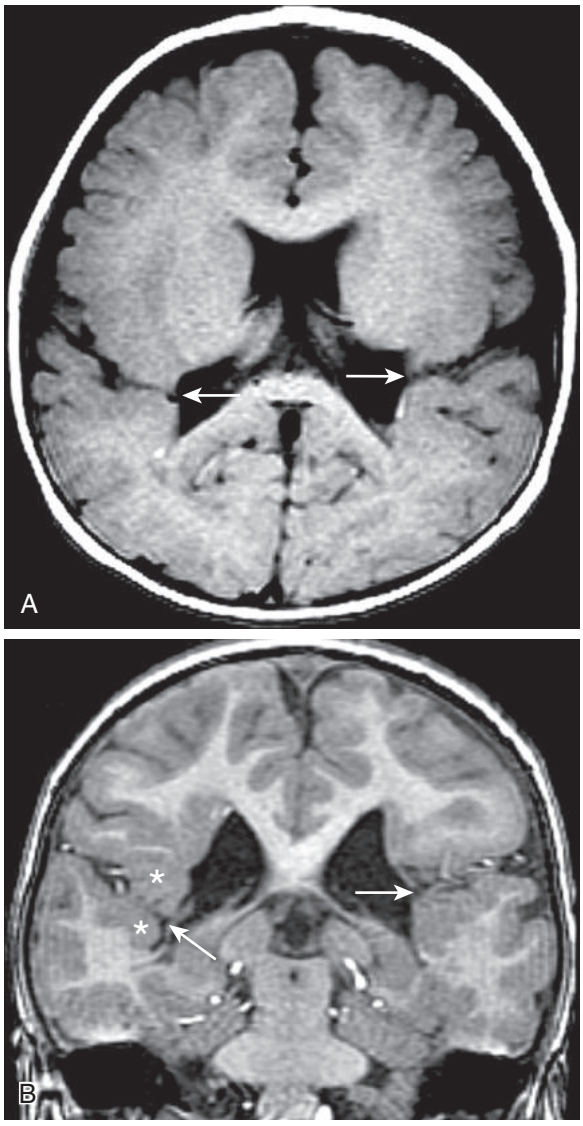
Destructive Lesions

Schizencephaly, porencephaly, and hydranencephaly are descriptive terms for malformations that result from destructive lesions during development. Schizencephaly may be unilateral or bilateral and "open-lipped" or "closed-lipped" (Fig. 53.8). When it is severe, both lateral ventricles communicate widely with the extra-axial space (bilateral open-lipped). Unsurprisingly, patients with schizencephaly have significant cognitive, motor, and communication difficulties, and epilepsy is common.[135] The severity of the epilepsy is not correlated with the severity of the brain malformation, and two studies found that patients with unilateral schizencephaly had an earlier age of seizure onset and more refractory epilepsy than those with bilateral schizencephaly.[136,137] Diagnosis is by MRI, and findings may include other brain malformations, such as cortical dysplasia, corpus callosum dysgenesis, and absent septum pellucidum. Presumed causes of schizencephaly include destructive events such as ischemia and infection, however, familial cases are known.[94] Schizencephaly may also result from an extreme failure of neurons to migrate. This is supported by histology showing that the schizencephalic cleft has features of a migrational disturbance, such as large neuronal heterotopia bordered by adjacent PMG.[138] Pituitary insufficiency is seen in a substantial subset of patients with schizencephaly, particularly if optic nerve hypoplasia is also present.

Porencephaly is a fluid-filled cavity within the cerebral hemispheres, caused by loss of tissue secondary to trauma, infection, or hemorrhage. Porencephaly can develop prenatally and postnatally. Pathogenic variants in *COL4A1* are associated with an autosomal dominant form of hereditary porencephaly. Pathogenic variants in *COL4A1* can also cause lesions of the kidneys, eyes, cardiac muscle, or skeletal muscle.[139] *COL4A2* has been associated with familial and sporadic porencephaly. These two genes encode collagen subunits that help form the basement membrane. Most hypothesize that collagen-related porencephaly is secondary to vascular events. *COL4A1* and *COL4A2* pathogenic variants can be associated with stroke and other highly variable manifestations in family members with the same pathogenic variant, so the affected infant and at-risk family members should undergo neurologic, ophthalmologic, renal, and cardiac screening.

Hydranencephaly is due to the replacement of the hemispheres with a fluid-filled sac and may be considered an extreme form of porencephaly. This rare condition has several proposed mechanisms, including infarction, leukomalacia, necrosis, infection, and thromboplastic material from a cotwin. In addition to the etiologies previously mentioned, there may be an association of destructive lesions, such as porencephaly, hydranencephaly, and schizencephaly, with in utero exposure to vasoactive drugs such as cocaine, heroin, and methamphetamine.[140]

A rare genetic disorder called *Fowler syndrome*, caused by pathogenic variants in *FLVCR2* causes severe hydrocephaly and also hypokinesia, CNS vasculopathy, and arthrogryposis. This disorder is also called *proliferative vasculopathy and hydranencephaly–hydrocephaly (PVHH) syndrome*. Although case reports have described two siblings that survived beyond infancy, most infants do not live beyond the first year.[141,142] Hydrocephalus and feeding difficulties are frequent complications, and decisions about



• **Fig. 53.8** Magnetic Resonance Image Scan Demonstrating Bilateral Open-Lip Schizencephaly. (A) Axial T1-weighted image. Note that the bilateral clefts *(arrows)* extend to the lateral ventricles. (B) Coronal T1-weighted image. Note that the clefts *(arrows)* are lined with gray matter *(asterisks)*. (Courtesy Dr. A. James Barkovich, Department of Radiology, School of Medicine, University of California, San Francisco.)

whether to treat the infant with CSF diversion or tube feeding can be difficult given the limited developmental potential and life span.

Malformations of Structures in the Posterior Fossa

Normal Midbrain and Hindbrain Development

The brainstem comprises the midbrain, pons, and medulla, while the cerebellum is composed of a vermis and hemispheres. The development of these structures occurs after the closure of the neural tube and coincides with prosencephalic development.[143] The development of the cerebellum, like that of the cortex, is influenced by both intrinsic (genetic) and extrinsic (inductive/environmental) factors.[144] The midbrain structures derive from the mesencephalon and myelencephalon, and the hindbrain derives from the rhombencephalon by gestational week 4. The cerebellar vermis derives from both the caudal third of the mesencephalon and the hindbrain.[145] Cerebellar development continues into the second postnatal year, making the cerebellum particularly vulnerable to injury in premature infants.[146] The posterior fossa is evaluated by ultrasonography or MRI during the second and third trimesters of pregnancy, but MRI is the modality of choice if abnormalities are suspected.[147]

For this section, we will characterize posterior fossa malformations on the basis of imaging findings.[148] Embryonic and genetic classifications have also been used.[149,150] Alternatively, posterior fossa malformations can be classified on the basis of inherited (metabolic, structural, neurodegenerative, or the spinocerebellar ataxias) versus acquired causes (vascular, hypoxic-ischemic encephalopathy infection, disrupted development, toxic, neoplastic, or teratogenic).[151] Clinical features of posterior fossa abnormalities are nonspecific but include hypotonia, developmental delay, nystagmus, and decreased visual attention. Depending on the malformation, seizures and apnea may also be present, as may cranial nerve dysfunction. The combination of clinical, imaging, and genetic testing information is required for accurate diagnosis.

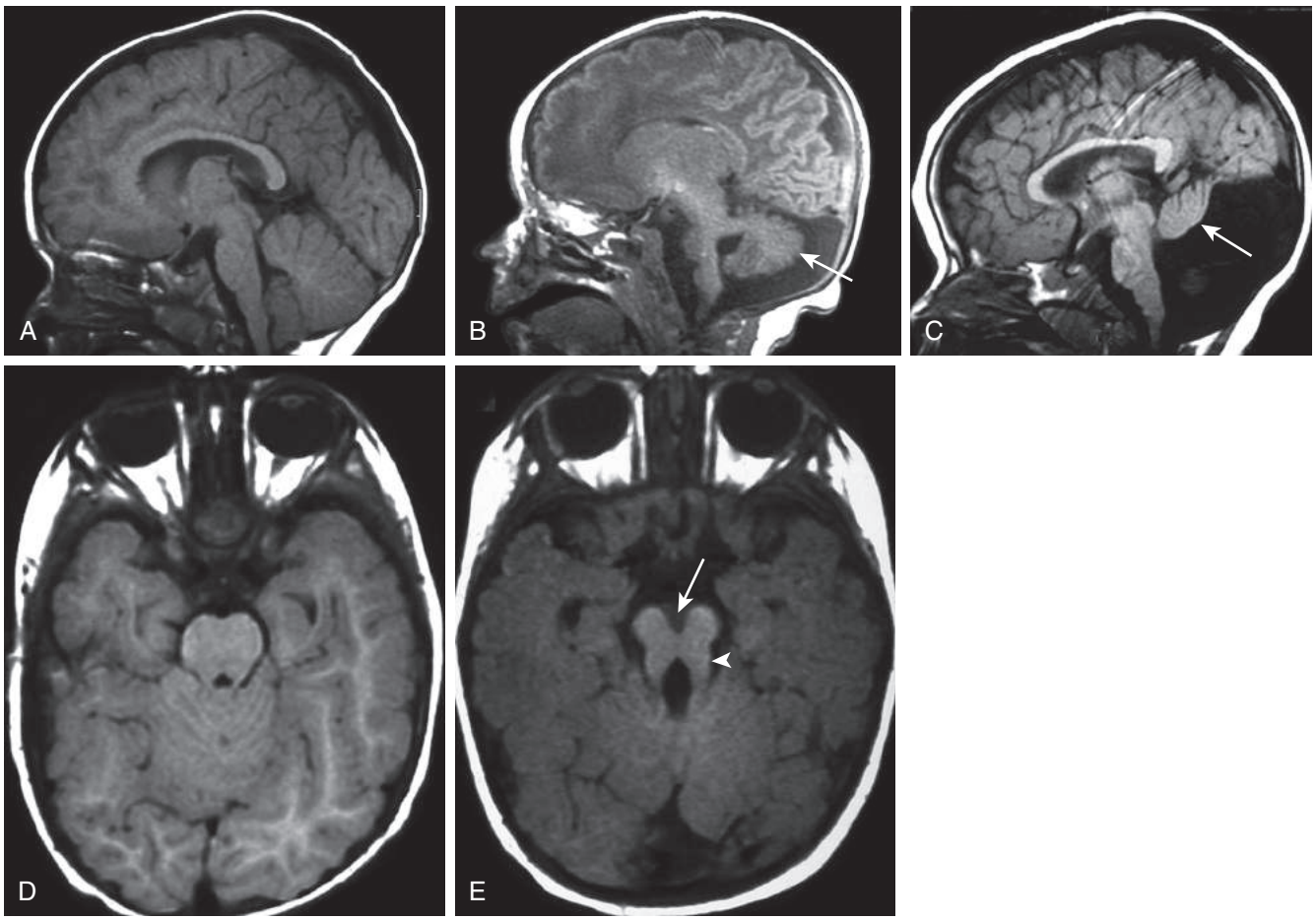
Malformations With Major Cerebellar Involvement

Dandy–Walker Malformation

Dandy-Walker malformation (DWM) is typified by aplasia or hypoplasia of the vermis of the cerebellum, cystic dilation of the fourth ventricle, and enlargement of the posterior fossa with an upward displacement of the lateral sinuses, tentorium, and torcula (Fig. 53.9).[152–155] The pons may also appear hypoplastic given the interconnectedness of these structures.[145] Diagnosis may be made in utero by ultrasonography and fetal MRI. Prenatal ultrasonography and MRI are increasingly used, but both continue to be limited in the precise anatomic definition of these malformations.[156] The enlargement of the posterior fossa and displacement of its contents may be related to communication of the fourth ventricle with a retrocerebellar cyst, often of considerable size. Hydrocephalus is also a common feature that may present in the neonatal period with macrocephaly.[154,157]

Epidemiology and Etiology

Estimates of the prevalence of DWM are affected by the lack of agreement regarding the definition. While some estimates are as high as 1 in 5000 liveborn infants,[158] the prevalence is likely substantially lower. DWM may be isolated or associated with a number of syndromes (e.g., Walker–Warburg syndrome, Meckel syndrome) as well as trisomies 9, 13, and 18. Given the low recurrence risk of 1% to 3%, DWM is likely due to de novo pathogenic variants, environmental insults, and vascular and multifactorial causes. Using exome sequencing, pathogenic variants in seven different genes were identified in 16% of DWM patients without a known diagnosis, highlighting the etiologic heterogeneity of DWM.[159]



• **Fig. 53.9** Posterior Fossa Abnormalities. Sagittal images of a normal brain (A), the brain of a child with Joubert syndrome (B), and the brain of a child with Dandy-Walker malformation *(DWM)* (C) illustrate the features of these hindbrain malformations. Note the presence of cerebellar vermis hypoplasia *(arrow)* in both (B) and (C). By contrast, the patient with Joubert syndrome (B) has only a slightly enlarged fourth ventricle, whereas in the DWM (C) the fourth ventricle is massively dilated. (D, E) Molar tooth sign. Comparison of axial images from a normal brain (D) with that of a child with Joubert syndrome (E). Note two key features of the molar tooth sign: a deepened interpeduncular fossa *(arrow)* and the elongated superior cerebellar peduncles *(arrowhead)*. (A–C, Courtesy Dr. Joseph G. Gleeson, Department of Neurology, School of Medicine, University of California, San Diego; and Dr. William B. Dobyns, University of Chicago School of Medicine, Chicago; D, E, Courtesy Dr. Joseph G. Gleeson, Department of Neurology, School of Medicine, University of California, San Diego.)

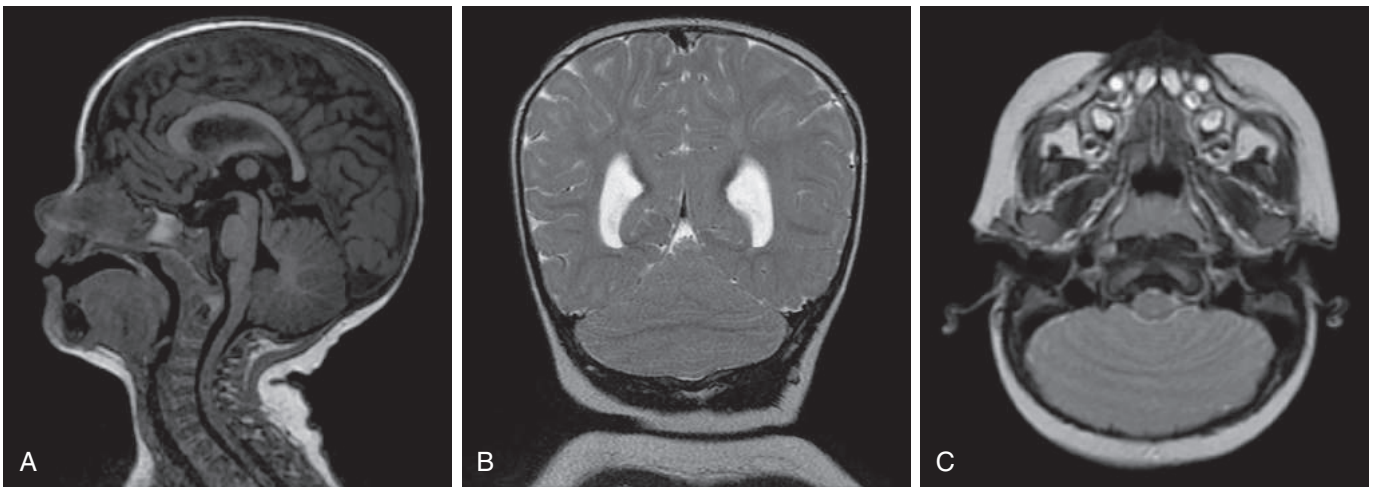
Clinical Features and Management

DWM can be associated with other brain imaging abnormalities, particularly ACC and hydrocephalus. In addition, cardiac and other organ malformations can be present. Management is similar to that for other neurodevelopmental conditions and can include surgical correction of hydrocephalus with shunt placement within the lateral ventricles and/or posterior fossa. The outcome is broad: patients with isolated DWM often have mild developmental issues, while those with additional malformations and specific genetic conditions are at much higher risk.[160]

Rhombencephalosynapsis

Rhombencephalosynapsis (RES) is unique among the cerebellar vermis hypoplasias. Instead of the cerebellar hemispheres being widely separated, the hemispheres, white matter, and deep cerebellar nuclei are variably fused (Fig. 53.10).[161] Clinically, patients with RES may present with ataxia, muscle hypotonia, abnormal eye movements, dysarthria, head shaking, and developmental delay with variable severity. RES may be isolated or seen in combination with other syndromes such as vertebral, anal, cardiac, tracheoesophageal, renal, and limb anomalies (VACTERL) and may be associated with other CNS abnormalities, such as hydrocephalus.[162–165] Additional proposed causes include other genetic defects (particularly postzygotic-mosaic variants) and environmental factors (such as maternal diabetes and other exposures).[163]

RES can be diagnosed prenatally by fetal MRI and should be suspected in fetuses with ventriculomegaly and cerebellar hypoplasia identified by ultrasonography. In the absence of ventriculomegaly or other imaging abnormalities, RES is rarely diagnosed prenatally. In addition, when aqueductal stenosis is suspected prenatally or postnatally, the cerebellum should be closely scrutinized for RES.[162]



• **Fig. 53.10** Rhomboencephalosynapsis as Demonstrated by Magnetic Resonance Imaging. (A) T1-weighted sagittal image showing excessive white matter centrally in the cerebellum and absent primary and horizontal fissures. The T2-weighted coronal (B) and axial (C) show continuous folia across the midline and the smooth dorsal cerebellar surface caused by vermian agenesis and hemisphere fusion.

Malformations With Both Cerebellar and Brainstem Involvement

Joubert Syndrome and Related Disorders

Joubert syndrome (JS) is a recessive disorder usually presenting with hypotonia, abnormal eye movements, and developmental delays. The prevalence has been estimated to be 1 in 100,000. Apnea and tachypnea may be notable in infancy and early presentation may include head titubation, which is a subtle nodding of the head seen in JS and other posterior fossa malformations.[166–168] JS is defined by the "molar tooth sign" on imaging (see Fig. 53.9): cerebellar vermis hypoplasia/aplasia, deep interpeduncular fossa, and thick, elongated superior cerebellar peduncles. Historically, several other names have been used to refer to JS, including cerebellar ocular renal syndrome, cerebellar hypoplasia/aplasia, oligophrenia, cerebellar vermis hypoplasia/aplasia, oligophrenia, congenital ataxia, ocular coloboma, and hepatic fibrosis (COACH) syndrome, and Varadi–Papp syndrome.[169] Pathogenic variants in more than 35 genes have been identified in more than 70% of patients with JS, most commonly in five genes *AHI1, CC2D2A, CEP290, TMEM67*, and *CPLANE1*.[170,171] Distinguishing JS from other hindbrain malformations is essential, since the recurrence risk can be as high as 25%, and substantial subsets of patients with JS develop progressive retinal dystrophy, fibrocystic kidney disease, and liver fibrosis, requiring lifelong monitoring and treatment.[172] Some genetic causes are associated with a higher risk of complications (e.g., loss of *CEP290* is associated with retinal dystrophy). Therefore, genetic testing can be used to inform family planning to guide the monitoring of affected patients.[171]

Pontocerebellar Hypoplasias

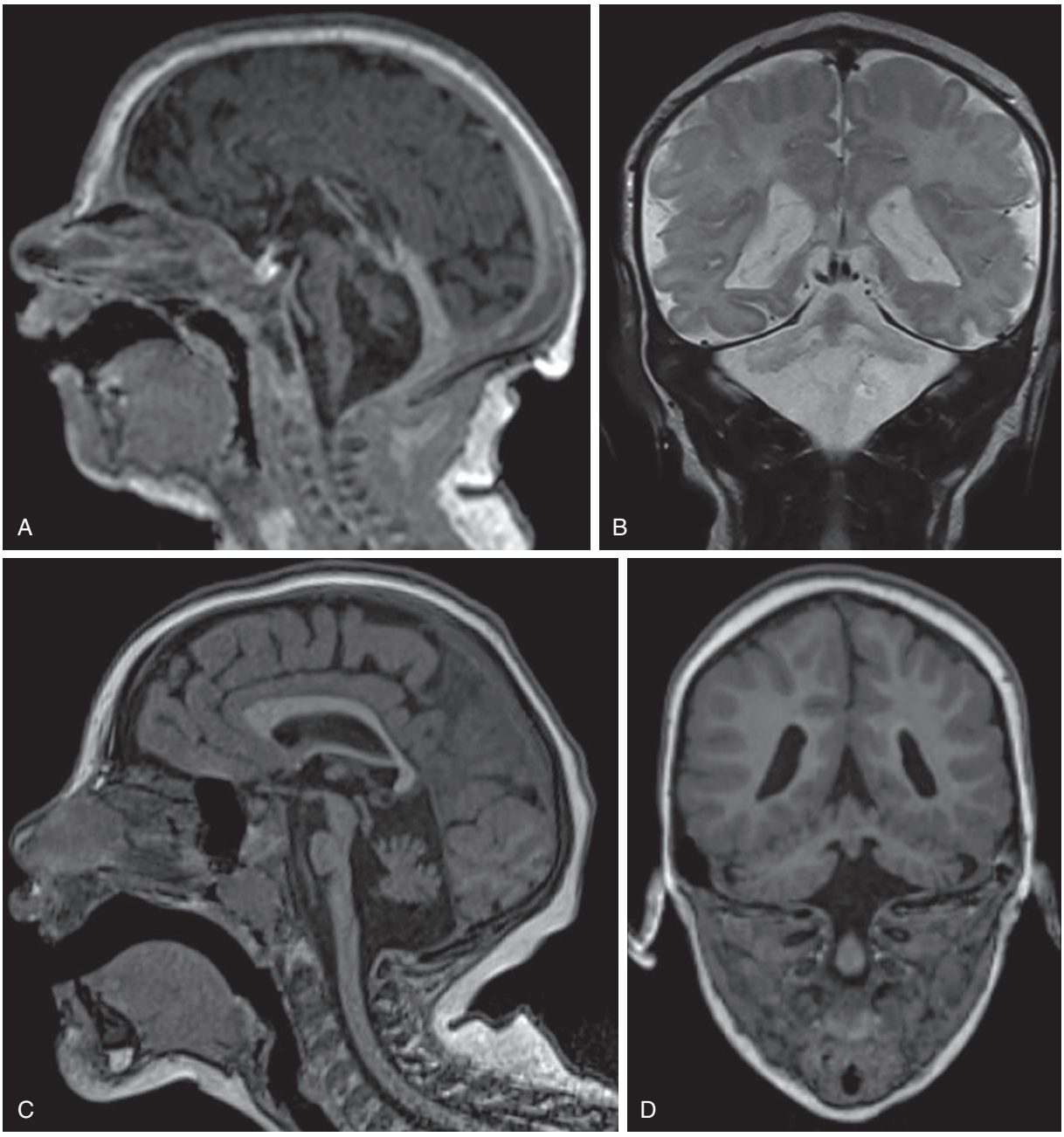
Pontocerebellar hypoplasia (PCH) is a diverse group of largely autosomal recessive disorders characterized by a variable degree of pontine and cerebellar hypoplasia (Fig. 53.11). PCH can occur in association with congenital disorders of glycosylation, mitochondrial diseases, and congenital muscular dystrophies. At least 13 overlapping subtypes of PCH have been proposed, many presenting in the neonatal period.[150] Multiple genetic causes of PCH have been identified, most commonly pathogenic variants in *TSEN54*, but up to 40% of patients do not have an identified cause.[173,174] Given the phenotypic and genetic overlap in these subtypes, the use of a combination of genetic, clinical, and imaging findings is more specific in describing these disorders (Table 53.1).[175] In patients with *TSEN*-related PCH, the vermis is often relatively preserved compared with the hemispheres, in contrast to most other PCH disorders. Outcome data are sparse and depend on the type of PCH, but typically patients have severe developmental delays, tone abnormalities, feeding issues, and respiratory compromise.[175] A number of PCHs have distinctive imaging features that point to specific diagnoses and should be sought through careful review of clinical and radiologic features. For instance, the combination of PCH, asymmetric brainstem, bulbous asymmetric basal ganglia, and cerebellar dysplasia, with or without lissencephaly, in the absence of other malformations, is strongly suggestive of tubulinopathy conditions caused by dysfunction of one of the nervous system specific tubulin genes.[176] *MACF1*-related conditions combine PCH and some degree of lissencephaly without brainstem asymmetry or other malformations.[177] Pontine tegmental cap dysplasia is diagnosed by a characteristic "cap" on the dorsal surface of the pons, with absent inferior cerebellar peduncles, hypoplastic middle cerebellar peduncles, and prominent cranial nerve deficits including sensorineural hearing loss, trigeminal anesthesia, facial palsy, and swallow dysfunction.[178] Improvements in MRI and genetic testing will continue the remarkable progress in our understanding of human hindbrain malformations.

Malformations With Brainstem Involvement

Chiari Malformations

Historically, three numbered types of Chiari malformations have been defined, but they are not etiologically related.

Chiari type I malformation is characterized by inferior ectopia of the cerebellar tonsils into the spinal canal, often with compression of the tonsils and restricted CSF flow. It is uncommonly



• **Fig. 53.11** Pontocerebellar Hypoplasia. (A) T1-weighted sagittal and (B) T2-weighted coronal images of a patient with *TSEN45*-related pontocerebellar hypoplasia *(PCH)*. *TSEN54* pathogenic variants have been identified in PCH types 1, 2, 4, and 5 and can be associated with additional malformations, including atrophic cortex and cerebellar hemisphere hypoplasia more than vermin hypoplasia, cerebellar hemisphere more than vermian hypoplasia. (C) T1-weighted sagittal and (D) T1-weighted coronal images of a patient with *CASK*-related PCH. *CASK*-related PCH may be associated with mild small pons and cerebellum, with proportionate hypoplasia of the cerebellar vermis and hemispheres.

diagnosed in neonates. Diagnosis is based on clinical and imaging findings; patients may have headache and lower cranial nerve, cerebellar, and brainstem dysfunction. Herniation of the tonsils greater than 5 to 10 mm in the presence of symptoms is diagnostic, but similar herniation can be seen in the absence of symptoms. Other findings include syringomyelia and scoliosis.[179] Mild inferior tonsillar ectopia without compression of the tonsils or restricted CSF flow is common and is not usually associated with symptoms. Chiari type I malformation may be either congenital or acquired. In acquired Chiari malformations the posterior fossa size is normal.

Chiari type II malformation is seen almost exclusively in association with open neural tube defects (NTDs) and is defined by displacement of the cerebellar vermis, medulla, and the fourth ventricle into the spinal canal and is associated with a small posterior fossa, "beaked" tectum, and frequently, hydrocephalus. MRI

TABLE 53.1 Pontocerebellar Hypoplasia*

Pontocerebellar Hypoplasia	Clinical Findings	Radiologic Findings	Associated Genes (Major Listed First)
Type I	Spinal muscular atrophy	Mild hypoplastic pons, with proportional hypoplasia of the vermis and hemispheres	*EXOSC3, RARS2, TSEN54, VRK1*
Type 2	Neonatal encephalopathy, progressive microcephaly, increased tone, dyskinesia, seizures, cortical visual impairment	Postmigrational microcephaly, small pons and cerebellum, atrophic cortex with thin corpus callosum, vermis less affected than hemispheres	*TSEN54, TSEN2, TSEN34*
Type 3	Hypotonia, hyperreflexia, infantile seizures, developmental delay	Small pons and cerebellum, reduced amount of cerebral white matter	*PCLO*
Types 4 and 5	Severe type 2	As type 2	*TSEN54, TSEN2, TSEN34*
Type 6	Elevated CSF lactate level	Small pons and cerebellum, vermis affected more severely than hemispheres	*RARS2*
Type 8	Acquired microcephaly, increased tone and contractures, moderate to severe developmental delay	Small pons and cerebellum with proportionate hypoplasia of the vermis and hemispheres	*CHMP1A*
Type 9	Progressive microcephaly, seizure onset in infancy, hypertonia, and visual impairment. Severe developmental delay	Small cerebellum with atrophy, pons with ventral flattening, mega cisterna magna and brainstem with a figure-of-eight appearance, atrophy of cerebral cortex, and corpus callosum hypoplasia	*AMPD2*
Type 10	Progressive microcephaly and neurodegeneration by 6 months, absent or delayed speech, progressive spasticity, spontaneous seizures, severe developmental impairment	Small cerebellum, pons, and corpus callosum with atrophy	*CLP1*

Modified from Aldinger KA, Doherty D. The genetics of cerebellar malformations. *Semin Fetal Neonatal Med.* 2016;21(5):321–332.
CSF, Cerebrospinal fluid.
*All with autosomal recessive inheritance.

can be used to confirm these findings and often demonstrates cerebellar hypoplasia and ACC, although these features have not been associated with a higher risk of neurodevelopmental issues. Rarely, migration defects, HPE, and interhemispheric cysts can also be seen.[179]

The term *Chiari type III malformation* has been used to refer to a variety of hindbrain malformations that include occipital or high cervical encephalocele, often in combination with kinked brainstem or cervical spinal cord. A review of 57 patients noted encephalocele in about half; symptom severity was largely in proportion to the type and amount of material in the encephalocele.[180] The medical literature on outcomes is extremely limited, but several reported patients have had limited life spans and substantial disabilities.

Neural Tube Defects and Spinal Cord Dysraphisms

Open NTDs result from a failure of primary neural tube closure during the fourth week of gestation.[42] Anencephaly results from failure of anterior neuropore closure, while myeloschisis and myelomeningocele (MMC) result from failure of posterior neuropore closure. Craniorachischisis totalis occurs with the complete failure of neural tube closure and typically results in pregnancy loss during embryogenesis or early fetal development. The remainder of the NTDs discussed here result in malformations of the CNS and the overlying axial skeleton, meninges, and skin that are associated with various degrees of viability in the newborn period.

Epidemiology and Etiology

NTDs are one of the most common congenital malformations encountered in newborns, with 0.5 to 2 in 1000 pregnancies affected worldwide.[181,182] The prevalence differs widely and is particularly influenced by ethnicity, geographic area, and socioeconomic status.[183] In the United States, for example, the risk of NTDs is higher in people of Hispanic descent but lower in people of African American descent. After the first affected pregnancy, the risk of recurrence increases with each subsequent affected pregnancy.[184]

A marked decline in the prevalence of both anencephaly and spina bifida has occurred in recent decades. In 1960 the prevalence for England and Wales was about 6 in 1000 births; in 1990 this rate dropped to about 1 in 1000. The lower rates reflect two major interventions: in utero diagnosis with the termination of affected pregnancies and maternal periconceptional folate therapy. Maternal folate therapy is estimated to prevent approximately 60% to 70% of NTDs, so a combination of interventions may

be required to optimally prevent NTDs, and other factors need to be considered.[183,185–192] Most countries now recommend a healthy diet and folate supplementation before conception through the 12th week of pregnancy, with additional supplementation based on risk.[193,194]

Gene–Environment Association of Neural Tube Defects

The etiology of NTDs is complex and multifactorial, and both genetic and environmental factors interact to determine risk; however, most defects occur sporadically.[182,195] Environmental risk factors linked to NTDs include maternal hyperthermia, hyperglycemia, lower socioeconomic status, dietary factors, and prenatal exposure to a number of drugs, including antiepileptic medications such as valproate and carbamazepine.[182,183,195–198] In addition, the fungal toxin fumonisin has been associated with NTDs. A fourfold to fivefold higher prevalence of NTDs occurs in a Mexican-American population because of fumonisins in corn flour used to make tortillas.[198,199] NTDs have been associated with genes implicated in diabetes mellitus, obesity, and glucose and oxidative stress. The genetics influences on NTDs are also complex, encompassing chromosomal abnormalities (e.g., trisomies 13 and 18), single gene disorders (e.g., Waardenburg syndrome), and risk alleles (*MTHFR* and *VANGL1* variants).[200] Approximately 200 genes are known to be required for neurulation, many of which are involved in folic acid metabolism or transport.[201] Epigenetic mechanisms (such as histone modification, methylation, and nucleosome remodeling) may also play a role in neurulation.[198]

Fetal Diagnosis of Neural Tube Defects

Diagnosis of open NTDs may be suspected in the case of maternal serum elevation of alpha-fetoprotein (AFP) level and can be confirmed by fetal ultrasonography or MRI (Fig. 53.12).[202] AFP screening is designed to detect open NTDs and Down syndrome.[203] The optimal time for determination in maternal serum is 16 to 18 weeks' gestation and in amniotic fluid is 14 to 16 weeks' gestation. In open NTDs (anencephaly, open spina bifida, and open encephalocele), fetal AFP leaks directly into the amniotic fluid, indirectly increasing maternal serum protein levels. By contrast, skin-covered NTDs are not associated with elevated maternal serum AFP levels.[204] A false-positive AFP result can occur with misdating of the fetus (if older than predicted) and a multiple-gestation pregnancy.[205] Other causes of a high maternal serum AFP level include contamination of the amniotic fluid by fetal blood (which may occur in cases of esophageal and duodenal atresia), omphalocele, gastroschisis, congenital nephrosis, polycystic kidneys, renal agenesis, annular pancreas, or fetal demise.

Ultrasonography can be used as early as the end of the first trimester for evaluation of the radiographic signs of NTDs, but more typically the diagnosis is made during the second trimester. Abnormal head and cerebellar shape (the "lemon" and "banana" signs) are more sensitive ultrasound indicators for NTD diagnosis than spinal imaging findings. Historically, fetal MRI has not been superior to ultrasonography for identifying the spinal cord defect; however, for those patients electing to have in utero surgery, MRI is used for surgical planning.

Open Neural Tube Defects

Typically, NTDs are characterized as either open lesions (craniorachischisis, anencephaly, MMC, or myeloschisis) or closed lesions (encephalocele, meningocele, and occult spinal dysraphism).

Anencephaly

Anencephaly results after failed anterior closure where exencephaly converts to anencephaly by degradation of the neural tissue.[206] This is the most severe disorder of anterior neural tube closure. Anencephaly accounts for roughly half of all open NTDs.[182] As an early neurulation defect, it occurs no later than 24 days' gestation. Anencephaly can be diagnosed by fetal ultrasound examination (Fig. 53.13) during the first or second trimester[207,208]; it is frequently associated with polyhydramnios.[209]

Anencephaly most commonly involves the forebrain and upper brainstem. It is characterized by absence of the calvaria, and the intracranial contents are replaced by vascularized, disorganized glial tissue (area cerebrovasculosa).[42,210] The hypothalamus and cerebellum are usually malformed, the anterior lobe of the pituitary is present, and the internal carotid arteries are hypoplastic, which may be secondary to reduced brain parenchyma. Because the anencephalic infant has a period of exencephaly, where brain tissue extrudes through the unformed calvaria and is degraded by exposure to the amniotic fluid, some investigators have hypothesized that the primary defect is abnormal skull formation. There are some cases in which remnants of calvarial bones are present, with brain tissue under the protective bones.[42]

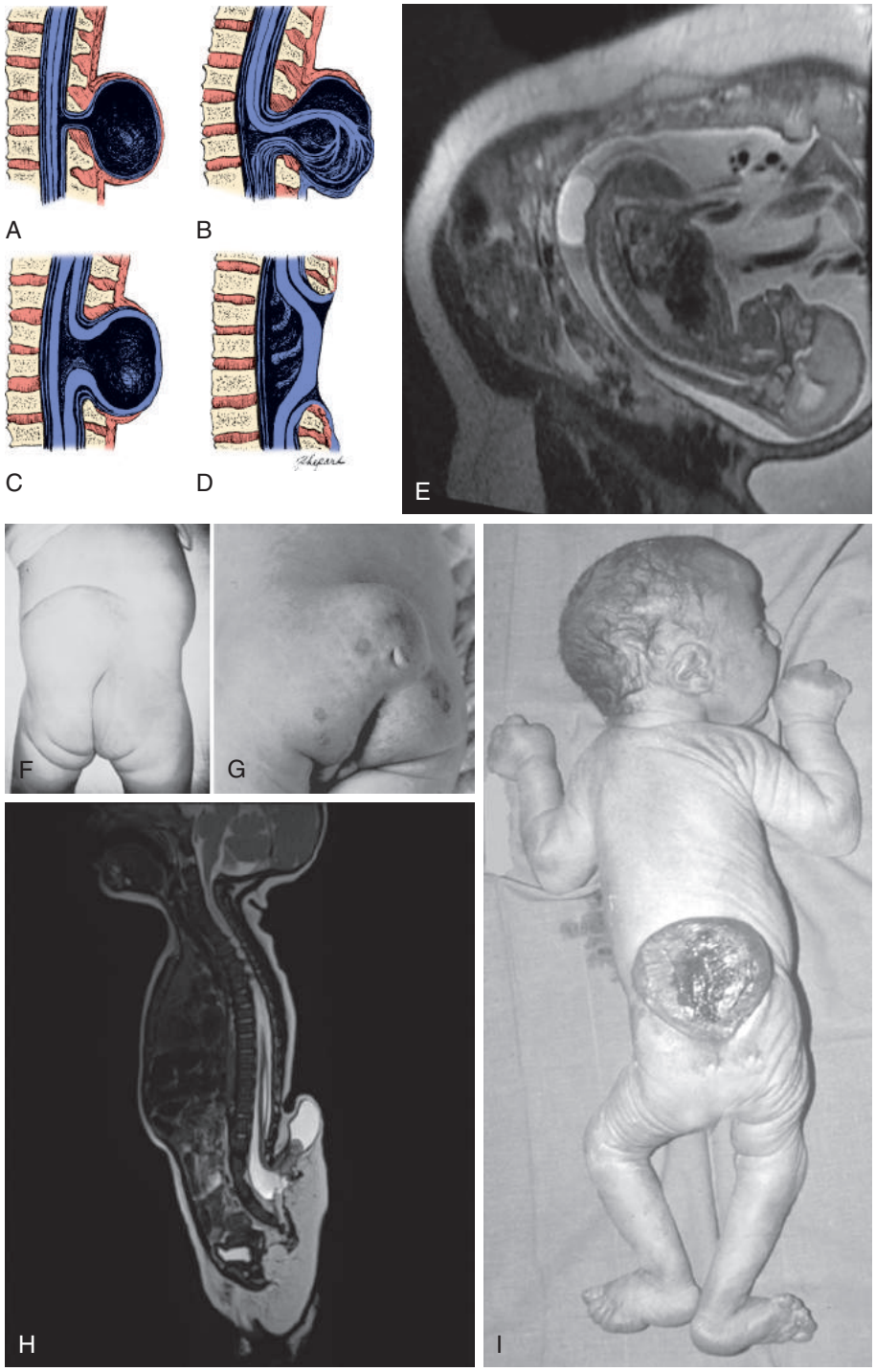
Most neonates are stillborn, and the physical examination is notable for a lack of the brain and cranial vault.[192] Liveborn neonates live less than 1 or 2 months at the most.[211,212] Without intensive care, survival has not been reported beyond the neonatal period. In a series of 211 women who reported choosing to deliver fetuses with an in-utero diagnosis of anencephaly, 72% were liveborn, approximately 67% of the liveborn infants died within 24 hours, and six infants lived for 6 days or more (maximum of 28 days). This study suggested that continuation of pregnancy is a preferred option for some women after they have been counseled about the expected outcomes.[213]

Myelomeningocele

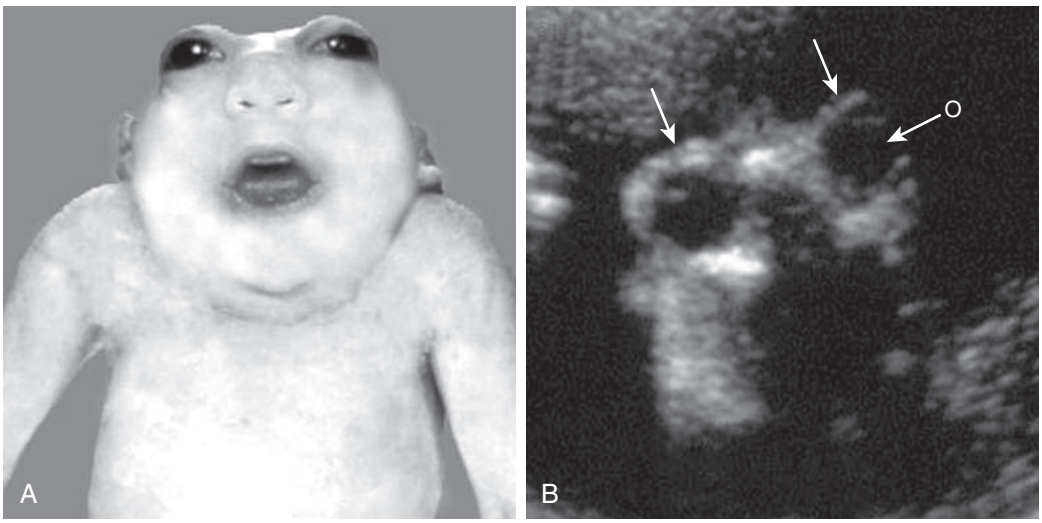
MMC and similar malformations of the spinal cord arise from the failure of posterior neuropore closure, probably no later than day 26 of gestation. Open spinal NTDs are exposed lesions on the back without vertebral or dermal covering (see Fig. 53.12).[210] MMC is characterized by herniation of the meninges and spinal cord at the site of the defect, and most often occur in the lumbar or lumbosacral regions.[155,210]

The diagnosis of MMC is usually made prenatally by ultrasonography and/or increased AFP level.[206] MMC is usually accompanied by other clinically significant CNS abnormalities, including Chiari II malformation and obstructive hydrocephalus.[214] Based on historical data, 60% of patients with occipital, cervical, thoracic, or sacral lesions develop hydrocephalus, in contrast to 90% of those with thoracolumbar, lumbar, or lumbosacral lesions.[215] Because of decompression caused by leakage of CSF from the MMC at birth, hydrocephalus and increased pressure may become evident only after surgical closure of the back.[216]

Clinical management of the newborn with an NTD must be individualized. At present, surgical closure is advocated for



• **Fig. 53.12** Neural Tube Defects. (A) Meningocele. Through the bony defect (spina bifida), the meninges herniate and form a cystic sac filled with spinal fluid. The spinal cord does not participate in the herniation and may or may not be abnormal. (B) Myelomeningocele. Spina bifida with myelomeningocele; the spinal cord is herniated into the sac and ends there or may continue abnormally further downward. (C) Myelocystocele or syringomyelocele. The spinal cord shows hydromyelia; the posterior wall of the spinal cord is attached to the ectoderm and undifferentiated. (D) Myelocele. The spinal cord is araphic; a cystic cavity is in front of the anterior wall of the spinal cord. (E) Fetal magnetic resonance image of lumbosacral dysraphism. Note the ventriculomegaly and crowded posterior fossa. (F–H) Three examples of skin-covered neural tube defects. (F, G) Lesion presented in the left buttock as a firm, well-circumscribed, lobulated tumor that became tense when the infant cried. (G) Macular erosions and a congenital skin tag and dimple over the surface. This last feature may be a pilonidal dimple displaced by the tumor. (H) T2-weighted image of a lipomyelomeningocele. (I) Newborn with a large thoracolumbar myelomeningocele. The distal musculature in the lower extremities was weak. (A–D, From Benda CE. Developmental Disorders of Mentation and Cerebral Palsies. New York, NY: Grune and Stratton; 1952; F–H, Courtesy Dr. Marjorie Grafe, Department of Pathology, Oregon Health and Science University, Portland, OR.)



• **Fig. 53.13** Anencephaly. (A) Infant with anencephaly. (B) Ultrasonogram of a fetus with anencephaly. Note the absence of the normal cranial structures *(arrows)* superior to the orbits *(O)*. (Courtesy Dr. Marjorie Grafe, Department of Pathology, Oregon Health and Science University, Portland, OR.)

most infants, resulting in decreased infection, improved cognitive abilities, ambulation, a lower prevalence of incontinence, and lower mortality.[217-219] To prevent infection, surgical closure of the spinal defect should occur within 48 hours after birth. The involvement of a plastic surgeon should be considered, particularly with wide or complex lesions. CSF diversion may be needed concurrently or after the back has healed. Additional surgical procedures may be required for shunt malfunction or infection, sequelae of Chiari II malformation (apnea, stridor, dysphagia), tethered cord, or syringohydromyelia. Initial evaluation of all neonates with a suspected NTD should include assessment of lower extremity sensory and motor function. Attention should be paid to spontaneous movements, response to touch and pain, and voiding and stooling pattern (particularly urinary retention and dribbling). Babies should be closely evaluated for other malformations, including congenital heart disease unless these have been excluded by prenatal ultrasonography. Monitoring neonates for Chiari symptoms (apnea, stridor, poor feeding) and signs of increased intracranial pressure is also required. The overall approach should be multidisciplinary, with the involvement of the neurosurgery, urology, and physical therapy departments at a minimum. Long-term care of the child with MMC also requires symptom management for neurogenic bladder and bowel, as well as endocrine, orthopedic, rehabilitative, and neuropsychiatric care.[220] Developmental pediatric, neurologic, or rehabilitation medicine specialists are often the primary point of contact for long-term management, depending on the resources available.

Fetal surgery has become an option for MMC repair before birth. A randomized trial demonstrated that fetal surgery was associated with a lower rate of shunt placement, possibly better motor function, and slightly higher quality of life scores through school age but was also associated with premature delivery and maternal complications.[221–224] In addition, mothers can require prolonged bedrest during the pregnancy and cannot deliver subsequent children vaginally. No improvements in neurogenic bowel/bladder have been observed as compared with those with standard postnatal care.[225] Fetoscopic back closure holds promise for benefiting the fetus while minimizing risks associated with hysterotomy.[226] In light of these positive results under ideal research conditions, some centers promote fetal surgery as the standard of care; however, the American College of Obstetrics and Gynecology recommends an approach that balances the risks and benefits to both the fetus and the mother.[227]

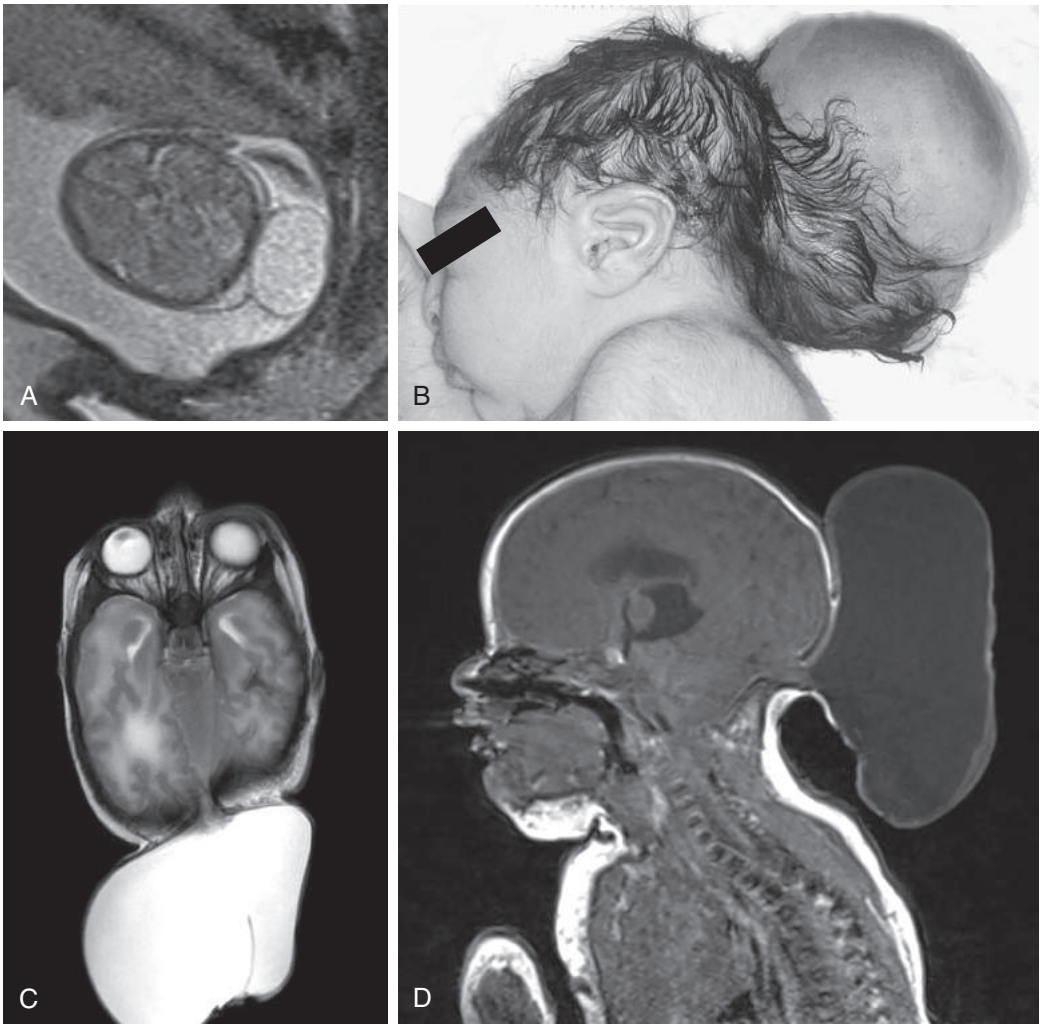
Myeloschisis

Myeloschisis defects also lack overlying vertebrae and skin but differ from MMC lesions in that there is no overlying CSF-filled sac. This results in a continuous CSF leak. Similar to MMC, myeloschisis is associated with neurogenic bladder and bowel, and their mobility depends on the anatomic level of the defect. Closure may be more difficult than with MMC, and, as with MMC, postclosure hydrocephalus may result.[228] Prenatal diagnosis is by elevated AFP level and/or prenatal ultrasonography.

Skin-Covered Neural Tube Defects

Encephalocele

Encephalocele is considered a postneurulation disorder and likely occurs by mechanisms distinct from those for open NTDs.[192] Encephaloceles typically present as cranial skull defects through which brain tissue protrudes. Less severe defects include cranium bifidum, where there is a failure of midline fusion of the skull, and cranial meningoceles that contain meningeal but not neural tissue. In up to 80% of patients, encephaloceles are occipital (Fig. 53.14), with the remainder in the parietal, frontonasal, intranasal, or nasopharyngeal regions. Geographic or ethnic–genetic factors influence the location of the lesion. Frontal encephaloceles are more prevalent in Southeast Asia, while occipital lesions are more common in Western populations.[229] A role for genetic factors is supported by increased risk in patients with chromosomal disorders and a number of recessive syndromes (e.g., Meckel and Joubert syndromes; see earlier).[230] Overall, about half of infants with encephaloceles have other major congenital anomalies, including microcephaly, arrhinencephaly, anophthalmia, cleft lip or palate, craniosynostosis, complex congenital heart disease, and other malformations.[230]



• **Fig. 53.14** Fetal and Postnatal Images of Occipital Encephalocele. (A) Fetal axial magnetic resonance image. (B) Newborn with a large occipital encephalocele. (C) Postnatal T2-weighted axial magnetic resonance image of a patient with an occipital encephalocele. (D) Postnatal T1-weighted sagittal magnetic resonance image of a patient with an occipital encephalocele. (A, B, Courtesy Dr. Marjorie Grafe, Department of Pathology, Oregon Health and Science University, Portland, OR.)

Encephaloceles are also associated with various other CNS defects that influence surgical management and the severity of the outcome. In a series of 129 patients with anterior encephaloceles (including frontoethmoidal, orbital, transethmoidal, transsellar, and interfrontal), 22 had associated hydrocephalus, and ACC was seen in 16.[231] In addition to hydrocephalus, other anomalies include anomalous draining veins and nodular heterotopia.[210,232] The occurrence of lower occipital lobe encephalocele with skull base defects and malformations of the cerebellum and lower brainstem characterizes Chiari type III malformations.

Evaluation of the infant with an encephalocele can be aided by transillumination, skull radiographs, cranial ultrasonography, computed tomography scan, and MRI. Typically, MRI with magnetic resonance angiography and magnetic resonance venography is the study of choice, particularly for surgical planning. Frontonasal encephaloceles pulse or bulge with brief bilateral jugular vein compression, indicating communication with the subarachnoid space. Nasal gliomas, dermoids, and teratomas can all occur in the same region. Intranasal encephalocele should be suspected when an intranasal mass is found in a child with a broad nasal bridge and widely spaced eyes. Some of these children may also present with recurrent meningitis.[210] Basal encephaloceles are not usually diagnosed in infants and can be located in the nasopharynx, sphenoid sinus, or posterior orbit.

In most patients with encephalocele, neurosurgical management is indicated during infancy; however, large lesions or other severe CNS anomalies may preclude intervention, since typically, brain tissue exterior to the skull cannot be salvaged. Early treatment is imperative for those infants at high risk of meningitis caused by lesions that externally communicate and leak CSF. Surgical repair goals include dural closure and improving cosmesis. Complications can include postoperative CSF leakage or pseudomeningocele formation.[233] For patients who are not surgical candidates, a palliative course should be pursued, although it can be quite challenging to decide which interventions are appropriate for a given patient. Ongoing discussions involving the family, subspecialists, and, ideally, dedicated palliative care providers are essential for good care of these patients.

Encephaloceles can be associated with medically intractable seizures, which may be responsive to surgical resection.[234] Survival

and outcome remain difficult to predict because of the variability of presentation and surgical selection bias. One study of a series of children with encephaloceles reported overall mortality of 29% (45% in infants with posterior defects; 0% in infants with anterior defects).[230] Neurologic deficits were severe in 33% of survivors. Mild neurologic deficits were found in 17% of survivors with anterior defects and in 50% of survivors with posterior defects. Developmental prognosis depends on the amount of neural tissue within the defect,[233] and the outcome can be quite favorable in patients with normal brain anatomy.

Meningocele

Meningoceles are skin-covered lesions containing meningeal tissue that typically herniate through the posterior skull or vertebral column.[192] This occurs more commonly in the lumbosacral spine than in the cervical spine, and while the spinal cord does not extend into the defect, it may still be tethered and eventually cause symptoms. Postnatally, meningoceles may be evaluated by spinal ultrasonography or MRI to evaluate the spine for tethering, syringohydromyelia, and diastematomyelia.[235] At centers without extensive ultrasound expertise, MRI is the study of choice, and computed tomography is used mainly to delineate bony landmarks. Chiari II malformation is not typically seen with posterior meningocele.[236]

Occult Spinal Dysraphisms

Occult spinal dysraphisms are closed defects of the distal part of the spinal cord with an intact dermal covering and are a result of defects of caudal neural tube formation (secondary neurulation). In some instances, these defects are truly occult without any overlying abnormalities of the skin and may go undetected until they become symptomatic. Prenatally, they may be detected by fetal imaging as a mass.[237] In most newborns, an occult spinal dysraphism is accompanied by cutaneous stigmata. Such stigmata include abnormal hair tufts, hemangiomas, pigmented spots, skin tags, aplasia cutis congenita, cutaneous dimples or tracts (particularly with CSF leak), or a subcutaneous mass, often apparent because of an asymmetric gluteal cleft.[237–239] Sacral dimples or deep gluteal clefts between the buttocks without other features are rarely associated with spinal dysraphism, while dimples clearly above the gluteal cleft are associated with a higher risk of spinal dysraphism.

Spinal dysraphisms are classified by a clinical, imaging, and developmental approach.[240–242] MRI is indicated for evaluation and diagnosis, when available. For closed dysraphisms, the initial distinction is based on the presence of a subcutaneous mass.[243] Skin-covered lesions without a subcutaneous mass should be examined clinically for skin tags, evidence of spinal cord dysfunction, and anorectal malformations. Defects with subcutaneous masses are classified by the tissue types involved: lipomyelocele (fat and meninges), lipo-MMC (fat, nerve, and meninges), meningocele (meninges only), and terminal myelocystocele, a complex lesion with persistence of the terminal syringohydromyelic cavity.[237]

Other spinal cord lesions associated with occult dysraphism include diastematomyelia–diplomyelia, lipoma, teratoma, and other tumors, dermal sinus with or without dermoid or epidermoid cyst formation, and tethered cord.[203,210] More severe lesions include neurenteric cysts, anterior meningocele, and caudal regression syndrome (dysraphia of sacrum and coccyx, atrophy of muscles and bones of the legs, fusion of spinal nerves and sensory ganglia, or agenesis of the distal part of the spinal cord).[244] As with other NTDs, infants of diabetic mothers are at increased risk of these lesions.[245] Abnormal conus and a thickened filum are usually present, and a symptomatic tethered cord is a common presentation after surgical repair of both skin-covered lesions and MMC.[246] Thickened filum with or without a small amount of fat can be seen frequently on spinal MRI and is usually not significant in the absence of a low-lying conus and neurologic symptoms. Hydrocephalus is rare in patients with occult spinal dysraphisms.

Clinical Features and Diagnosis

Progressive functional impairment can occur as the cord is stretched against the fixed filum,[247] but neurologic impairment rarely presents in the neonatal period.[248] Atypical voiding and stooling (particularly continuous dribbling) should prompt bladder volume measurement and urologic and neurosurgical referral. Delay in walking, disturbed sphincter control, contractures of the feet or legs, and pain in the back or legs may present in infancy or childhood, while gait and sphincter abnormalities, foot deformities, and scoliosis are more common in older patients. Rarely, recurrent meningitis and acute loss of function are seen.

Because prophylactic surgical intervention may prevent deterioration, early diagnosis is a necessity.[242,248,249] In neonates this evaluation may be facilitated by spinal ultrasonography, which permits a dynamic evaluation of lower spinal cord mobility, and MRI to define structural anomalies of the cord. Evaluation should occur as soon as a closed spinal dysraphism is suspected. The timing of surgical management depends on the presence or progression of neurologic symptoms and signs. The care of these children requires a multidisciplinary approach for the management of neurosurgical issues and comorbidities.[250]

Acknowledgment

We especially acknowledge and thank Stephen Back, Lauren Plawner, and Sarah Bauer Huang, who authored the previous two versions of this chapter. Their text was used as the basis of this update/revision.

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Brain Injury in the Preterm Infant

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KEY POINTS

- Intraventricular hemorrhage (IVH) remains a common cause of chronic neurologic morbidity. Despite a gradual decline in the incidence of most grades of IVH, the increased survival of very low birth weight infants has resulted in an increase in the absolute number of infants with IVH.
- Preterm infants are currently at much lower risk of severe white matter injury (WMI), which typically results in focal cystic necrosis and secondary gray matter degeneration. Cystic WMI is commonly associated with cerebral palsy, cortical visual impairment, and a spectrum of cognitive and learning disabilities.
- Preterm infants commonly display less severe diffuse WMI that results primarily in myelination disturbances related to the death of oligodendrocyte progenitors (preOLs). As applied clinically, both diagnostic cranial ultrasonography and magnetic resonance imaging (MRI) appear to underdiagnose the full extent of diffuse WMI.
- Chronic diffuse WMI is accompanied by reduced cerebral white matter growth related to a series of dysmaturation events that result in the regeneration of preOLs that fail to differentiate into myelinating oligodendrocytes. Chronic diffuse WMI is also accompanied by reduced cerebral gray matter growth that appears to be related to widespread disturbances in neuronal maturation rather than loss of developing neurons. These changes are evident in experimental models and in preterm neonates studied with quantitative MRI.
- Diffuse WMI, often reflected in punctate WMI on diagnostic MRI, is linked to a broad spectrum of persistent neurobehavioral disabilities that include impairments in motor and cognitive skills. Punctate WMI is most readily diagnosed on early-preterm MRI scans, becoming harder to detect at term-equivalent age. The burden of punctate WMI, referring to lesion volume *and* location, predicts motor and cognitive dysfunction. The spectrum of neurodevelopmental impairments that follow WMI in the preterm neonate are consistent with the dysmaturation in white matter and cerebral gray matter.
- These recently recognized forms of cerebral gray and white matter dysmaturation present new challenges for diagnosis and suggest new therapeutic strategies to promote the reversal of the processes that cause dysmaturation of neurons and preOLs.

General Principles of Preterm Brain Injury

The preterm brain is susceptible to a broad spectrum of injury that ranges from diffuse nonnecrotic lesions to hemorrhage to severe necrotic tissue destruction. Brain injury is initiated by two major *upstream mechanisms—hypoxia* and *ischemia*—with the potential for infection/inflammation to interact with these triggers and potentiate each other. Because the developing brain is rapidly evolving, the susceptibility to injury is critically related to the timing and severity of the insult. As brain development progresses, distinct populations of cells are selectively more vulnerable to injury, whereas others display greater resistance. Moreover, a wide variety of additional factors may sensitize the brain's susceptibility to injury. The preterm brain may be exposed to a variety of subclinical factors that in isolation may not be injurious but in combination may synergize to potentiate injury. Such factors include nutritional status, systemic illnesses, exposure to glucocorticoids, analgesics, and sedatives, the burden of painful procedures, and other sources of neonatal stress. Equally important to consider is the concept of tolerance in which an antecedent subinjurious insult may reduce the severity of a subsequent one. For example, a low-grade fetal infection or chronic hypoxia in a child with congenital heart disease may be protective against a subsequent more severe hypoxic-ischemic insult.

This chapter addresses three common and frequently overlapping forms of preterm cerebral injury: intraventricular hemorrhage (IVH), white matter injury (WMI), and gray matter injury. The impact of preterm cerebral injury is considerable. Among children born very preterm, even with modern neonatal intensive care, 5% to 10% have major motor deficits, including cerebral palsy related to significant WMI, and more than half have significant cognitive, behavioral, or sensory deficits.[1] These cognitive and neurobehavioral deficits are increasingly observed in the absence of significant motor impairments or cerebral palsy,[2] which has also suggested *primary* involvement of multiple gray matter structures. Gray matter injury was previously attributed to cystic necrotic WMI that led to *secondary* cortical and subcortical gray matter degeneration. Although contemporary cohorts of preterm survivors commonly display less severe injury, these milder forms of injury are associated with both reduced cerebral gray matter growth and reduced cerebral white matter growth.

WMI is tightly linked with brain dysmaturation. The primary mechanism of myelination failure in preterm neonates is dysmaturation, a disrupted cellular response whereby pre-oligodendrocytes fail to differentiate, that is, maturation arrest.[3] This is often accompanied by *neuronal* dysmaturation, a process that may also be initiated by hypoxia alone.[4–9] There is emerging evidence that neonatal brain dysmaturation is the most important predictor of neurodevelopmental impairments in preterm neonates in the first years of life.[10,11] Dysmaturation leads to impairment in cerebral growth that arises from complex and disparate responses of neurons and glia that fail to fully mature during a critical window in the development of neural circuitry.[8] Thus, preterm children are at increased risk for a broad range of cognitive impairments,

including reduced IQ, processing deficits in attention and executive functions (e.g., processing speed, working memory), and challenges with information processing and language that impact educational performance and behavior[12,13] and persist through childhood.[14] Adult survivors of prematurity have increased rates of cognitive, behavioral, and psychological problems that correlate with altered markers of brain development on MRI.[15–18] Hence, these highly prevalent neurocognitive impairments that span early development to adulthood support widely distributed disturbances in brain growth and connectivity that involve both gray matter and white matter.[19]

Intraventricular and Periventricular Hemorrhage

Pathogenesis

IVH is a common injury in the preterm brain, originating in the subependymal germinal matrix, a highly vascularized region with highly active angiogenesis during this period in development.[20] Cortical neuronal and glial progenitors develop from the germinal matrix and adjacent ventricular germinal zone during the late second and early third trimesters. The consequences of IVH are thus potentially several fold. IVH triggers degeneration of progenitor populations that are actively establishing cerebral connectivity.[21] Hemorrhage disrupts the blood-brain barrier exposing the preterm brain to toxic and pro-inflammatory substances, which may potentiate the risk for further hemorrhage by disrupting pathways that stabilize the vasculature.[22,23] Moreover, disruption of the germinal matrix disrupts neonatal neurogenesis, with a particular impact on later maturing populations of neurons including interneurons, which are key to the regulation of synaptic activity.[24,25] Severe IVH with parenchymal white matter extension additionally targets vulnerable glial progenitors leading to disturbances in myelination.

Involution of the germinal matrix occurs with advancing gestation. The subependymal germinal matrix derives its arterial supply from the anterior and middle cerebral arteries as well as the anterior choroidal artery. These arteries feed an elaborate capillary network of thin-walled vessels that is continuous with a deep venous system that terminates in the vein of Galen. The terminal, choroidal, and thalamostriate veins course anteriorly to form the internal cerebral vein, which courses posteriorly to join the vein of Galen. Several of these veins make a pronounced U-shaped turn as they join with the internal cerebral vein. This turn may influence venous drainage and pressure in the germinal matrix. Visualization of subependymal venous anatomy using susceptibility-weighted venography recently identified five anatomical variants that differ from the classical anatomical pattern and were associated with increased risk for IVH.[26]

The predisposition of the preterm infant to IVH is due to several hemodynamic factors.[27] A pressure-passive state exists because of the lack of autoregulation of blood flow in the cerebral arterioles of the preterm brain. In the presence of a highly vascularized subependymal germinal matrix, the risk of IVH is enhanced by the lack of a supporting basement membrane for the germinal matrix blood vessels, an increased amount of fibrinolytic activity, and a decrease in extravascular tissue pressure in the first few days of extrauterine life. Thus, IVH may occur in the setting of elevated venous pressure or an increase in fluctuations in cerebral blood flow (CBF) velocity triggered by factors that include respiratory distress, pneumothorax, asphyxia, myocardial failure, patent ductus arteriosus, arterial hypotension, hypothermia, and hyperosmolarity.[28] Fluctuating pressure passivity is common in preterm infants and may be associated with and precede IVH.[29–31]

• BOX 54.1 Pathogenic Factors Leading to Intraventricular Hemorrhage

Increase in cerebral blood flow
Fluctuation in cerebral blood flow
Increase in cerebral venous pressure
Endothelial injury
Vulnerable germinal matrix capillaries
Coagulation disturbances
Increased fibrinolysis

IVH has been produced experimentally when hypotension is followed by reperfusion.[32,33] These studies support the finding that IVH is more likely when an early period of prolonged hypotension is followed by an increase in blood pressure.[34,35] Isolated hypertension associated with seizures, intubation, and suctioning also predisposes the brain to IVH.[36,37] Even gavage feeding and surfactant administration can lead to changes in cerebral hemodynamics, as measured by near-infrared spectroscopy, that lead to IVH.[38–40] Box 54.1 lists key factors that may interact to produce IVH.

Cellular injury in infants with grade III or grade IV (periventricular hemorrhagic infarction [PVHI]) IVH may occur from antecedent ischemic injury, a decrease in cerebral blood flow, increased intracranial pressure, or vasospasm. More severe IVH is associated with cerebral WMI, cerebellar injury, and less frequently, pontine neuronal necrosis.[41] In this setting, venous infarction leads to neuronal as well as glial death. In a contemporary multi-center cohort, grade II-III intraventricular hemorrhage was associated with a higher risk of punctate WMI, suggesting links to the less severe spectrum of brain injury.[42]

The contribution of coagulation disorders and genetic factors to the pathogenesis of fetal or neonatal IVH remains unresolved. Fetal IVH occurs infrequently and in association with a wide variety of conditions, which include fetal bleeding disorders, anticoagulation, and conditions associated with thrombocytopenia including alloimmunization and hypoxemia.[43] Fetal IVH is also associated with apparent remote perinatal strokes in term newborns who present with hemiparesis related to periventricular venous infarction.[44] Mechanisms for coagulopathies in neonatal IVH may include thrombocytopenia related to platelet consumption or destruction in the setting of sepsis or pro-inflammatory states.[45] Roles for factor V Leiden or prothrombin variants in IVH also are of unclear significance.[46] Currently under study are many potential IVH risk genes including those involved in collagen-mediated vascular stabilization (e.g., COL4A1), nitric oxide-mediated vasomotor function (e.g., NOS3, encoding endothelial nitric oxide synthase), and vasoconstriction-mediated cerebral autoregulation (e.g., END1, encoding endothelin 1).[47]

Site, Incidence, and Timing of Hemorrhage

In preterm infants, germinal matrix hemorrhage is most commonly seen at the junction of the terminal, choroidal, and thalamostriate veins in the germinal matrix overlying the body of the

caudate nucleus at the level of the foramen of Monro. Parenchymal hemorrhage occurs most commonly in the frontoparietal regions, where it appears not to be an extension of IVH but rather a separate process—a hemorrhagic infarction. The hemorrhage is more often unilateral or, in less than a third of cases, asymmetrically bilateral.

The incidence and severity of IVH increase with decreasing gestation and peak at the limit of viability.[48,49] IVH occurs infrequently in term newborns, but often in association with birth-related complications.[50] The overall incidence of grade III IVH and PVHI in VLBW infants varies among centers and generally ranges from 10% to 20% for the majority of population-based studies worldwide.[48,49,51] Recent data from over 50,000 VLBW infants found an overall incidence of IVH of 24.6% and 8.1% for grade III IVH and PVHI.[52] Importantly, in very preterm neonates, even low-grade IVH is associated with an increased risk of WMI.[42] Although there has been a decline in the incidence of most grades of IVH, the increased survival of VLBW infants has resulted in an increase in the absolute number of infants with IVH who are at risk for adverse neurodevelopmental outcomes,[53,54] which include cerebral palsy, developmental delay, hydrocephalus, and epilepsy.

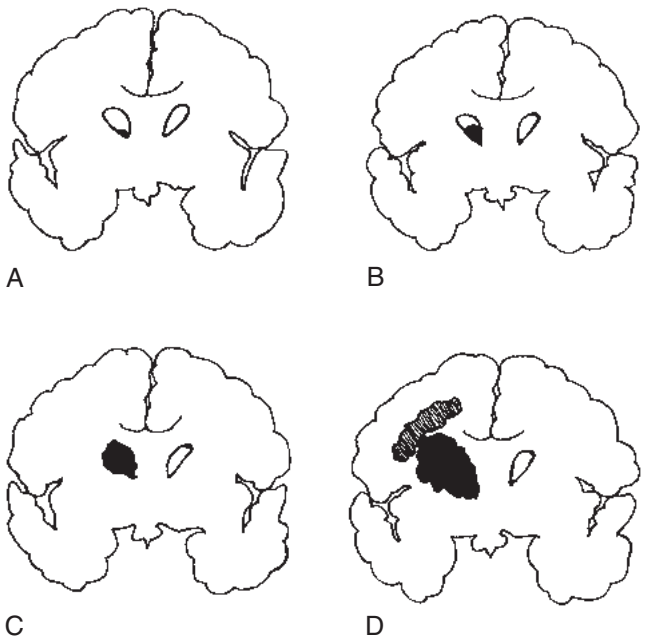
The high-risk period for IVH is the first 3 or 4 days of life. Hemorrhage is rarely seen at birth, although it has been reported as early as the first hour of life.[55] Around half of neonatal hemorrhages occur by the sixth hour of life, and only about a third occur after the first 24 hours of life.[56] Less than 5% of newborns develop IVH after the fourth or fifth day of life with a small percentage occurring by 7 to 10 days of life.[28] Both early and later onset of hemorrhage may occur because of systemic hypoperfusion that results in disturbances in cerebral blood flow.[57]

Clinical Presentation

The clinical presentation of IVH in the newborn depends on the extent of the hemorrhage.[58] It may range from *asymptomatic* to a sudden and catastrophic deterioration that manifests itself with neurologic signs such as stupor or coma, seizures, decerebrate posturing, or apnea. A tense fontanel together with a sudden drop in hematocrit, hyperglycemia, hyperkalemia, hypotension, or bradycardia may herald an IVH. Inappropriate secretion of antidiuretic hormone may occur. The more common presentation, however, is that of a gradual clinical deterioration with an altered level of consciousness, hypotonia, abnormal extremity, or eye movements. In 25% to 50% of cases, clinical signs are lacking and IVH is identified on routine cranial ultrasound screening.[59]

Grading of Intraventricular Hemorrhage

Ultrasound examination is a reliable and sensitive bedside technique for the evaluation of IVH. Papile et al.[60] adapted the standard grading system originally applied to computed tomographic images of IVH to ultrasound images. They classified IVH into four grades of severity related to the location and extent of the hemorrhage (Fig. 54.1, Table 54.1). This classification system was previously used widely for outcome studies. Currently, the more widely used grading system is that proposed by Volpe et al.,[41] which relies on the cranial ultrasound examination to define the extent of ventricular hemorrhage. Because parenchymal involvement is a distinct process, it is not included in the continuous grading of IVH severity (Table 54.1). PVHI appears to arise from venous infarction of the periventricular white matter rather than from a direct extension of the IVH



• **Fig. 54.1** The Progressive Grades of Intraventricular Hemorrhage from Mildest to Most Severe. (A) Grade I hemorrhage involves less than 10% of the ventricular volume of the lateral ventricles. (B) Grade II involves 10% to 50% of the ventricular volume. (C) Grade III involves more than 50% of the ventricular volume and is frequently associated with ventricular dilatation. (D) Hemorrhage is associated with parenchymal infarction.

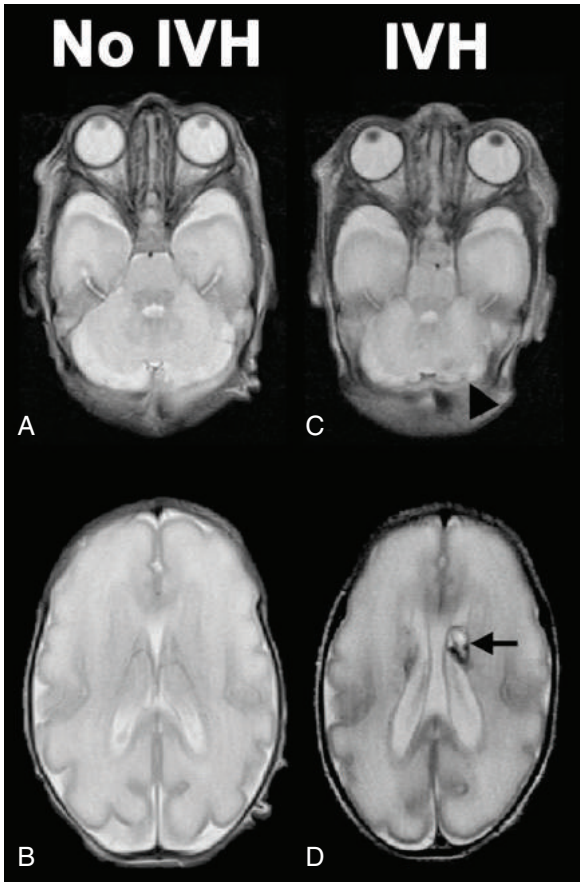
TABLE 54.1 Grading of Intraventricular Hemorrhage by Cranial Ultrasound

PAPILE GRADING SYSTEM[60]		VOLPE GRADING SYSTEM[41]	
Grade	**Findings**	**Grade**	**Findings**
I	Subependymal hemorrhage with minimal or no IVH	I	Germinal matrix hemorrhage IVH <10% of the ventricular volume
II	Definite IVH without distention of the ventricles	II	IVH 10%–50% of the ventricular volume
III	Enlargement of the ventricles secondary to distention with blood	III	IVH >50% of the ventricular volume, usually with distention of lateral ventricle
IV	Extension of the hemorrhage into the parenchyma along with IVH and enlargement	Periventricular hemorrhagic infarction (PVHI)	Periventricular echodensity signifying parenchymal lesion

IVH, Intraventricular hemorrhage.

into the parenchyma.[41] Hence the presence of intracerebral hemorrhage or parenchymal lesions is described separately and is not designated as grade IV. Intraparenchymal hemorrhage is followed in 1 to 8 weeks by tissue destruction and the formation of a porencephalic cyst. Serial ultrasound examinations are especially important in linking the severity of IVH with neurodevelopmental outcomes.[61–63]

In addition to various forms of cerebral WMI, IVH may cause graded injury to the cerebellum (Fig. 54.2) that manifests as magnetic resonance imaging (MRI) defined changes in microstructure[64] and reductions in cerebellar growth.[65] This reduced growth appears to be related to a large population of proliferative external granule cells that are the progenitors that generate the internal granule cell layer, and which account for most of the cells in the human cerebellum. These cerebellar progenitors may be particularly vulnerable to the toxicity of IVH-derived blood products, which are detected by MRI as hemosiderin deposition on the surface of the brainstem and cerebellum.[66,67] The heightened vulnerability of cerebellar progenitors is consistent with serial neuroimaging studies of human preterm survivors that demonstrated disrupted cerebellar growth in response to postnatal glucocorticoid exposure.[68] Furthermore, with the increasing use of MRI with "blood-sensitive" sequences such as susceptibility weighted images, primary hemorrhage in the cerebellar germinal matrix is increasingly recognized. Neurodevelopmental assessments at 4.5 years show that the size and location of cerebellar hemorrhages are associated with dose-dependent disturbances in motor and visuomotor function and increased externalizing behaviors.[69] Given this, the volumetric quantification and localization of a cerebellar hemorrhage, even when small, may allow for targeted rehabilitation interventions.



• **Fig. 54.2** Intraventricular Hemorrhage is Associated with Impaired Growth of the Cerebellum. (A, B) The normal appearance of the brain and cerebellum in a preterm neonate born at 28 weeks' gestational age who was imaged by magnetic resonance imaging (MRI) at 30.3 weeks. (C, D) Smaller cerebellum *(arrowhead)* associated with intraventricular hemorrhage (IVH) *(arrow)* in a preterm neonate born at 28 weeks' gestational age who was imaged by MRI at 30.1 weeks.

Outcome and Prognosis

Isolated grade I and II hemorrhages generally resolve without evolution. Grade III hemorrhages evolve over a period of 1 to 3 weeks and may produce a fibrotic reaction that obliterates the subarachnoid space with subsequent ventricular dilatation and hydrocephalus. Clinical symptoms of progressive hydrocephalus, such as rapid head growth, a full anterior fontanel, or separation of cranial sutures, often appear days or weeks after the onset of ventricular dilatation. The delayed onset of clinical symptoms is related to the presence of a large subarachnoid space as well as the paucity of myelin in preterm infants.

Outcomes differ between studies, but in general, mortality rates are not significantly increased in infants with a grade I or grade II hemorrhage. Grade III hemorrhages and PVHI are associated with increased mortality. In 15% of cases of IVH, areas of PVHI occur that are associated with mortality rates that approach 50% greater than those in infants without IVH.[54,70,71]

Several studies have focused on the relationship between IVH and subsequent neurodevelopmental outcomes. In extremely low birth weight (ELBW, <1000 g) survivors, even grade I and grade II IVH have been associated with a modest increase in morbidity relative to those infants without IVH in some,[72–74] but not all studies.[75,76] However, given the considerable morbidity associated with extreme prematurity, it can be challenging to independently assess the increased risk of neurologic complications associated with lower grades of IVH. One study of nearly 1500 ELBW survivors found that grade I and grade II IVH were associated with a 7% greater rate of poor neurodevelopmental outcomes after the exclusion of other cranial ultrasound abnormalities such as WMI or porencephaly.[54] Long-term outcome studies at 8 years of age found that even low-grade IVH was associated with higher rates of CP compared to infants without IVH.[77] The prognosis for infants with grade I and grade II IVH thus may not be benign if there is a comorbid brain injury. This increased risk of abnormal outcome may be related to underrecognized WMI or gray matter injury.[42,78,79] Follow-up studies have shown that the degree of IVH at birth and the presence of ventriculomegaly are predictors of neurologic status at a corrected age of 24 months. Neurologic sequelae occur in up to 35% of infants with grade III IVH and 55% with grade IV IVH.[70] The persistence of ventriculomegaly in grade III or grade IV IVH is associated with a greater risk of more severe neurologic sequelae that include seizures, cerebral palsy, and severe impairment of vision or hearing.[80] Posthemorrhagic ventricular dilatation (PHVD) is a common sequela of severe IVH and of PVHI and may precede posthemorrhagic hydrocephalus (PHH). Variation in management in PHVD likely contributes to the wide variance in outcome following PHVD. The management of PHVD and its relationship with the outcome is addressed later in this chapter.

Prevention

Prevention of preterm birth is the most effective method of reducing the incidence of IVH. In the event of preterm labor, it is advisable that the neonate be born at a center specializing in high-risk deliveries. The risk of IVH is higher in neonates who are outborn and transported after birth.[81,82] Prenatal administration of steroids is associated with a decreased risk of mortality, IVH, and cerebral palsy.[49,83–85] Dexamethasone and betamethasone appear to be equally effective to reduce mortality, neurodevelopmental disabilities, and IVH.[86,87] Prenatal administration of magnesium sulfate reduces the combined risk of fetal/infant death and CP.[88,89] However, there is no clear benefit of magnesium sulfate to reduce the risk for IVH.[90,91]

Neonatal delivery room or resuscitation practices may be associated with increased IVH risk. At some gestational ages, VLBW infants were at risk for worse outcomes including higher rates of IVH after cardiopulmonary resuscitation.[49,92,93] Delivery of high tidal volumes during positive pressure ventilation carries an increased risk for severe IVH.[94] Umbilical cord milking was also associated with a higher rate of severe grades of IVH when compared with delayed umbilical cord clamping.[95] A relatively small trial found reduced rates of IVH with delayed cord clamping, potentially mediated by improved cerebral autoregulation.[96]

Optimization of neonatal nursing care to avoid abrupt fluctuations in systemic pressure also appear to decrease the risk of IVH.[97,98] Optimal neurologic intensive care of the preterm infant includes reduced exposure to hyperventilation, hypocarbia, or hypoxemia, as well as maintenance of adequate mean arterial pressure (MAP).[99–101] Abrupt elevations in CBF may be precipitated by excessive handling or tracheal suctioning. Other risks for IVH include pneumothorax, acidosis, rapid infusions of sodium bicarbonate, and volume expanders.

Because of the increased risk of adverse neurodevelopmental sequelae in infants with IVH, several clinical trials have been performed to evaluate the role of prolonged neuromuscular paralysis in preterm infants. A meta-analysis of five trials concluded that although neuromuscular paralysis with pancuronium may decrease the risk of IVH and pneumothorax in asynchronously breathing infants, its routine use was not recommended because of concerns about safety and long-term pulmonary and neurologic effects.[102]

A low MAP or increased fluctuations of blood pressure have been associated with an increased risk of IVH.[103] Although close monitoring of the MAP is recommended, there is no evidence that pharmacologic manipulation of systemic blood pressure (e.g., with pressors, steroids, or volume expanders) to achieve a set goal (e.g., MAP >30 mmHg) alters the incidence of IVH or improves neonatal outcome. In early studies, phenobarbital administration appeared to be beneficial by preventing fluctuations in blood pressure.[104,105] However, a subsequent multicenter trial of prenatal administration of phenobarbital (10 mg/kg) to 110 women provided no reduction in the postnatal frequency of IVH in preterm infants between 24 and 33 weeks' gestational age.[106] A larger trial confirmed these findings, and long-term follow-up at 18 to 22 months found no difference in neurodevelopmental outcomes.[107,108] A meta-analysis of 10 trials of postnatal administration of phenobarbital showed no difference in the rates of severe IVH or ventriculomegaly.[109] Therefore, postnatal administration of phenobarbital does not appear to be beneficial for the prevention of IVH.

Pharmacologic doses of vitamin E, an antioxidant, were associated with a reduction in the incidence of IVH in low birth weight infants when given intramuscularly.[110] However, after reports of the association of such large doses of vitamin E with sepsis and necrotizing enterocolitis, its use for prevention of IVH was curtailed.[111,112]

Indomethacin, a prostaglandin synthase inhibitor, was originally found to decrease the incidence of IVH in infants weighing less than 1250 g.[113] However, later studies on the long-term effects of indomethacin prophylaxis in ELBW infants showed no increase in the rate of survival without neurosensory impairment, despite a reduction in the rate of severe IVH.[114–116] A meta-analysis of 19 trials involving 2872 infants found an increased risk of oliguria, which was not associated with major renal impairment. There was no difference in the incidence of necrotizing enterocolitis, a modest reduction in the number of infants with severe IVH, and no difference in long-term neurosensory impairments.[117] Prenatal administration of indomethacin, as a tocolytic to arrest preterm labor, is associated with increased risks of severe IVH and WMI.[118] However, recent evidence suggests the decreased risk of WMI with prolonged postnatal indomethacin exposure in the preterm neonate.[119,120]

Prenatal administration of steroids[121–126] and surfactant replacement therapy[127,128] decrease the incidence of IVH as well as neonatal mortality in low-birth-weight infants. However, an increase in the incidence of IVH may occur with surfactant administration as a result of the mode of instillation[129–131] and a drop in $Paco_2$ with improved ventilation. Since a significant drop in MAP and CBF volume can occur during surfactant administration, attention should be paid to the speed and volume of instillation.

Mechanical ventilation may be associated with an increased risk of IVH.[132] Several studies evaluated the early use of high-frequency ventilation versus conventional ventilation for infants with respiratory distress syndrome. These studies found either an increased risk of IVH with high-frequency oscillatory ventilation[133–135] or no increase in IVH risk.[136,137] The results of a recent meta-analysis of 19 studies and 4096 infants found no increased risk of severe IVH or WMI in preterm infants treated with high-frequency versus conventional ventilation.[132]

It is important to avoid both hypocarbia (PCO_2 <30 mmHg) and hypercarbia (PCO_2 >55 mmHg) because of their significant effects on CBF.[138–143] Avoiding low PCO_2 has been shown to be neuroprotective in animal studies.[144,145] Hypocarbia is associated with hypotension and an increased risk of IVH and WMI.[146,147] Hypercarbia promotes increased cerebral blood flow, which in the presence of other therapies aimed at increasing blood pressure increases the risk of IVH.[147–149]

Free radicals and iron have been shown to be damaging to oligodendrocyte progenitors (preOLs) in both cell culture and animal studies,[150,151] and iron-chelating agents have been shown to be neuroprotective in animal models of IVH.[152,153] However, clinical studies are lacking to evaluate the benefit of antioxidants such as iron chelators in the setting of IVH. As 80% of iron transfer occurs in the third trimester, it is also important to emphasize that iron sufficiency is required for normal brain development, and so withholding or chelating iron may be detrimental.

Management

Because of the high risk of IVH and ischemic WMI in the early perinatal period, short-term management should be focused on the elimination of factors shown to promote excessive fluctuations

in CBF[103] and the prevention of nosocomial infections.[154,155] Clinical practices to limit fluctuations in CBF include delayed cord clamping, maintenance of blood gases and metabolic status within a normal range, avoidance of excessive suctioning and handling, and detection and treatment of seizures. Systemic blood pressure should be maintained with particular attention to the rate of administration of fluids.

Infants with birth weights of less than 1500 g or a gestational age of less than 32 weeks should undergo a screening ultrasound examination to detect IVH in the perinatal period when the risk of IVH is the highest.[156] An initial routine cranial ultrasound scan is optimally done within 4 to 7 days of age, as an ultrasound scan on the fourth postnatal day detects 90% of lesions.[157] If normal, a repeat scan at 4 to 6 weeks of age improves detection of WMI and at term-equivalent age may inform risk for neurodevelopmental disabilities.[158,159] If IVH is detected, a repeat ultrasound examination after 5 to 7 days may be necessary to establish the IVH grade because the hemorrhage may extend during the next several days. Serial imaging is often necessary because about half of infants with ventricular enlargement from IVH develop rapidly progressive ventricular dilatation during the next 4 to 8 weeks. In the setting of severe IVH, regular screening ultrasound examinations every 1 to 2 weeks to reevaluate the neonate for progressive ventricular dilation and WMI should complement measurement of head circumference, examination of the fontanel, and assessment of clinical status until 36 weeks of life.[160] Clinical recommendations for the surveillance and management of PHVD have recently been revised.[161] The decision to continue intensive care support is partly informed by the severity of the IVH and associated brain injury as assessed by a combination of cranial ultrasound and MRI examinations. MRI is the optimal imaging modality to detect smaller cerebellar hemorrhages and potentially more subtle forms of noncystic WMI.[160]

There is a lack of broad consensus for evidence-based guidelines on the optimal clinical management of PHVD,[162] though more recent evidence favors earlier intervention. Serial lumbar punctures or ventricular taps were previously used in the nonsurgical management of PHVD to temporarily prevent the progression of hydrocephalus and potentially reduce the need for shunt placement. A systematic review of four controlled trials found that the early removal of debris from liquefied blood clots did not reduce mortality, developmental disabilities, or the risk of permanent shunt dependence, and was associated with an increased risk of central nervous system (CNS) infection.[163,164] However, other studies suggest that increasing ventricular size predicts more adverse neurodevelopmental outcomes and that earlier intervention with lumbar punctures and/or reservoirs may reduce the eventual need for ventriculoperitoneal (VP) shunts.[165,166] In preterm infants with PHVD, those with early intervention, had outcomes indistinguishable from those without intervention, even when eventually requiring a shunt. Remarkably, outcomes in this group were all within the normal range.[167] In infants managed with a late approach to intervention, the need for intervention predicted worse outcomes.[167] In a post hoc analysis of a randomized control trial examining the treatment threshold for lumbar puncture to manage PHVD, earlier intervention was associated with lower odds of death or severe neurodevelopmental disability in preterm infants with progressive PHVD.[168] The accumulating evidence suggests that earlier intervention approaches for PHVD support better neurodevelopmental outcomes and outweigh potential risks.[161]

A randomized controlled trial of the combined use of furosemide and acetazolamide in 177 infants with PHVD was ineffective in reducing VP shunt placement and was associated with an increased risk of adverse neurologic outcomes.[169] Several studies also found no benefit of early intraventricular delivery of a fibrinolytic agent (tissue plasminogen activator, urokinase, or streptokinase) to reduce complications associated with PHH.[163,170] A multicenter randomized clinical trial of 77 patients compared drainage, irrigation, and fibrinolytic therapy (DRIFT) with the removal of excess cerebrospinal fluid (CSF) via a reservoir. DRIFT reduced severe cognitive disability in survivors at 2-year follow-up as well as overall death or severe disability, but was associated with a much greater increase in secondary intraventricular bleeding.[171] At 10-year follow-up, the benefit of DRIFT to improve cognition persisted, supporting the need for a larger more balanced trial.[172]

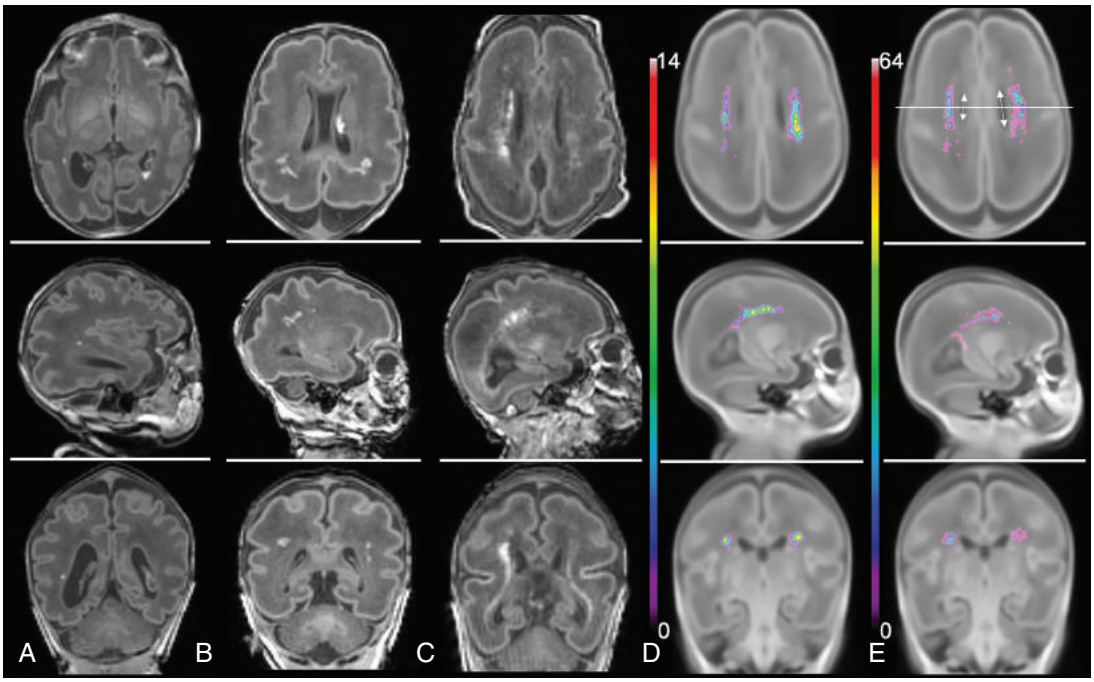
Definitive management by the early placement of a VP shunt that diverts cerebrospinal fluid from the lateral ventricles to the peritoneal cavity in a preterm infant weighing less than 2.5 kg is problematic because of the risk of skin breakdown, shunt obstruction, and infection. Placement of a ventricular access device (e.g., Ommaya/Rickham reservoir), an external ventricular drain, or a ventriculosubgaleal shunt is a temporary measure for the early management of PHVD. A small retrospective historical cohort study found that ventriculosubgaleal shunts compared with ventricular access devices may reduce the need for daily CSF aspiration.[173] However, both approaches had similar complication rates, and similar numbers of infants ultimately required VP shunt placement.[174] Either placement of VP shunts or endoscopic third ventriculostomy are long-term treatment options for PHVD or PHH, and both have similar clinical outcomes.[175]

White Matter Injury

Spectrum of White Matter Injury

The spectrum of WMI includes three major identifiable forms of disease: focal cystic necrosis, punctate WMI with focal microscopic necrosis, and diffuse nonnecrotic lesions. Focal cystic necrosis or cystic-PVL is characterized by necrotic lesions greater than 1 mm in diameter with the corresponding loss of all critical tissue elements, including glia, axons, vasculature, and neural progenitors.[176] Both ultrasound and MRI imaging modalities can adequately detect focal cystic necrosis, which characteristically occurs within the deep periventricular white matter.[41,177] Cysts form in the WM two to six weeks after injury or birth and can resolve after several additional weeks.[159] These cysts can be late in onset and may be missed if imaging is performed only in the first weeks of life or soon after an injurious episode.[157,178] Large cystic necrotic lesions greater than about 1 mm in diameter are the most severe, but their incidence has decreased markedly in recent years (Fig. 54.3C). In several series, focal cystic lesions were detected by MRI in less than 5% of cases.[179–185] In fact, the incidence of all forms of necrotic WMI were found to have decreased by approximately 10-fold in contemporary cohorts of autopsy cases relative to retrospective cases from earlier decades.[186]

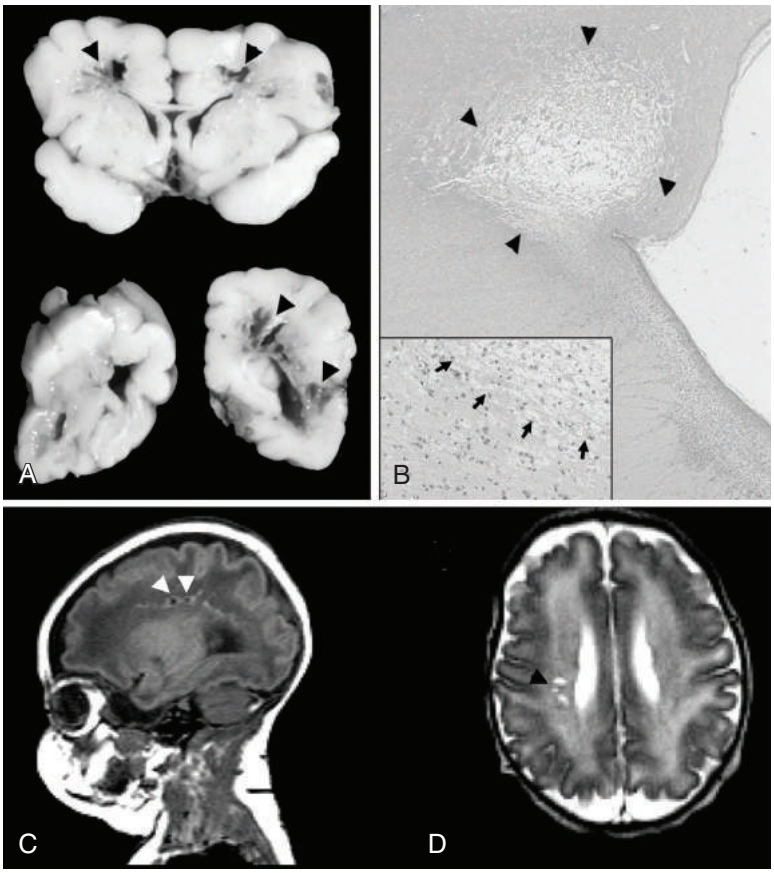
Although the incidence of large necrotic lesions has decreased markedly, discrete small foci of microscopic necrosis (microcysts) less than 1 mm in diameter appear to be much more common.[187] Similar to focal cystic necrosis, microcysts are destructive lesions



• **Fig. 54.3** Spectrum of White Matter Injury (WMI) and Relationship with Neurodevelopmental Outcome. Axial *(top)*, sagittal *(middle)*, and coronal *(bottom)* early-life T1-weighted MRI images from three very preterm neonates demonstrating a range of WMI. (Column A) *Minimal WMI* reflected in a punctate focus of T1-hyperintensity in the right occipital lobe. Note is made of left sided grade 2 IVH with T1 hyperintensity in the occipital horn of the lateral ventricle. (Column B) *Moderate WMI* reflected in multiple confluent areas of T1-hyperintensity bilaterally, involving the parieto-occipital lobes predominantly. On the axial image, note is made of prior Grade 2 IVH with T1 hyperintensity in the body of the left lateral ventricle. (Column C) *Severe WMI* reflected by confluent areas of T1 hyperintensity bilaterally, involving all lobes, including areas of T1 hypointensity reflecting cystic lesions. (Column D) *Cumulative WMI* map: Probabilistic WMI map of 58 very preterm neonates overlaid on a T1-weighted early preterm brain template. Punctate WMI that occurred at a homologous region in two or more very preterm neonates is displayed. The color bar on the left indicates the color coding of the WMI summation. The maximum value on the map is 14 on the brain template. (Column E) *Odds ratio (OR) map of WMI for adverse motor outcome* at 18 months on the brain template. OR maps of punctate WMI for motor outcomes overlaid on the T1-weighted neonatal brain template. The maximum OR value on the motor OR maps is 64. Note that lesion location is an important predictor of motor outcome. The mid-ventricle line from the simple imaging prediction rule based on these maps is also shown here, with lesions anterior to this line being predictive of adverse motor outcomes. (Images courtesy of Dr. Jessie Guo, The Hospital for Sick Children, Toronto, ON; modified from Guo T, Duerden EG, Adams E, et al. Quantitative assessment of white matter injury in preterm neonates: association with outcomes. *Neurology*. 2017;88(7):614–622 and Cayam-Rand D, Guo T, Grunau RE, et al. Predicting developmental outcomes in preterm infants: a simple white matter injury imaging rule. *Neurology*. 2019;93[13]:e1231–e1240.)

enriched in cellular debris, degenerating axons, and phagocytic macrophages (Fig. 54.4A–C). Because of their small size, these areas evolve into focal collections of astrocytes and macrophages but may not form persistent fluid-filled cysts.[186,188] Microcysts are typically not detected on clinical MRI scans at field strengths of 3 Tesla (T) but can be detected experimentally at higher magnetic field strengths of 12T (Fig. 54.4D).[188] In a study of human archival and contemporary autopsy cases, microcysts were observed in at least 30% of cases, but they constituted approximately 1% to 5% of the total burden of WMI.[186] Hence, although microscopic necrosis occurs with high incidence, the burden is typically low. It should nevertheless be emphasized that the clinical significance of these small necrotic lesions remains an important but clinically inaccessible question since microcysts are not readily detected by clinical MRI. It is thus possible that microcysts may be clinically silent or a significant contributor to motor or cognitive disabilities, depending on the extent to which they localize to functionally important regions of white matter.

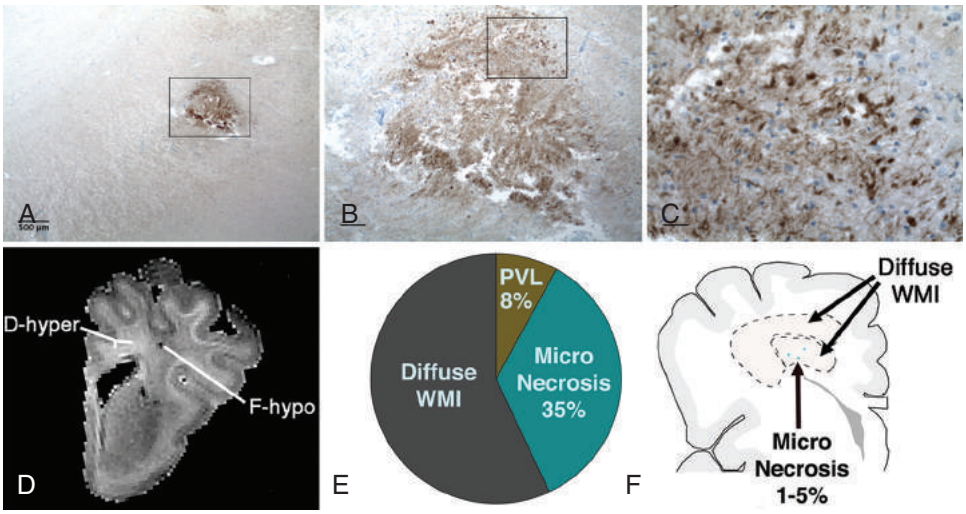
Punctate WMI is an increasingly recognized pattern of injury in preterm survivors. The pathological correlate of punctate WMI is not clearly defined and may include a pathological spectrum between focal cystic and microscopic necrosis.[41] Lesions characterized by small (1 to a few mm) discrete necrotic foci that do not evolve into true cysts, but rather result in focal gliotic changes have been described in an experimental model of moderate preterm brain injury.[188] In contrast to microscopic necrosis, these lesions are detectable at clinical MRI field strength as punctate areas of hyperintensity on T1-weighted MRI images (Fig. 54.3A and B).[189,190] Ultrasound, however, is not of adequate sensitivity to detect these lesions. Early-life MRI is the preferred method and timing to detect the maximal extent of this WMI pattern.[11,191] The distribution of punctate lesions on MRI varies



• **Fig. 54.4** Severe White Matter Injury Results in Focal or Diffuse Tissue Destruction (Periventricular Leukomalacia). (A) This autopsy brain, from an infant who died of complications of prematurity, shows large foci of severe cystic necrosis *(arrowheads)* in frontal *(upper specimen)* and parietal *(lower specimens)* periventricular white matter. (B) Histologic analysis of the frontal lesion (stained with hematoxylin and eosin) shows a large focus of necrosis *(arrowheads)* adjacent to the lateral ventricle. The *inset* shows a high-power detail of the edge of the lesion *(arrows)*, where marked rarefaction of the tissue can be appreciated adjacent to a region of gliosis at the *lower left*. (C) Appearance of cystic necrotic white matter injury on magnetic resonance imaging. Images from a preterm infant born at 33 weeks' gestational age and scanned at 5 weeks of age (38 weeks' adjusted gestational age). (D) Small areas of cavitation *(arrowhead)* are appreciated as hypointensity on the sagittal T_1-weighted image and as hyperintensity on the axial T_2-weighted image. (Courtesy Dr. Marjorie Grafe, Oregon Health & Science University, Portland, OR, and Dr. Ken Poskitt, Children's and Women's Hospital, University of British Columbia, Vancouver, BC.)

along a characteristic topology.[11] Lesions clustering within WM areas are suspicious for an ischemic parenchymal injury, while those with a linear pattern are more likely to have associated susceptibility changes, and reflect microhemorrhage or venous congestion that may not have a parenchymal injury component.[192] There is, however, unexplained variability in the nature of lesions detected at different centers, which may reflect differences in management, clinical acuity, or modes of detection. In a single center, the incidence of punctate WMI appears to be decreasing over time; in this cohort prolonged exposure to indomethacin predicted reduced WMI.[120]

Diffuse WMI is the most frequently observed form of injury in contemporary cohorts of preterm newborns.[186] Diffuse WMI is much more widely distributed than previously appreciated and comprises activated astrocytes and microglia that can occur independently from or extend widely beyond foci of necrosis (Fig. 54.4E and F). In contrast to necrotic injury, diffuse WMI is defined by selective degeneration of preOLs, whereas axons are mostly spared except in necrotic foci.[193,194] Human preOLs are a population of late oligodendrocyte progenitors, which are the precursors of all the myelinating cells of the CNS. They are particularly susceptible to significant oxidative damage of a magnitude seen with hypoxia-ischemia (HI) in the clinical context.[195] The susceptibility to preterm WMI peaks at approximately 23 to 32 weeks' postconceptional age and coincides with a developmental window when preOLs predominate in human cerebral white matter. Paradoxically, it was shown experimentally that ischemia is necessary but not sufficient to cause WMI. Even under conditions of moderately severe ischemia, some regions of white matter may be relatively spared. The regions of particular vulnerability to WMI are defined by both the timing of the appearance and the distribution of susceptible preOLs.[196] Although cranial ultrasound examination is the preferred bedside imaging technique for diagnosing necrotic WMI, it has limited sensitivity for diagnosing diffuse WMI.[160] MRI is the preferred method to visualize diffuse WMI (Fig. 54.5A–C),[197] but much of the widespread WM abnormalities within injured WM are not detected by conventional MRI techniques.[41] Experimental studies (Fig. 54.5D–F) demonstrated that early WMI is particularly well visualized at high magnetic field strength (11.7T),[188]



• **Fig. 54.5** Microscopic Necrosis Occurs Commonly but Constitutes a Small Fraction of the Total Burden of Preterm Cerebral White Matter Injury. (A) Typical sparse distribution of microcysts visualized with a marker of degenerating axons in a human autopsy brain at 32 weeks' postconceptional age. (B, C) Higher-power detail of the degenerating axons in the microcyst seen in the *box* in (A). (D) A microcyst visualized by high-field (12T) ex vivo magnetic resonance imaging as a focal hypointense lesion *(F-hypo)* on T2. This lesion was detected 2 weeks after global cerebral ischemia in a fetal sheep model of preterm white matter injury (WMI) (Riddle et al., 2011). Note the surrounding diffuse WMI, which is seen as a diffuse hyperintense signal *(D-hyper)*. (E) Pie chart showing the relative percentages of human diffuse WMI, cystic periventricular leukomalacia (PVL), and microscopic necrosis. Note that microcysts often overlap in distribution with regions of diffuse WMI. (F) The relative burden of human microcysts *(blue dots)* compared to diffuse WMI *(tan shading)*, which typically constitutes more than 80% of the total burden of WMI. Scale bars: (A) 500 μm; (B) 100 μm; (C) 25 μm. (From Back SA, Miller SP. Brain injury in premature neonates: a primary cerebral dysmaturation disorder? *Ann Neurol*. 2014;75:469–486.)

which suggests that currently used clinical MRI field strengths of 1.5 to 3T may be a limiting factor to detect both diffuse and microscopic WMI. However, diffuse WMI may be detected with advanced MRI techniques, even at current clinical field strengths. In particular, diffusion tensor imaging (DTI) and magnetic resonance spectroscopic imaging (MRSI) can be used to detect disturbances in WM maturation.[198,199] DTI measures the three-dimensional spatial distribution of water movement along axons in the white matter. Fractional anisotropy is a DTI parameter that describes the directional preference of water diffusion and varies between 0, equal diffusion of water in all directions (isotropic, e.g., CSF), and 1, all diffusion is along a single vector (anisotropic, e.g., mature axons). FA values increase with WM maturation as immature oligodendrocytes progress toward early myelination during the preterm period.[200,201] DTI measurements, such as FA, can be used to assess microstructural changes in brain development that occur with the delay or failure of myelination within regions of diffuse WMI.[202] Similarly, MRSI, which measures the concentration of brain metabolites, has a typical pattern of development in the WM and disturbances in WM development may be detected as reduction in the expected increase in N-acetylaspartate (NAA)/choline ratio, for example.[154,203]

It should also be emphasized that preterm survivors frequently display diffuse abnormalities in gray and white matter maturation by virtue of the clinical morbidities associated with preterm birth that may be more common than focal or diffuse WMI.[197,204] These diffuse abnormalities are apparent on MRI as enlarged subarachnoid spaces, a reduction in the amount of white matter, ventriculomegaly, and impaired gyral development that persists to adulthood.[205,206] However, many preterm newborns do not have these dramatic abnormalities, and up to 20% with adverse outcomes do not have significant qualitative abnormalities on MRI, which highlights the need for quantitative MRI metrics of brain maturation.[191,207] Importantly, the regional differences in brain maturation in adults born preterm that predict cognition differ from those that predict mental health.[17]

Physiologic Factors Related to the Pathogenesis of White Matter Injury

Role of Hypoxia-Ischemia

HI contributes to the pathogenesis of preterm cerebral injury via several maturation-dependent mechanisms. Since direct measurements of CBF in WM are technically challenging in human preterm neonates, experimental studies have often been used to define the role of CBF disturbances in the generation of cerebral WMI. The developmental epoch when CBF disturbances occur is a critical factor that influences susceptibility to hypoxia-ischemia. For example, acute injury to the cerebral cortex is relatively low compared with that to the white matter in the preterm fetal sheep, even with prolonged ischemia, whereas severe panlaminar cortical necrosis occurs in term animals.[208,209] After global cerebral hypoperfusion, midgestation sheep displayed a predilection to periventricular and subcortical WMI, whereas near-term animals displayed predominantly parasagittal cortical neuronal injury.[208–210] Cerebral ischemia in conjunction with hypoxia appears to be a critical factor to generate significant preterm WMI. WMI was infrequently observed when a restriction in uteroplacental blood flow resulted in decreased oxygen delivery to and mild acidemia in the fetus without systemic hypotension or cerebral hypoperfusion.[211–213] Similarly, in models

of maternal-fetal infection, preterm ovine WMI was observed only when repeated systemic fetal endotoxin exposure resulted in both transient hypoxemia and hypotension.[214,215]

Pressure-Passive Circulation

Disturbances in cerebral autoregulation appear to be a key factor that predisposes preterm neonates to cerebral WMI from hypoxia-ischemia. *Cerebral autoregulation* refers to the maintenance of constant CBF over a range of changes in systemic arterial blood pressure or cerebral perfusion pressure.[216–218] Cerebral autoregulation appears to involve an intrinsic property of arterial smooth muscle cells that respond to changes in transmural pressure to modify muscle tone. Autoregulation may be mediated in part by a balance between endothelial cell-derived constricting and relaxing factors.[219] This autoregulatory range has both upper and lower limits. When blood pressure changes above or below these limits, CBF fails to remain constant and increases or decreases passively, along with changes in arterial blood pressure. Preterm infants are particularly prone to display a "pressure-passive" circulation, especially in the setting of critical illness and the presence of impaired autoregulation is associated with greater mortality and increased radiographically defined cerebral injury.[29,220–223] Cerebral autoregulation disturbances in preterm infants were initially studied by means of xenon clearance and Doppler and more recently by near-infrared spectroscopy and spatially resolved spectroscopy.[218,224] Severe perinatal asphyxia, hypoxia, growth restriction, congenital heart disease, head trauma, and hypercapnic acidosis, even when relatively mild, attenuate or even abolish autoregulation.[225–229] Delayed cord clamping has been associated with improved cerebral autoregulation, which may mediate some of its effect on IVH.[96] Fundamental questions regarding cerebral autoregulation remain unanswered, including the optimal clinical practices for blood pressure regulation.[218,230] Novel ultrafast Doppler technology may offer new insights into cerebral hemodynamics in the preterm neonate.[231,232]

Factors That Influence the Distribution of White Matter Injury

Multiple factors that influence cerebral blood flow (CBF) or white matter metabolism determine the severity of WMI. It was originally proposed that the topography of more severe WMI was determined by the distribution of arterial end and border zones within the white matter. This attractive hypothesis provided an explanation for the propensity of more cystic necrotic lesions to localize to the deeper periventricular white matter. It was proposed that when periventricular white matter flow falls below a critical threshold, periventricular white matter would sustain greater WMI relative to a putatively better-perfused cerebral cortex. It has been feasible to test this hypothesis experimentally, but not in humans. Quantitative measurements of fetal CBF were done in utero in histopathologically defined regions of the cerebral cortex and white matter in preterm fetal sheep.[233] In the setting of moderately severe ischemia, no pathologically significant gradients of fetal blood flow were detected between the cerebral cortex and periventricular white matter during either ischemia or reperfusion. Moreover, although WMI preferentially localized to deeper white matter regions, they were not susceptible to greater ischemia (Figs. 54.6 and 54.7). Neither were less vulnerable superficial regions of white matter characterized by greater blood flow during ischemia. As discussed earlier, the distribution of WMI was explained by the relatively higher density of preOLs in susceptible regions of white matter.

It is currently unclear if recurrent HI predisposes to more severe WMI. In preterm-equivalent neonatal rats, a pronounced increase in cell death was observed in chronic WMI after recurrent HI.[234] However, in preterm fetal sheep, whose lesions more closely resemble those of humans, recurrent HI did not trigger enhanced WMI, which suggested that recurrent HI may confer protection against more severe WMI through an ischemic tolerance-like mechanism.[235]

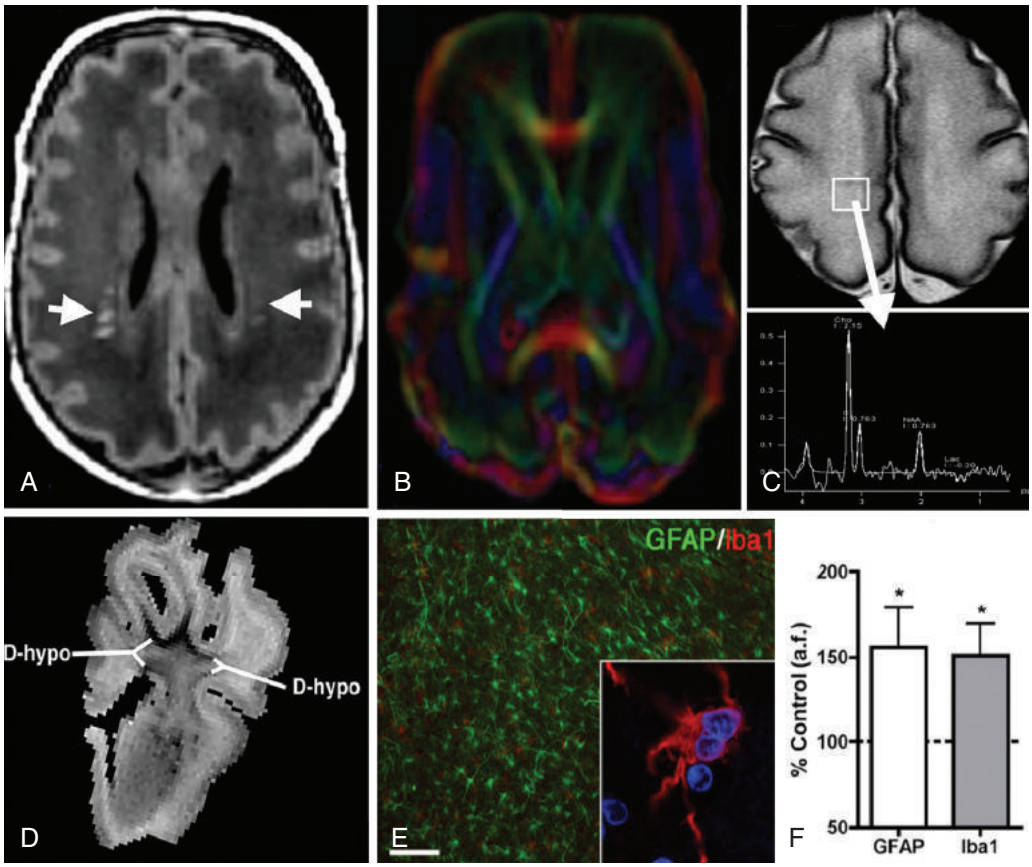
Clinical Factors Related to the Severity of White Matter Injury

Several common clinical conditions have been associated with an increased risk of more severe WMI (Box 54.2). Among these is neonatal sepsis, which occurs in up to 25% of preterm newborns who weigh less than 1500 g at birth[236–238] and is associated with a significantly increased risk of WMI.[239] Postnatal infections have been linked to altered development of white matter pathways[240,241] and widespread impairments in brain development,[154] especially when multiple.[155] Both culture-proven and culture-negative suspected infections have been associated with increased rates of cerebral palsy and other neurodevelopmental disabilities that are consistent with neonatal brain imaging findings.[154,191,242,243] Recurrent (≥3) postnatal infection, but not infection type or specific pathogen is associated with progressive WMI and adverse neurodevelopmental outcomes.[155,244,245] This suggests that exposure to infection may sensitize the preterm brain to subsequent episodes of infection, ischemia, or other insult.[246]

Necrotizing enterocolitis (NEC) has also been associated with an increased risk for adverse cognitive and motor outcomes.[247–250] In addition, in a large preterm cohort, infants with both NEC and sepsis were found to have the greatest risk for adverse neurodevelopmental outcomes.[242] This association yields further support for a two-hit hypothesis of brain injury.[251]

In contrast to postnatal infections, chorioamnionitis is not strongly associated with WMI in modern cohorts. Although studies as recently as 2000 found an association between cystic PVL and histological or clinical chorioamnionitis,[252] multiple subsequent studies using MRI-defined WMI have failed to show that histological chorioamnionitis is associated with WMI or adverse childhood outcomes.[202,239,253,254] This evolution is likely reflective of the shift in the spectrum of WMI from classic PVL to punctate and diffuse forms of WMI. Despite the lack of strong associations between prenatal chorioamnionitis and adverse neurodevelopmental outcomes, chorioamnionitis may indirectly increase the risk of postnatal infections and hypotension as independent risk factors for cerebral ischemia and WMI. Placental insufficiency has been associated with WMI in animal models of preterm brain injury.[212] Ongoing research continues to evaluate the role of the placenta in perinatal brain injury as new MRI technology yields novel insights into human fetal hemodynamics and brain development.[255,256]

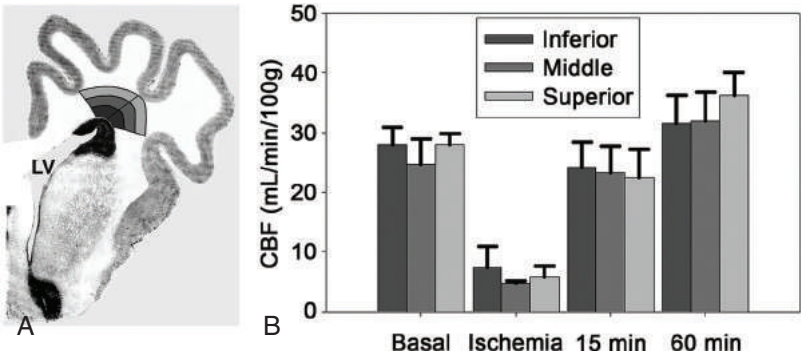
Bronchopulmonary dysplasia (BPD) and the duration of mechanical ventilation exposure have been associated with adverse white matter, cortical, and brainstem development.[257–260] Although a history of BPD is a strong predictor of cognitive outcome, even after birth weight and neurologic morbidity have been controlled for,[261] a causative role for isolated hypoxemia in WMI remains unclear from clinical or experimental studies[262] and multiple other factors related to the development of BPD may contribute to observed association with adverse neurodevelopmental outcomes.[116] Postnatal exposure to corticosteroids for



• **Fig. 54.6** Diagnostic and Experimental Magnetic Resonance Imaging Approaches to Define Dysmaturation Processes Related to Diffuse White Matter Injury. (A) Diffuse human white matter injury (WMI) on diagnostic magnetic resonance imaging (MRI) (1.5 T; T1) has the appearance of bilateral multifocal signal hyperintensities (*arrows*). (B) Diffusion tensor imaging defines the microstructure of white matter tracts and can be used to follow the long-term progression of diffuse WMI. (C) Magnetic resonance spectroscopic imaging can be applied to define biochemical and metabolic abnormalities associated with diffuse WMI. Both diffusion tensor imaging and magnetic resonance spectroscopic imaging detect abnormalities beyond the areas of signal abnormality on T1-weighted images. (D) Diffuse WMI defined by high-field ex vivo MRI at 12 T in a preterm fetal sheep model of global cerebral ischemia. Diffuse WMI was visualized as extensive hypointense regions *(D-hypo)* on a T2-weighted image at 1 week after ischemia. (E) The typical histopathologic features of diffuse WMI seen on MRI. Note the pronounced staining of reactive astrocytes *(green)* and microglia/macrophages (*red* and *inset*) indicative of a diffuse inflammatory response quantified in (F); the *asterisks* indicate $P < .05$. *Scale bar* in (E) 100 μm. *GFAP*, Glial fibrillary acidic protein.

the prevention and treatment of chronic lung disease is linked to impaired brain growth and adverse neurodevelopment, and this effect may be more pronounced with dexamethasone treatment.[68,263–265] Ongoing research continues to evaluate the dose and timing of corticosteroid therapy for BPD in order to optimize neurologic and pulmonary outcomes.[266]

Hypoxemia, as an independent risk factor for WMI, was proposed on the basis of a suspected association between cystic WMI and BPD.[267] However, a direct association between hypoxemia from chronic lung disease and severe WMI is difficult to establish, because cystic WMI is also commonly associated with IVH and PVHI. The response of the fetus to acute hypoxemia without ischemia has been extensively studied in preterm fetal sheep. Transient hypoxemia was found to cause variable degrees of combined WMI and gray matter injury in the midgestation or near-term sheep.[211,268] However, WMI was notably more severe when significant hypotension was observed, which resulted in *both* cerebral hypoxia and ischemia.[269] Midgestation chronic hypoxemia, generated by chronic placental insufficiency, caused disturbances in hippocampal, cerebellar, and white matter development.[213,268] Exposure of neonatal rodents to chronic hypoxia results in a spectrum of somatic and cerebral growth retardation that is accompanied by disturbances in white matter development that include ventriculomegaly, reduction in white and gray matter volumes, and disrupted myelination.[267,270–272] Interestingly, acute moderate hypoxia with or without ischemia has been recently shown to cause disruptions in the maturation of fetal subplate within the developing WM.[273] This transient population of neurons is important for the normal development of thalamocortical circuits and disruption may underlie indirect cerebral maturational changes. Several studies of moderate to severe intermittent hypoxia in neonatal rodents, which model apnea of prematurity, have shown disturbances in myelination that correlate with hypoxia severity but the relative contribution of ischemia secondary to bradycardia remains



• **Fig. 54.7** Periventricular White Matter Injury Is Not Explained by Gradients of Reduced Cerebral Blood Flow in Cerebral White Matter. (A) Analysis of fetal sheep cerebral blood flow (CBF) in preterm cerebral white matter during hypoxia-ischemia and reperfusion. CBF measurements were made in inferior, middle, and superior regions of the white matter, as illustrated (*gray gradient*). Typically, white matter injury localized to the inferior region of the white matter. (B) There were no differences between baseline CBF values and those obtained during ischemia or reperfusion at 15 or 60 minutes after ischemia in inferior, middle, and superior regions of the white matter. Hence white matter injury was not preferentially associated with regions that sustained the most severe ischemia. *LV*, Left ventricle. (From McClure M, Riddle A, Manese M, et al. Cerebral blood flow heterogeneity in preterm sheep: lack of physiological support for vascular boundary zones in fetal cerebral white matter. *J Cereb Blood Flow Metab*. 2008;28:995–1008.)

• BOX 54.2 Pathogenic Factors Contributing to More Severe White Matter Injury

Postnatal sepsis may promote postnatal cerebral hypotension or modify the response to hypoxia-ischemia.
More severe hypoxemia coupled with hypotension may increase the magnitude of energy failure mediated by hypoxia-ischemia.
Hypocarbia may exacerbate hypoxia-ischemia by promoting more severe hypoperfusion.
Hypoglycemia may exacerbate energy failure associated with hypoxia-ischemia.
Steroid exposure (e.g., dexamethasone) may increase the risk of white matter injury.
Exposure to multiple painful or stressful procedures may increase the risk of white matter injury.
Bronchopulmonary dysplasia is associated with multiple cerebral risk factors including hypoxemia, hypocarbia, mechanical ventilation, and steroid exposure.
Necrotizing enterocolitis may potentiate the interaction of inflammation and ischemia leading to increased white matter injury.
Nutrition deficiencies in calories, lipids, or parenteral administration may predispose infants to impaired cerebral repair and growth.

unclear.[274,275] However, central features of human preterm WMI, such as preOL degeneration and reactive gliosis, are not observed in rodents. In addition, in children with cyanotic heart disease, WMI may occur due to antenatal factors[155,276] independently of the perioperative risk associated with surgical correction of cyanotic congenital heart disease.[277,278] It thus appears that hypoxemia is related to disturbances in white matter maturation that are distinct from the injury generated by acute hypoxia-ischemia.

Hyperoxia is also a potential complication of neonatal resuscitation and ventilation of the preterm infant during intensive care, but a direct association with human preterm WMI has not been demonstrated. A rabbit model of neonatal resuscitation found no increase in IVH or cerebral injury in response to hyperoxia.[279] Tissue culture models of hyperoxia have demonstrated the enhanced vulnerability of preOLs to cell death from oxidative stress, and transient disturbances in myelination in neonatal rodents were observed.[262]

WMI is a prominent and common feature of symptomatic hypoglycemia in term infants that has a predilection for the posterior limb of the internal capsule and posterior cerebral regions. WMI often occurs in association with cortical or subcortical gray matter abnormalities seen on MRI.[280] Experimental studies support the observation that even mild hypoglycemia exacerbates cerebral injury from HI.[281,282] Studies in preterm fetal sheep found that hypoglycemia was the most significant factor associated with more severe WMI generated by global cerebral HI.[283]

Preterm infants are often routinely exposed to multiple painful and stressful procedures that have been linked to altered brain maturation that involves white and gray matter structures, as well as impaired brain function.[284] A higher number of painful procedures in the NICU is associated with delayed white matter maturation and adverse cognitive outcomes into early childhood.[285–287] Procedural pain has also been linked with impaired postnatal growth,[288] a predictor of poor cortical development,[289] and interacts to predict cognitive outcomes at school age.[290] Further studies have provided support for the association between procedural pain and anomalous gray matter development in multiple regions including the thalamus,[5,7] subcortical gray matter,[286] and cerebral cortex.[291]

Importantly, analgesic and sedative medication exposure in the preterm neonate have also been linked to regional dysmaturation that is specific to the exposure: increasing midazolam exposure predicts slower growth of the hippocampus,[6] whereas increasing morphine exposure predicts slower growth of the cerebellum.[4] Exposure to painful procedures is also associated with more adverse neurodevelopment, which may be modulated by more optimal parent-child interactions,[292,293] as well as parental stress and anxiety.[294–296] Importantly, experimental models and human studies support the potential of parent-infant interactions to compensate for compromised early brain maturation

and adversity.[293,294,297–300] In addition to these medical factors, social factors such as socioeconomic status are recognized as robust predictors of brain maturation and outcomes, and most recently as a modifier of cognitive outcomes in response to early-life WMI.[301–303]

There is increasing attention to the role of biological sex in pathways related to brain injury and neurodevelopmental outcome in children born preterm.[304] Meta-analyses reveal a consistent female advantage in cognitive and motor development in children born preterm.[305,306] Male preterm neonates are at higher risk of developing IVH than females.[307–310] However, in contrast with other clinical risk factors, female preterm neonates demonstrate a stronger association of pain with abnormal brain development and slower basal ganglia growth with exposure to glucose for analgesia.[5] Sex is also recognized as a predictor of brain volumes and microstructural development at term equivalent age[311,312] and in adolescence in preterm children.[313,314] Yet, volumetric difference between preterm and term-born female children narrows by age 8 to 12 years, whereas that in males persists.[315,316] Additional studies are needed to more definitively delineate how biological sex modifies longer-term responses to preterm birth and brain injury.

Pathogenesis of Chronic White Matter Injury

Emerging Roles for Myelin in Brain Development, Learning, and Memory

Myelination disturbances are one of the central features of chronic WMI. Myelination begins in the preterm brain and normally progresses in well-defined sequences that continue for years postnatally. The central role of myelin is to wrap axons to ensure optimal nerve conduction throughout the CNS. However, emerging studies are challenging the long-held belief that myelin is a stable structure that simply provides static insulation to nerve fibers. Recent studies report that myelin sheaths are dynamic structures that contribute importantly to learning and memory by remodeling the thickness of myelin sheaths so as to enhance or diminish the relative strength of nerve conduction.[317,318] Hence adaptive myelin plasticity appears to provide a mechanism whereby the nervous system can strengthen or weaken the flow of information along competing pathways so as to optimize new learning.[319] Whereas synaptic plasticity operates on millisecond dynamic timescales to fine-tune learning and memory, myelin plasticity occurs on longer timescales to enable the nervous system to solidify the acquisition of new skills. For example, in adult animals, the generation of new oligodendrocytes and myelin is required for the learning and acquisition of new motor skills.[320–322] Several studies in humans have shown a microstructural change in WM tracts after exercise and skill training,[323,324] including in children,[325,326] and provide indirect evidence of myelin plasticity in humans.

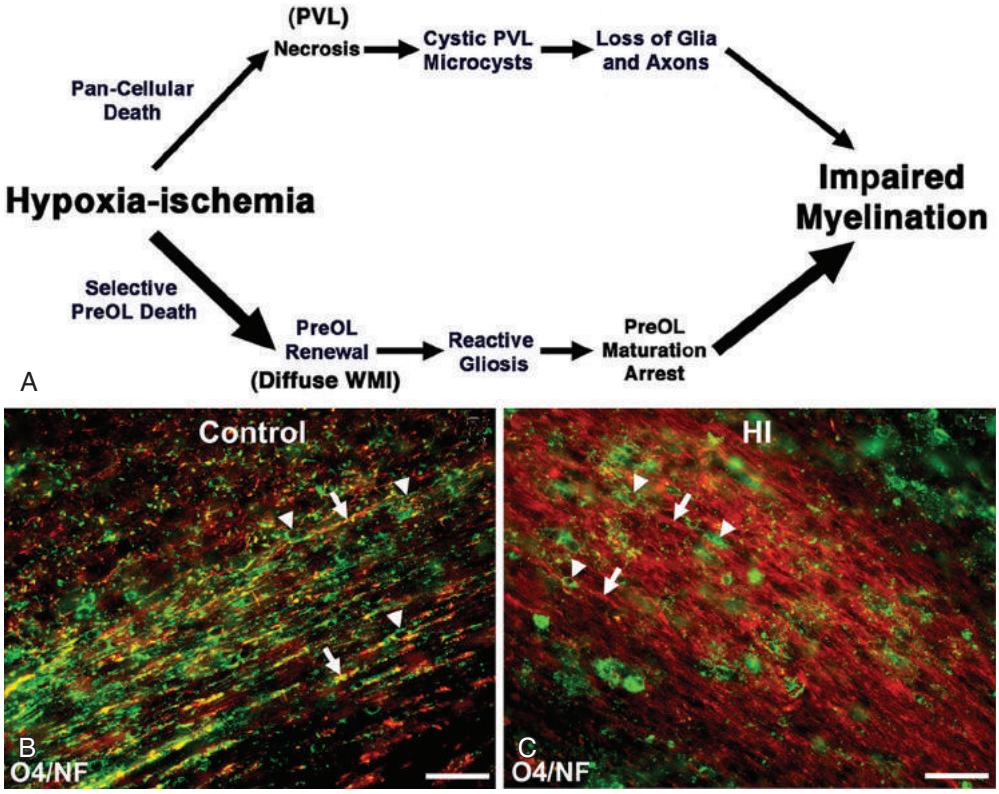
Hence the definition of these new roles for myelin underscores the potential impact that WMI may have on the disruption of the timing and sequences of myelination during a critical period in preterm brain development. Preterm brain development involves multiple cell maturational and activity-dependent events that coincide with sequential waves of late neurogenesis, gliogenesis, glial and neuronal maturation, synaptogenesis, and myelination. Aberrant myelination is likely to disrupt both gray matter and white matter development by causing enduring disturbances in the ultimate establishment of neural networks and connectivity that are integral to normal brain function.

Pathogenesis of Chronic Myelination Failure and Potential Therapeutic Strategies

Myelination disturbances are related to aberrant regeneration and repair responses to acute death of preOLs. The preterm developing brain contains a population of early oligodendrocyte progenitors that serve as an apparent reservoir of cells that can mount repair responses after WMI. In response to injury, early progenitors rapidly proliferate and partially differentiate to regenerate preOLs that are lost in early WMI.[3,234] Thus, the preterm human white matter is capable of mounting a robust repair response to injury. Despite this inherent plasticity, the injured white matter displays robust astrogliosis, which diffusely extends beyond the core of injury as a functional "glial scar" that blocks the normal maturation of preOLs to myelinating cells.[3,327] Hence the pathogenesis of chronic diffuse WMI leads to a disruption of the normal progression of myelination through a series of dysmaturation events that result in regeneration of preOLs that accumulate in chronic lesions but fail to differentiate into myelinating oligodendrocytes (Fig. 54.8). By contrast, focal necrotic WMI results in a loss of both glia and axons that causes an irreversible block on myelination (Fig. 54.8).

The mechanism by which this glial scar contributes to myelination failure is an active area of investigation. It appears likely that pronounced disturbances in the composition of the extracellular matrix result in the generation of inhibitory molecules that block preOL maturation. One widely studied molecule is hyaluronic acid, which is generated by reactive astrocytes within the glial scar and is digested by hyaluronidases that are upregulated in response to WMI.[235,328,329] These hyaluronidases generate bioactive fragments of hyaluronic acid that block preOL maturation.[330] Preclinical studies demonstrated that functional remyelination was promoted by a broad-spectrum hyaluronidase inhibitor.[328] The downstream signaling involved in chronic WMI appears to include Wnt pathway members as well as epidermal growth factor receptor and toll-like receptor-mediated pathways.[272,331,332] It is currently unknown whether this chronic injury environment will have a negative influence on stem cell-based therapies to promote myelination.[333–335]

Since multiple signaling pathways appear to be involved in the pathogenesis of chronic WMI and myelination failure, one potential therapeutic strategy is to target multiple signaling pathways via the pleiotropic growth factor erythropoietin (Epo), which is widely used to stimulate neonatal erythropoiesis. Epo is currently under evaluation as a protective agent for WMI, because of its demonstrated actions to promote angiogenesis, neurogenesis, and gliogenesis during normal brain maturation.[336] A randomized controlled trial of repeated intravenous administration of recombinant high-dose Epo to preterm neonates in the first 2 days of life was found to be safe and was associated with enhanced white matter maturation as defined by diffusion tensor imaging,[337,338] but 2-year neurodevelopmental outcomes were not improved.[339] Building on these overall positive but small studies, the Preterm Erythropoietin Neuroprotection (PENUT) trial, which randomized 741 neonates born between 24 and 27 6/7 weeks gestation to high-dose Epo therapy for 2 weeks followed by maintenance Epo therapy until 32 completed weeks, was undertaken and showed no reduction in severe neurodevelopmental disability or death.[340] Despite the failure to prevent neurodevelopmental disability in this well-designed study, outstanding questions remain about the potential of Epo to enhance preOL maturation in diffuse WMI if administered for longer durations.[341,342] Potential benefit for



• **Fig. 54.8** Pathogenesis of Myelination Failure in Chronic Diffuse White Matter Injury. (A) Distinctly different pathogenetic mechanisms mediate abnormal myelination in focal necrotic lesions (periventricular leukomalacia [PVL]; *upper pathway*) versus lesions with diffuse white matter injury (WMI; *lower pathway*). When it is most severe, hypoxia-ischemia (HI) triggers white matter necrosis (*upper pathway*) with pancellular degeneration that depletes the white matter of glia and axons. Severe necrosis results in cystic PVL, whereas milder necrosis results in microcysts. Milder HI (*lower pathway*) selectively triggers early oligodendrocyte progenitor (preOL) death, but preOLs are rapidly regenerated in chronic lesions enriched in reactive astrocytes that contribute to a block in preOL differentiation to myelinating oligodendrocytes. Myelination failure in diffuse WMI thus results from preOL arrest rather than axonal degeneration, as occurs with white matter necrosis. Note that the lower pathway is the dominant one for many contemporary preterm survivors, whereas the minor upper pathway reflects the declining burden of white matter necrosis. (B) Typical appearance of normal early myelination in neonatal rodents. Axons are visualized in *red* and early myelination of axons is in *green*. (C) Arrested maturation of preOLs in a chronic white matter lesion where numerous preOLs (*green*) are seen, but the axons (*red*) are diffusely unmyelinated. Scale bars 100 μm. *HI*, Hypoxia-ischemia; *PreOL*, oligodendrocyte progenitor; *PVL*, periventricular leukomalacia; *WMI*, white matter injury. (B, C adapted from Segovia KN, McClure MM, Moravec M, et al. Arrested oligodendrocyte lineage maturation in chronic perinatal white matter injury. *Ann Neurol* 2008;63:517–526.)

neonatal encephalopathy in term infants was demonstrated in a recent double-blinded, placebo-controlled trial of Epo administered with therapeutic hypothermia. A significant reduction in MRI-defined brain injury was observed in the perinatal period as was improved motor function at 1 year of age in 50 infants with hypoxic-ischemic encephalopathy (HIE).[343] Several phase III trials to test the efficacy of Epo as a neurotherapeutic for HIE in combination with hypothermia are ongoing or planned. The effectiveness of therapeutic hypothermia in infants ≥36 weeks' gestational age with HIE and in preterm animal models[344] has prompted investigation of potential benefits in preterm infants. Results in smaller trials have been mixed,[345,346] and a randomized controlled trial of 168 infants 33 to 36 weeks' gestational age has finished recruiting, but results will not be available until neurodevelopmental follow-up is completed.

Preterm Cerebral Gray Matter Injury

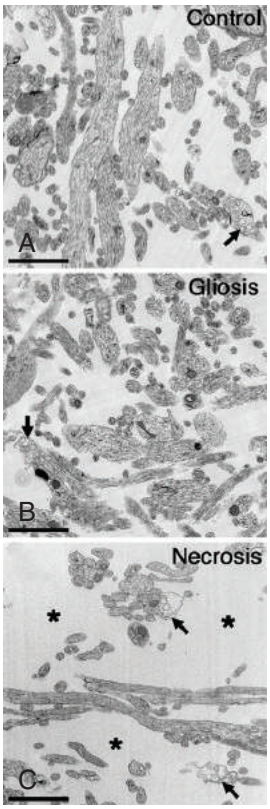
During the last trimester, the cerebral gray matter structures undergo dramatic growth and maturation. Several neuroimaging studies have identified significant reductions in the growth of human cortical and subcortical gray matter structures, including the basal ganglia, thalamus, hippocampus, and cerebellum.[64,198,204,289,347,348] Consistent with the findings of these structural studies, preterm newborns also exhibit reduced functional connectivity between the cortex and thalamus on functional connectivity MRI.[349–351] Children and adults born preterm with normal neurocognitive function nevertheless express altered cortical activation and functional connectivity during language and visual processing.[19,352–354] Thalamocortical connections are also disrupted in preterm newborns with WMI, resulting

in visual dysfunction.[355] Regionally specific altered functional connectivity in children and adolescents born preterm is thus a significant risk factor for multiple adverse neurocognitive outcomes.[352,356–359] Importantly, functional connectivity is dependent on both the relevant gray matter structures and their white matter connections.

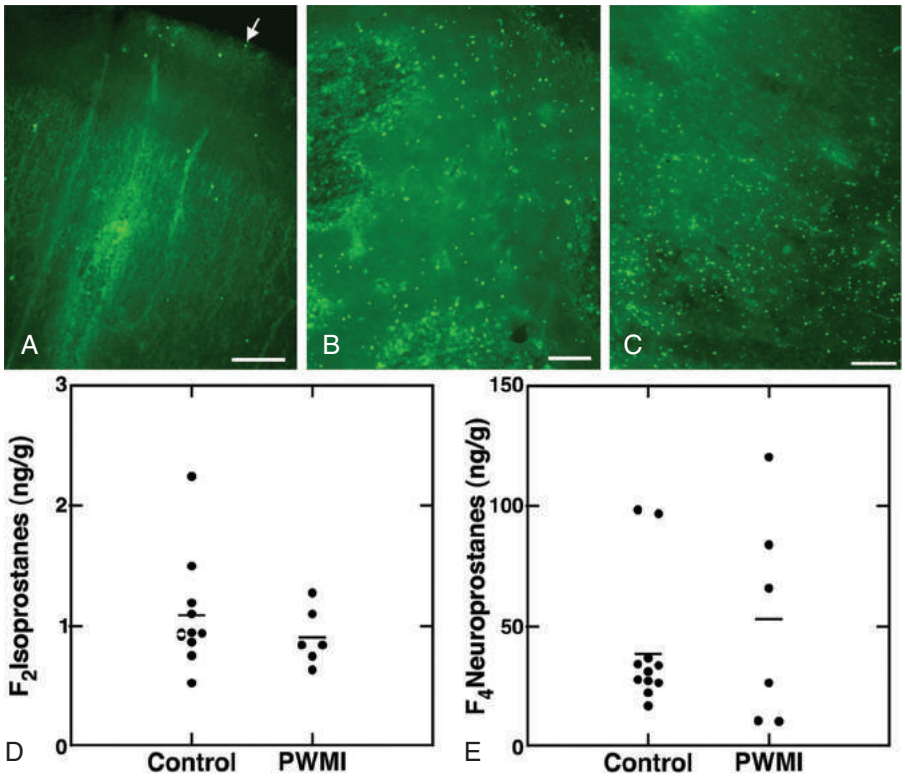
The relative contributions of destructive versus dysmaturational processes to these common structural, functional, and neurobehavioral abnormalities are under active investigation. These two complementary potential mechanisms may explain impaired cerebral growth in preterm survivors. The first may involve primary degeneration of neurons in multiple cortical and subcortical gray matter structures or secondary neuronal degeneration related to axonal injury in foci of white matter necrosis. Subplate neurons, for example, were proposed to be particularly vulnerable to HI in neonatal rodents. Subplate neurons are a transient population required to establish cortical circuitry through guidance cues for thalamocortical connections. They were reduced in a number of human autopsy cases with diagnosed necrotic WMI.[360] These findings are consistent with the link between impaired thalamocortical connectivity and cognitive delays in preterm children.[361] Similarly, in rodents, significant subplate neuron loss appears to occur only in association with more severe cortical neuronal degeneration[362,363] and was not observed in preterm fetal sheep, where neither severe gray nor white matter injury was sustained.[273] Human autopsy studies also found neuronal loss in the cortex, basal ganglia, thalamus, and cerebellum in association with necrotic WMI and axonal degeneration.[187,360,364,365] The apparent mechanism relates to retrograde degeneration of neurons in response to axonal injury in the white matter. As discussed earlier (Fig. 54.4A–C), axonal injury is a prominent feature of WMI where necrosis is present.[193,366] Significant axonal degeneration has not been observed in diffuse nonnecrotic WMI (Fig. 54.9) in either human or experimental models.[3,194] Hence primary or secondary neuronal degeneration appears to occur in the setting of more severe cerebral injury, where cystic necrotic WMI would also be common.

White matter necrotic lesions are a minor component of diffuse WMI in experimental models, and in contemporary cohorts of human WMI cases dysmaturational processes predominate.[3,188] In human autopsy cases, with early diffuse WMI and preOL degeneration, neither the preterm gray matter nor the preterm white matter displayed evidence of significant oxidative stress to degenerating neurons or axons (Fig. 54.10).[195] Significant early neuronal loss was not observed in association with human nonnecrotic diffuse WMI.[187,195] Diffuse WMI thus triggers selective preOL degeneration but appears to spare axons and migrating neurons in the white matter.

These observations support the notion that significant neuronal loss may be uncommon in contemporary cohorts of preterm survivors and that altered gray matter morphology may instead be related to altered development in surviving neurons. While there is a strong relationship between region and extent of WMI and gray matter abnormalities,[350,354] preterm infants can have more extensive gray matter abnormalities than "injuries" identified by MRI signal abnormalities.[6,204,351] The underlying cellular responses that accompany impaired cerebral gray matter development have been studied in a model of global preterm ischemia in preterm fetal sheep. This model closely reproduces the spectrum of WMI and gray matter injury in humans.[367] In fact, diffuse WMI in preterm fetal sheep resulted in reduced growth of the cerebral cortex,[368] caudate nucleus,[369] and hippocampus[370] without loss of neurons. Interestingly, reduced hippocampal growth was also observed in animals treated with hypoxia alone, which did not induce significant WMI. These experimental findings are consistent with observations of microstructural dysmaturation of the cerebral cortex in preterm neonates with restricted postnatal growth,[289] who also show a predilection for WMI.[202,371] Reduced cerebral growth was accompanied by a significant *reduction* in the complexity of the dendritic arbors of the major populations of cortical and caudate projection neurons (Fig. 54.11). Importantly, during normal fetal sheep and human development, significant cerebral cortical growth is accompanied by a pronounced *increase* in the dendritic arbor of projection neurons. These morphologic disturbances in the dendritic maturation of dysmature projection neurons were accompanied by significant reductions in the density of dendritic spines, which are the major sites where synaptic transmission occurs. Regional specificity remains important as hippocampal CA1 neurons show an increase in basal dendrite



• **Fig. 54.9** Axon Degeneration Is Uncommon in Chronic Diffuse White Matter Injury. White matter injury resulted from hypoxia-ischemia in preterm fetal sheep. Injured axons were identified by electron microscopy since optimal markers of axon degeneration have not been developed for light microscopy. (A) Normal preterm white matter contained numerous axons with normal axoplasm and intact microtubules. A swollen degenerating axon (*arrow*) was rarely seen in control white matter. (B) Regions of diffuse white matter injury were very similar to normal controls in the paucity of degenerating axons. (C) By contrast, regions of focal white matter necrosis (i.e., periventricular leukomalacia) contained large areas devoid of axons (*asterisks*) as well as numerous degenerating axons (*arrows*). Scale bars: 2 μm. (From Riddle A, Maire J, Gong X, et al. Differential susceptibility to axonopathy in necrotic and non-necrotic perinatal white matter injury. *Stroke*. 2013;43:178–184.)

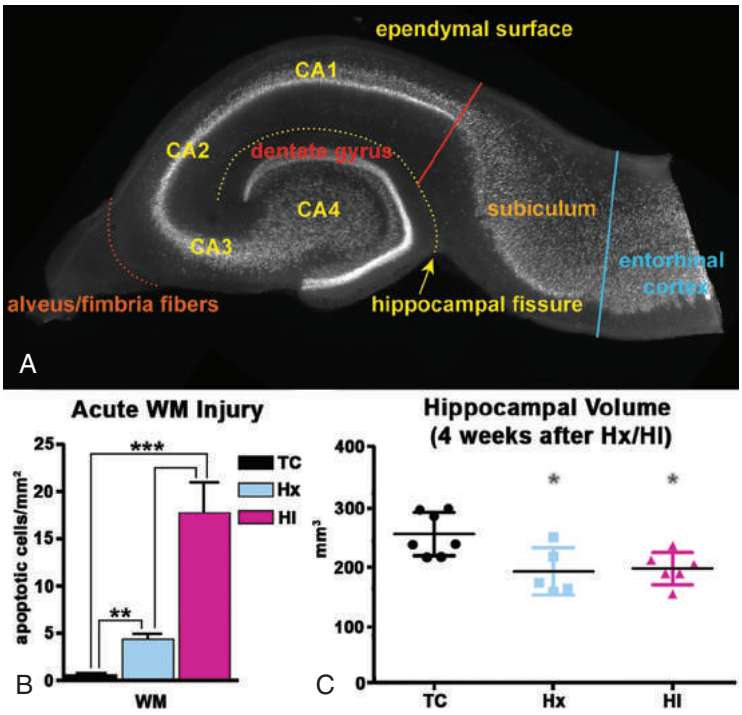


• **Fig. 54.10** Neurons and Axons Are Markedly More Resistant to Oxidative Injury in Gray and White Matter than Oligodendrocyte Progenitors. (A) Typical paucity of cell death in the overlying cerebral cortex from a preterm infant with diffuse white matter injury at autopsy. Cell death is visualized in (A–C) by TUNEL staining, which detects DNA fragmentation of apoptotic and necrotic cells. The *arrow* marks the pial surface. (B, C) The periventricular white matter from the same case showed extensive oligodendrocyte progenitor degeneration. The individual degenerating cells appear as *bright green dots*. (D, E) When compared with controls, no significant oxidative injury to the cerebral cortex was observed in autopsy brains where significant preterm white matter injury was observed. Oxidative injury was measured with two stable markers optimized for preterm human autopsy brains: F_2-isoprostanes are a general marker of oxidative injury to cells and F_4-neuroprostanes specifically detect injury to neurons and axons. Scale bars: (A, C) 200 μm; (B) 100 μm. *PWMI*, Preterm white matter injury. (Adapted from Back SA, Luo NL, Mallinson RA, et al. Selective vulnerability of preterm white matter to oxidative damage defined by F2-isoprostanes. *Ann Neurol*. 2005;58:108–120.)

complexity in response to HI, likely due to homeostatic developmental regulation driven by functional changes within hippocampal circuits.[370]

Electrophysiologic studies found significant abnormalities in excitatory synaptic function at 1 month after preterm HI in multiple brain regions.[369,370] These findings are consistent with the notion that dysmaturation of the dendritic arbor of projection neurons is accompanied by persistent disturbances in neuronal function.[372] Hence these findings support the observation that milder forms of cerebral injury nevertheless result in impaired cerebral growth that involves disturbances in neuronal maturation that affect large distinct populations of neurons in multiple cortical and subcortical gray matter structures.

Since disturbances in the maturation of preterm neurons occur during a critical window in the establishment of human neuronal connections, even transient neuronal dysmaturation may have persistent global effects on the subsequent development of CNS circuitry. Consistent with this notion, recent neuroimaging studies have evaluated whether reduced cerebral growth is accompanied by disturbances in the microstructure of the developing cerebral cortex. During normal development, the cerebral cortex displays a normal progressive loss of cortical fractional anisotropy.[373] This loss of anisotropy is related to the progressive maturation of cortical projection neurons, which are the major population of neurons in the cortex. Immature cortical projection neurons display an "anisotropic morphology" related to their simplified dendritic arbor, which causes asymmetric diffusion of water along their dendrites. As neuronal maturation progresses, the dendritic arbor assumes an "isotropic morphology" in which water diffusion along dendritic processes is similar in all directions. Thus, dysmature cortical projection neurons have a simplified dendritic arbor that results in greater "anisotropic morphology."[368] This reduced dendritic arbor complexity accounted for the greater anisotropy that was observed experimentally in chronic gray matter lesions relative to the reduced anisotropy in normal gray matter (Fig. 54.12). Delayed maturation of regional cortical microstructure in preterm survivors[374] and related macrostructural markers such as cortical surface area and curvature[206,375] correlate with neurodevelopmental outcomes in preterm survivors. In the setting of reduced cortical growth,



• **Fig. 54.11** Hippocampal Growth is Reduced Similarly after Fetal Hypoxia *(Hx)* and Hypoxia–Ischemia *(HI)*. (A) Neuronal staining of a transverse slice through the hippocampal formation reveals the neuronal subfields of the fetal sheep hippocampus and dentate gyrus in the stereotypical "jelly-roll" configuration. Area calculations included the CA1–CA4 subfields and the dentate gyrus of each slice and excluded the subiculum, entorhinal cortex, and alveus. (B) Peak cellular degeneration in the white matter *(WM)* 25 hours after HI analyzed by apoptotic marker caspase-3 reflects the vulnerability of the WM to hypoxic-ischemic injury. Minimal injury was noted after Hx without ischemia. No increase in cellular injury was found in the hippocampus in either condition (data not shown). (C) After 4 weeks, the hippocampal volume of fetuses exposed to Hx or HI was similarly reduced versus controls *(TC)*. $*p < .05$, $**p < .01$, $***p < .001$. (Adapted from McClendon E, Wang K, Degener-O'Brien K, et al. Transient hypoxemia disrupts anatomical and functional maturation of preterm fetal ovine CA1 pyramidal neurons. *J Neurosci*. 2019;39[40]:7853–7871.)

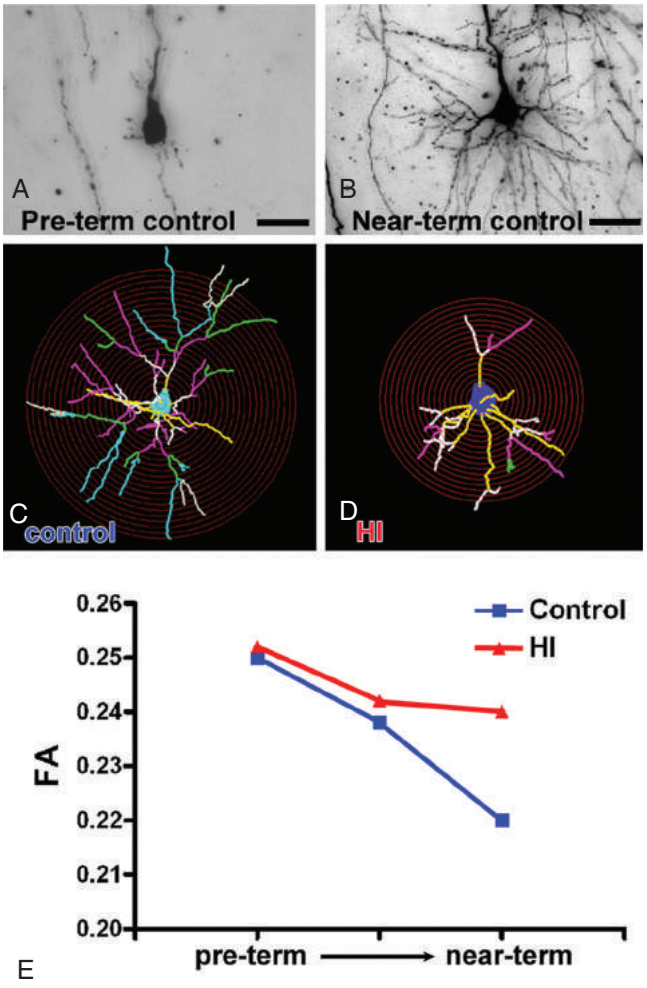
the gray matter of human preterm survivors was found to display increased anisotropy, consistent with greater dysmaturity.[9,289] Disturbances in cortical fractional anisotropy were associated with impaired somatic growth (weight, length, and head circumference), even after coexisting brain injuries on MRI (e.g., WMI) and other aspects of systemic illness (e.g., infection) had been accounted for.[289]

Recent studies in very preterm neonates highlight the critical link between nutrition in the first 2 to 4 weeks of life with subsequent growth of the brain to term-equivalent age and neurodevelopmental outcomes in early childhood.[376,377] While these studies point to the importance of early life energy and lipid intake, even more specific aspects of nutrition are being investigated. Improved microstructural white matter development and less IVH are linked to higher postnatal docosahexaenoic acid levels, suggesting the potential of nutrition strategies to promote optimal brain maturation.[378] Consistent with these observations, higher serum docosahexaenoic acid levels in the first 28 days of life are positively associated with brain volumes at term-equivalent age in extremely preterm neonates.[379] More recent evidence from preterm born children studied at 7 years of age with functional MRI measures of thalamic network connectivity, suggests that optimizing early protein intake may promote long-term brain maturation.[380]

These studies support that dysmaturation of cerebral neurons and circuits are major underlying mechanisms that lead to various neurocognitive impairments seen in survivors of preterm birth and that these dysmaturation events do not require significant cell death or classic neuropathological injury. As in developing WM, multiple factors including nutritional status and exposure to cerebral hypoxia or ischemia, or infection may contribute to the pathogenesis of neuronal dysmaturation in preterm survivors. It is unclear, however, the extent to which dysmaturation occurs secondary to or independent from diffuse WMI, or, perhaps most likely, both. This distinction has important implications for the development of neurotherapeutic approaches. Further studies are needed to define how disturbances in neuronal maturation arise and contribute to the widespread disturbances in cerebral connectivity that appear to underlie the enduring neurocognitive and behavioral deficits observed in preterm survivors.

Summary

Advances in neonatal neurologic intensive care have been accompanied by considerable progress in the reduction in the incidence and overall severity of IVH in preterm neonates. These advances



• **Fig. 54.12** After hypoxic-ischemic white matter injury, the preterm brain is enriched in immature neurons that do not degenerate but are highly susceptible to impaired maturation that manifests itself as a less complex dendritic arbor. (A) A typical pyramidal projection neuron from the preterm cerebral cortex of a control fetal sheep that was visualized with a Golgi silver stain. Note the paucity of processes and the simplified appearance of this typical preterm neuron. (B) Neurons undergo a dramatic increase in complexity of the dendritic arbor in near-term animals. (C, D) Four weeks after preterm ischemia, cortical projection neurons display disrupted maturation. A typical control neuron (C) displays highly arborized dendrites, whereas a brief preterm exposure to hypoxia-ischemia *(HI)* resulted in neurons with a significantly more simplified dendritic arbor (D). The reduced complexity of the dendrites can be appreciated from the overlay of the red concentric Scholl rings, which illustrate that the processes of the dysmature neurons intersect less frequently with the rings. The *yellow, white, pink, green*, and *blue lines* represent first-order, second-order, third-order, fourth-order, and fifth-order branches respectively from the soma. Note the overall reduction in the size and complexity of the branching pattern of the ischemic neurons in (D). (E) Reductions in cortical growth also manifest themselves as disturbances in cortical fractional anisotropy (FA), a magnetic resonance imaging measure of maturation and complexity of the white matter microstructure. Note the normal progressive decline in FA in controls *(blue)* between preterm and near-term cortical development. In response to ischemia, higher cortical anisotropy (more restricted water diffusion) was observed in response to ischemia *(red)* relative to controls *(blue)*, which was related to the reduced complexity of the dendritic arbor of the ischemic neurons, for example, in (D) versus controls, for example, in (C). Scale bars: (A, B) 20 μm. (From Back SA, Miller SP. Brain injury in premature neonates: a primary cerebral dysmaturation disorder? *Ann Neurol*. 2014;75:469–486.)

have contributed to similar reductions in the incidence of more severe cystic necrotic cerebral WMI and gray matter injury. Unexpectedly, despite these pronounced reductions in injury severity, neurodevelopmental morbidity persists at very high rates[381] while the number of preterm births continues to rise.[382] Preterm neonates are surviving with an evolving constellation of motor, cognitive, and behavioral disabilities that appear to be related to a broad spectrum of injury that ranges from relatively uncommon severe forms of injury to more moderate injury that is accompanied by widespread disturbances in cellular maturation. This translates to large numbers of brain cells that fail to fully mature during a critical window in the development of neural circuitry. These recently recognized forms of cerebral gray and white matter dysmaturation raise new diagnostic challenges and support the urgent need for new therapeutic strategies focused on regeneration and repair to reverse the processes that promote dysmaturation.

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Neonatal Encephalopathy

ULRIKE MIETZSCH AND SANDRA E. JUUL

KEY POINTS

- Neonatal encephalopathy is a clinical syndrome that can result from a variety of underlying causes.
- Hypoxic-ischemic encephalopathy (HIE) is a major cause of neonatal brain injury and mortality worldwide.
- Magnetic resonance imaging and magnetic resonance spectroscopy are useful imaging tools to identify brain injury and help predict long-term neurodevelopmental outcomes in neonates with HIE.
- Cerebral arterial or venous thrombosis may be the cause of unexplained seizures.
- Infections acquired in utero can present with neonatal encephalopathy.
- Metabolic disorders, genetic conditions, and brain malformations can present as neonatal encephalopathy.

Neonatal Encephalopathy

Neonatal encephalopathy describes a clinical syndrome unique to the term and near-term newborn in the immediate perinatal period.[1] The clinical findings may include seizures and are characterized by an impairment of one or more components of the central nervous system (CNS) including mental status, tone, primitive reflexes, autonomic functions including respiration, as well as abnormal feeding.[2–4] The causes of neonatal encephalopathy are diverse and include infections, stroke, genetic conditions, metabolic disorders, and brain malformations (Table 55.1).[2,3,5] The incidence of neonatal encephalopathy in high-income countries ranges from 2 to 6:1000 term live births with a combined average of 3.9:1000; the exact incidence, however, is difficult to determine due to the heterogeneous nature of the presenting clinical picture and might be much higher than currently described in the literature.[6–10] Factors associated with neonatal encephalopathy are plentiful and can be divided into maternal, utero-placental, and fetal in origin (Table 55.2).

Recent placental pathology studies have given insight into pathophysiologic changes associated with neonatal encephalopathy. Commonly observed findings in placentas from newborns with neonatal encephalopathy can be acute or chronic, and include infection, inflammatory changes, and vasculopathies, particularly those altering fetal vascular perfusion.[11,12] It is understood that findings consistent with fetal vascular malperfusion take at least 48 hours to evolve[13] and therefore can be an indicator of the acuity of neonatal encephalopathy. Changes consistent with global fetal vascular malperfusion, including avascular villi and fetal thrombotic vasculopathy (FTV), occurred in 20–24% of cases diagnosed with neonatal encephalopathy, 3 to 4× higher than in control newborns.[14–16] The severity of clinical encephalopathy can be linked to the severity of fetal vascular placental changes.[17] In some series, thrombosed placental vessels and fetal vascular malperfusion have been demonstrated in up to 50% of cases with perinatal arterial-ischemic stroke.[15] The impact of placental inflammatory changes is less clear. Those affecting the fetal side of the placenta, histologically known as funisitis, are six times higher in neonates with encephalopathy than in healthy term newborns (31.4% vs. 4.4–5.4%); however, alterations affecting the maternal side, also referred to as chorioamnionitis, are only twice as common.[16] Based on these observations, abnormal fetal vascular perfusion and inflammatory changes can be associated with neonatal encephalopathy.[18–20]

TABLE 55.1 Disorders Associated With Neonatal Encephalopathy

1. Hypoxic-Ischemic Encephalopathy
   - Acute perinatal event (sentinel event)
   - Subacute event
2. Vascular/Inflammatory
   - Arterial ischemic stroke
   - Acute hemorrhagic stroke/parenchymal hemorrhage
   - Cerebral venous sinus thrombosis
   - Severe maternal or fetal anemia
3. Metabolic
   - Hyperbilirubinemia
   - Electrolyte disturbances:
     Hypoglycemia
     Hyponatremia
     Hypocalcemia
   - Inborn errors of metabolism with toxic substrate accumulation
   - Inborn errors of metabolism leading to energy deficient state
4. Genetic
   - Malformations
   - Copy number variations
   - Single gene mutations
5. Infectious
   - Meningitis
   - Encephalitis
   - Ventriculitis

TABLE 55.2 Risk Factors Associated With an Increased Risk for Neonatal Encephalopathy and Hypoxic-Ischemic Encephalopathy

Maternal

Advanced maternal age
Pre-eclampsia/eclampsia
Prolonged second stage of labor
Shock
Cardiac arrest

Uteroplacental

Uterine hyperstimulation
Chorioamnionitis
Chronic vasculitis
Funisitis
Placental insufficiency
Placental chronic villitis
Fetal-maternal hemorrhage

Intrapartum Sentinel Event

Placental abruption
Uterine rupture
Prolapsed cord
Tight nuchal cord
Shoulder dystocia

Fetal

Category III fetal heart rate tracing
Congenital heart disease
Inborn errors of metabolism
Perinatal stroke or thrombosis
Hemolytic disease
Infection
Genetic syndromes
Small for gestational age/intrauterine growth restriction
Large for gestational age

Hypoxic-Ischemic Encephalopathy

Epidemiology

Birth asphyxia is one of the leading causes of neonatal mortality worldwide, accounting for an estimated 20–30% of neonatal deaths.[21,22,24] Hypoxic-ischemic encephalopathy (HIE) accounts for an estimated 25–45% of neonatal encephalopathy and is the most common form of acute brain injury in newborns.[25] The incidence of HIE is approximately 1–3:1000 term/near-term live births[26–28] in high-resource countries and as high as 31:1000 live births in low-resource settings.[29]

Motor disabilities and long-term neurodevelopmental impairment (NDI) including cognitive, neuropsychological, epilepsy, educational, and behavioral problems are common in surviving neonates with moderate or severe HIE.[30,31] Despite advances in therapeutic intervention, 31–55% of neonates with moderate or severe HIE still experience significant sequelae and either don't survive the neonatal period (17–38%) or suffer from significant neurodevelopmental impairment (14–35%), including intellectual disabilities, cerebral palsy (13–32%), and seizures.[32–40] Brain injury secondary to hypoxia-ischemia during labor can be seen in up 24% of term-born children with cerebral palsy.[41] The emotional impact and costs associated with medical and rehabilitative care of infants with HIE are substantial.[42] The Centers for Disease Control and Prevention estimated the lifetime costs for all people with cerebral palsy born in 2000 to be US $14.7 billion, which equals approximately US $1.2 million per person with cerebral palsy.[43]

Pathophysiology

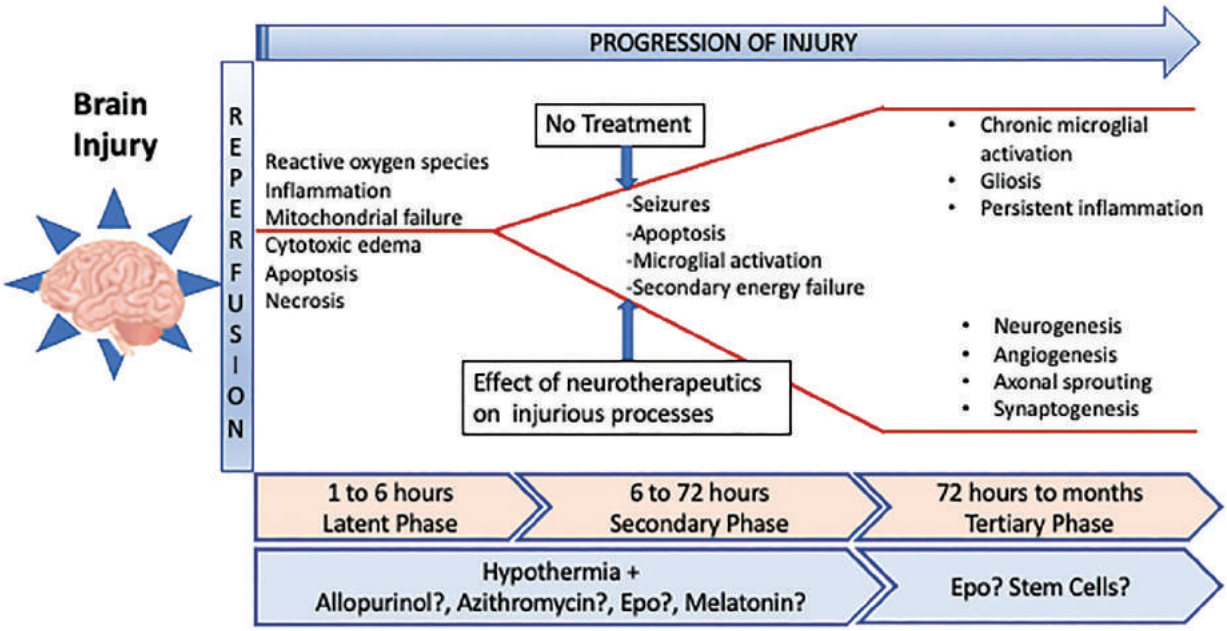
HIE results from a significant intrapartum hypoxic-ischemic sentinel event (acute or prolonged). Factors contributing to such events are plentiful and can be divided into maternal, utero-placental, and fetal in origin (see Table 55.2). Maternal and utero-placental causes are often sudden and severe and are estimated to occur in one-third of HIE cases.[43a]

Factors that increase the risk of a sentinel event include maternal medical conditions associated with impaired placental perfusion such as chronic hypertension, diabetes, autoimmune diseases, preeclampsia, and alterations originating from within the placenta leading to inflammatory changes, such as chorioamnionitis, villitis, vasculitis, and funisitis.[16,44,45] Intrapartum clinical inflammatory factors, such as maternal fever, chorioamnionitis, and prolonged rupture of membranes, may also increase the risk and severity of HIE.[27]

Regardless of the cause, significant hypoxia-ischemia can lead to cardiac and vascular compromise, with subsequent diminished cerebral perfusion and oxidative metabolism, energy failure, cell death, and depending on duration and extent, may result in hypoxic-ischemic brain injury.

The pathophysiologic changes after an acute hypoxic-ischemic event follow characteristic phases defined as primary injury, latent, secondary, and tertiary phases (Fig. 55.1).[46] The primary injury phase is characterized by decreased cerebral oxygen and glucose delivery as the immediate result of compromised cerebral perfusion.[47–49] Glycolysis in an oxygen-poor environment leads to depletion of adenosine triphosphate (ATP), and accumulation of lactic acid. Cellular mechanisms dependent on ATP, such as Na^+/K^+ ATPase pumps, can no longer maintain their functions. This results in depolarization of neuronal cell membranes with a subsequent increase in intracellular sodium and calcium concentration, which generates osmotic and electrochemical gradients across the cell membrane and leads to cytotoxic edema.[48] Induction of destructive enzymes (e.g., phospholipases, proteases, and endonucleases) also occurs. Activated phospholipases (e.g., phospholipase A2) hydrolyze cellular membrane phospholipids and release free fatty acids such as arachidonic acid, which can further increase glutamate release, perpetuate membrane peroxidation, and activate N-methyl-D-aspartate (NMDA) receptors, which results in a further increase in intracellular calcium. Abnormally high intracellular calcium concentrations have toxic effects on intracellular organelles, particularly the mitochondria,[50] and stimulate the activation of nitric oxide synthase resulting in increased production of reactive oxygen species (ROS), including nitric oxide (NO). NO damages the mitochondrial membrane leading to devastating mitochondrial dysfunction.[51] The consequences for cellular health and metabolism are significant and include the induction of necrotic and apoptotic pathways as well as stimulation of the inflammatory cascade.[47,52]

The next phase of injury, referred to as the latent phase, is characterized by reperfusion with active suppression of cerebral



• **Fig. 55.1** Pathophysiologic stages, mechanisms, and opportunities for therapeutic intervention in hypoxic-ischemic encephalopathy in relation to evolution over time.

metabolism. A partial rebound of neuronal oxidative energy metabolism occurs by meeting a decreased demand, leading to improvement of cytotoxic edema, and increased cerebral tissue oxygenation.[53,54] While the neurotoxic cascade is suppressed during this stage, increased perfusion and oxygenation continue to contribute to additional ROS production.[55,56] This phase occurs between 1 and 6 hours after insult and is the optimal window in which to initiate therapeutic hypothermia.[51]

The following, secondary phase, is characterized by secondary energy failure, ongoing inflammation, oxidative injury, and excitotoxicity. This phase may be observed as early as 6 hours after the initial insult and typically lasts for multiple days. While some repletion of energy sources takes place during the latent phase, rapid depletion of these resources occurs again during the secondary phase leading to cytotoxic edema, mitochondrial failure, and accumulation of excitotoxins and cytokines, ultimately resulting in delayed cell death.[57,58] Ongoing injury during secondary energy failure is thought to be directly related to the extent of primary energy failure,[59] partially mediated by neuroglial activation after early neuronal cell death. This leads to the release of proinflammatory cytokines and ROS, which attracts peripheral immune cells that can enter the parenchyma via the disrupted blood-brain barrier and facilitate cell death.[27] The degree of secondary energy failure and degree of cellular energy deficit determines the extent and pathway of neuronal death, for example, necrosis, apoptosis, and autophagy, all of which occur on a continuum.[58,60]

The tertiary phase is characterized by reparative and restorative processes. This phase can last weeks to years. The immune response shifts towards the healing of the neuroinflammatory process, with microglia exerting their phagocytic capabilities alongside penetrating peripheral macrophages. Phagocytosis of dead cells triggers the release of anti inflammatory cytokines and the production of neuronal growth factors, an essential component of the remodeling process that includes neurogenesis, angiogenesis, and axonal sprouting.[27,59]

Clinical Presentation

Newborns who have undergone hypoxic-ischemic compromise often present with encephalopathy, although other organ systems may be affected. Obtaining a careful pregnancy and labor history to identify any acute sentinel events complicating labor and/or delivery such as decreased fetal movement, an abnormal fetal heart rate pattern (bradycardia or category III tracing), or meconium passage prior to delivery may provide important information regarding the timing of the insult.

Following delivery, newborns with HIE commonly present with respiratory failure requiring positive pressure ventilation for a prolonged period of time (≥10 minutes), often needing endotracheal intubation with assisted ventilation. Hypoxia also leads to cardiac and vascular compromise, and newborns with HIE may therefore present with persistent bradycardia or even asystole requiring cardiopulmonary resuscitation including epinephrine administration. The cardio-respiratory compromise is reflected in persistent low Apgar scores (≤5) by 10 minutes of age. Laboratory biomarkers of hypoxia-ischemia include significant fetal acidemia (pH ≤ 7.0 and/or a base deficit of ≥12) and an elevated serum lactate diagnosed from either arterial or venous umbilical cord blood samples, or an arterial or venous sample obtained from the newborn within the first postnatal hour.

At birth, neonates with HIE are commonly depressed and manifest clinical symptoms consistent with neurologic injury. The clinical picture evolves significantly over the first hours after birth. When assessing a depressed neonate for therapeutic intervention, the worst exam between one and six hours of life is the most informative regarding the severity of HIE and eligibility for therapeutic hypothermia. It is, however, important to note that the exam continues to evolve over the first week of life and becomes more predictive for long-term outcomes towards the end of the first week. The Modified Sarnat Exam (Table 55.3A) and the Thompson Encephalopathy Score (Table 55.3B) are the standardized neurologic assessments used to assess the severity of

TABLE 55.3A Modified Sarnat Exam

Category	Normal	Mild	Moderate	Severe
Level of Consciousness	Normal	Hyperalert or irritable	Lethargic or poorly responsive	Minimal or no responsiveness
Spontaneous Activity	Normal	Slightly decreased	Decreased	Absent
Posture	Normal	Mild distal flexion	Distal flexion, complete extension	Decerebrate
Muscle Tone	Normal	Hypertonic	Hypotonic	Flaccid
Primitive Reflexes				
1. Suck reflex	1. Normal	1. N/A	1. Weak or bite	1. Absent
2. Moro reflex	2. Normal	2. Low threshold to elicit	2. Weak or incomplete	2. Absent
Autonomic Reflexes				
1. Pupils	1. Normal	1. N/A	1. Constricted	1. Dilated and either fixed or sluggishly reactive; asymmetric
2. Respirations	2. Normal	2. N/A	2. Periodic breathing	2. Intubated and ventilated

TABLE 55.3B Thompson Encephalopathy Score

Category	Score 0	Score 1	Score 2	Score 3
Muscle tone	Normal	Hypertonicity	Hypotonicity	Flaccid
Level of consciousness	Normal	Hyperalert, stare	Lethargic	Comatose
Seizures	None	Infrequent <3/day	Frequent >2/day	N/A
Posture	Normal	Fisting, cycling	Strong distal flexion	Decerebrate
Moro reflex	Normal	Partial	Absent	N/A
Grasp reflex	Normal	Poor	Absent	N/A
Suck reflex	Normal	Poor	Absent ± bites	N/A
Respirations	Normal	Hyperventilation	Brief apnea	Apnea, ventilated
Fontanel	Normal	Full, not tense	Tense	N/A

HIE and eligibility for therapeutic hypothermia.[61,62] The Modified Sarnat Exam, or alternatively the Thompson Encephalopathy Score, can then be followed sequentially over the first week of life to monitor changes in neurologic status.

Neonates with mild encephalopathy appear hyperalert and irritable with wide-open eyes, often with a "stunned look" or a blank stare, and dilated pupils. An increased muscle tone and a heightened Moro reflex reactive to tactile and sensory stimuli are observed. Neonates with moderate encephalopathy appear lethargic with low tone, a weak suck, constricted pupils, and a decreased Moro reflex. Neonates with severe encephalopathy appear stuporous, flaccid and have decerebrate posture, absent reflexes (suck, gag, and Moro), and poorly reactive pupils. Respiratory disturbances are also common with moderate and severe HIE, with periodic breathing and apnea present in most affected neonates.[63]

HIE is the most common cause of seizures in the neonatal period. Seizures are often subclinical or subtle in presentation, manifesting as apneic events, vital sign instability, or less commonly, with stereotypic movements such as abnormal eye movements (e.g., tonic, horizontal eye deviation with or without jerking, eyelid blinking), oral-buccal-lingual movements (e.g., sucking or lip smacking), or limb movements (e.g., finger flicking, swimming motions or bicycle pedaling). Seizures occur in 56–88% of patients with HIE,[64–69] are subclinical in 43–67%,[70,71] and progress to status epilepticus in 23–67%.[68,70,72,73]

Other organ system involvement is commonly seen in neonates with HIE including cardiac dysfunction, respiratory failure, acute kidney injury, abnormal glucose homeostasis, syndrome of inappropriate antidiuretic hormone secretion (SIADH), liver dysfunction, and disseminated intravascular coagulopathy (DIC).

Evaluation

Laboratory tests and ancillary studies are pertinent in assessing and treating newborns with suspected HIE, particularly since multisystem organ involvement is common. Metabolic derangements such as hypoglycemia, hypocalcemia, hyponatremia, hypomagnesemia, hypoxemia, decreased P_{CO_2} and metabolic acidosis are frequently seen in the first 24 hours after birth. Prompt corrective intervention may avoid further compromise. Serum lactate is often significantly elevated following hypoxia or ischemia, reflecting the anaerobic metabolism of glucose for energy in the setting of decreased tissue oxygenation. A complete blood count may demonstrate leukopenia or leukocytosis, which may suggest

infection, anemia (e.g., in cases of known or suspected abruption), or thrombocytopenia, which may also occur with DIC. Coagulation studies to assess for DIC and to guide clinical management should include prothrombin time (PT), activated partial thromboplastin time (aPTT), international normalized ratio (INR), and fibrinogen level. Liver function may be abnormal and should be assessed by monitoring serum alanine transferase (ALT), aspartate transferase (AST), particularly since many medications given to critically ill newborns are metabolized in the liver. ALT and AST typically don't reach their peak level until 24 to 48 hours after the insult, hence daily monitoring of liver function is crucial for appropriate medication adjustment. Initial newborn creatinine levels may reflect maternal values, but a rising creatinine, especially in the setting of poor urine output, is consistent with acute kidney injury (AKI) and provides pivotal information for managing renally cleared pharmacologic agents in the critically ill newborn. Myocardial perfusion is frequently compromised in neonates with HIE and can lead to decreased ventricular function and reduced cardiac output in as many as 75–100% of patients with HIE,[74,75] Electrocardiogram and cardiac enzymes (serum creatine kinase, creatine kinase-MB isoenzyme, and troponin I) are good markers to assess cardiac dysfunction after perinatal asphyxia, and abnormalities are seen in up to 90% of neonates with HIE; those changes are more significant as the severity of HIE increases.[76,77] However, echocardiography remains the preferred method for assessment of cardiac compromise in this population, allowing for serial examinations and individually targeted treatment.[75] Furthermore, neonates with HIE often have decreased heart rate variability on continuous electrocardiogram, which is associated with the severity of injury and outcome.[78,79]

Neuromonitoring

Electroencephalography

Given the high incidence of seizures and frequent lack of clear clinical expression in this high-risk population, the American Clinical Neurophysiology Society recommends that all neonates with HIE undergoing therapeutic hypothermia are monitored with electroencephalogram (EEG) throughout cooling and rewarming,[80] preferentially as continuous video-EEG (cEEG). Alternatively, in centers where cEEG is unavailable, amplitude-integrated EEG (aEEG) may be used.

Prompt recognition and treatment of HIE-related seizures are indicated, as increased seizure burden is associated with increased brain injury on magnetic resonance imaging (MRI) and worse long-term outcomes independent of the degree of HIE.[81–83] Moreover, acute symptomatic seizures become more difficult to treat the longer they last, which may lead to increased exposure to antiepileptic medications and associated side effects such as respiratory depression and hemodynamic compromise, in addition to worsening brain injury. Seizures in neonates with severe HIE tend to be longer in duration and more frequent, resulting in a higher seizure burden compared to neonates with moderate HIE.[84] Therapeutic hypothermia does not affect the incidence of seizures (54% vs. 60%),[85] but does significantly decrease the seizure burden, particularly in neonates with moderate HIE.[66,68,85,86]

Near-Infrared Spectroscopy

Near-infrared spectroscopy (NIRS) allows noninvasive, continuous bedside monitoring of cerebral hemodynamics and oxygenation by measuring the absorption spectra in the near-infrared region of light (700 to 1000 nm) of oxygenated and deoxygenated hemoglobin (Hgb) in the cortical tissue. The calculated percentage ratio of oxygenated Hgb to total Hgb (oxygenated plus deoxygenated Hgb) is commonly referred to as cerebral oxygenation.[87–89] Regional cerebral blood flow measured by MRI showed a strong correlation with mixed venous saturation values measured by NIRS in neonates with HIE undergoing therapeutic hypothermia.[90] Further observations in neonates with HIE showed that cerebral oxygenation decreases in the first 4 to 6 hours of life and then slowly increases within the first 24 hours.[91] The initial decrease is less pronounced in more severely affected newborns, likely due to a lack of metabolic activity within the severely injured brain.[92,93] Correlations with injury on MRI imaging and long-term outcome have shown that neonates who continue to have increased cerebral tissue oxygenation after 24 hours have more significant injury on MRI and less favorable neurodevelopmental outcome at 2 years.[87,94,95]

Neuroimaging

Ultrasound

Cranial ultrasonography (CUS) can be used as a first-line imaging tool in newborns with HIE since it can be performed at the bedside in critically unstable neonates. However, extra-axial structures are poorly visualized, and the sensitivity and specificity of CUS findings in HIE are low and correlate poorly with long-term outcomes.[96] White matter echogenicity detected by early CUS suggests antenatal events rather than acute injury at the time of birth.[97] The accuracy of CUS increases towards the middle and end of the first week of life.[98] Significant cerebral edema may be demonstrated as increased focal or diffuse brain parenchyma echogenicity, slit-like ventricles, and obliteration of the extracerebral cerebrospinal fluid (CSF) spaces and the interhemispheric fissure. Cerebral edema can be seen secondary to HIE, metabolic encephalopathies, seizures, or infections, among other causes. While signs of HIE may be difficult to detect with CUS, this imaging modality may provide preliminary information about brain malformations or other potential causes of neonatal encephalopathy that may clinically resemble HIE.[99] Although CUS is a valuable tool to assess neonates with suspected brain injury, MRI is the study of choice in the diagnostic assessment of neonates with HIE.

Magnetic Resonance Imaging and Magnetic Resonance Spectroscopy

In encephalopathic newborns, MRI done at 4 to 5 days after birth, and following rewarming from therapeutic hypothermia, can be used to assess for injury patterns consistent with HIE and rule out other causes, including neurogenetic, neurovascular, or inflammatory diseases.[100] A combination of conventional imaging sequences (T1- and T2-weighted images), diffusion-weighted imaging (DWI), diffusion tensor imaging, plus magnetic resonance spectroscopy (MRS) can provide information on brain microstructure, connectivity, and metabolism and can assist with determining the timing and severity of brain injury in newborns with HIE.[101,102] MRI performed in the newborn period has a high predictive value for subsequent neurologic impairment at 18 months of age. However, the timing is important, and MRI should ideally be performed on postnatal days 4 to 6. DWI, which measures the random Brownian motion of water molecules within a voxel of tissue, can identify cytotoxic edema and ischemic brain neonatal tissue days before T1 and T2 sequences.[104] Pseudo normalization of the brain MRI occurs after the first week and may underestimate the final extent of basal ganglia and thalamic

lesions.[97,103,105–107] MRS is a tool to assess brain metabolism and is usually focused on a region of interest most commonly the basal ganglia. Elevated lactate peaks on MRS have been associated with poor long-term outcome, while N-acetyl aspartate (NAA), a marker of neuronal viability, is commonly decreased when neuronal loss is observed. The ratio of lactate to NAA can be helpful in estimating the severity of injury and predict long-term outcome.[108]

Characteristic patterns of brain injury seen in HIE give valuable information about onset, extent, and evolution of brain injury, and can be predictive of outcome. The neuropathology and pathogenesis of neonatal brain injury associated with HIE have been thoroughly described by Volpe, who summarizes three major patterns of injury based on historic fetal monkey and human studies.[109–113] The three injury patterns include (1) diffuse, (2) cerebral cortical-deep nuclear, and (3) deep nuclear-brain stem.

A *deep nuclear pattern* of injury is observed in the case of abrupt onset (within 10 to 46 minutes) of complete asphyxia. An example of such a sentinel event is a cord prolapse. The *cerebral cortical-deep nuclear pattern* can be observed after partial and prolonged asphyxia. Sentinel events corresponding to injury in the cerebral cortex and deep gray matter (basal ganglia and thalamus) evolve over longer periods, often hours. If this insult is prolonged and/or profound, additional effect on the brain stem can be observed and this injury pattern is referred to as *deep nuclear-brainstem pattern*.

The parasagittal cerebral injury pattern, also referred to as a *watershed predominant pattern*, is found after subacute partial and prolonged intrapartum hypoxia and involves the parasagittal white matter, in severe cases also the cortical gray matter.

Computed Tomography

Computed tomography (CT) lacks the sensitivity to evaluate the characteristics and extent of brain injury in term encephalopathic infants and is therefore rarely used to assess newborns with HIE.[97,102] An advantage of CT over CUS is that it can detect hemorrhages in areas where CUS is difficult to apply and can visualize the extra-axial regions better. An advantage of CT over MRI used to be the speed at which it can be done. Newer limited-sequence (rapid) MRI protocols are now of similar duration and cost. MRI can detect hemosiderin depositions via susceptibility-weighted images (SWI) and ischemic stroke on DWI more accurately than CT while sparing the developing brain from radiation exposure.[97,114,115]

Management

Neonates with HIE are frequently critically ill and demonstrate signs of multiorgan involvement. For that reason, the initial management steps should focus on promoting adequate oxygenation, ventilation, circulation, and correction of metabolic derangements. Myocardial ischemia can result in a low cardiac output state. Cardiovascular instability and hypotension are commonly observed in affected patients.[49,75] Continuous blood pressure monitoring facilitates prompt recognition of systemic hypotension and timely treatment to avoid additional compromise. Treatment options include inotropic agents as well as support with hydrocortisone. Hydrocortisone may be beneficial given the common compromise of the adrenal glands in HIE patients.

The newborn with HIE oftentimes shows signs of respiratory compromise secondary to the ischemic effects on the lungs, impairment of the central respiratory system, and frequent exposure to meconium-stained amniotic fluid. The resulting clinical symptoms include apnea, periodic breathing, impaired gas exchange, and pulmonary hypertension. Hypoxemia is particularly profound in the initial phase and improves with amelioration of the acidosis. Despite respiratory depression, newborns with HIE are frequently hypocarbic because of respiratory compensation of the metabolic acidosis. This can result in further compromise of the cerebral autoregulation and exacerbation of brain injury. Therefore, tight management of respiratory status and blood chemistry is crucial in this critically ill population.

The fluid and electrolyte status may become imbalanced due to cerebral edema and secondary SIADH. Hypoxic injury to the kidneys can also lead to AKI, which presents with oliguria or anuria, fluid retention, and subsequent electrolyte abnormalities, particularly hyponatremia. When profound, hyponatremia can result in seizures, further exacerbate brain injury, and worsen the cerebral edema. Therefore, fluid and electrolyte management are critical to avoid additional compromise, and significant temporary fluid restriction might be necessary.[49]

Glucose homeostasis is often disrupted in neonates with HIE. Hypoglycemia is an emergency and requires prompt correction as it can exacerbate brain injury and induce seizures.

Seizures can manifest clinically, but more often lack a clinical correlate and can be detected by EEG only. Prompt treatment of the acute symptomatic seizures and correction of electrolyte derangements (hypoglycemia, hyponatremia, and hypocalcemia) is pivotal to avoiding further injury.[116]

Liver injury and DIC are frequently observed in neonates with HIE. DIC warrants monitoring and when associated with bleeding, requires immediate treatment. Liver dysfunction impairs the production of clotting factors and additional vitamin K might be necessary to assist with the production of vitamin K-dependent clotting factors until either the liver injury is recovering or DIC resolves. Abnormal renal and hepatic function have a significant impact on metabolism and clearance of medications commonly used in this critically ill population (e.g., phenobarbital, gentamicin, and furosemide).[117]

Hypothermia Therapy

Hypothermia is currently the only proven neuroprotective therapy for HIE. Hypothermia decreases cellular metabolism by 5–8%/°C,[118,119] attenuates the inflammatory reaction, stabilizes the blood-brain barrier, and reduces glutamate and oxygen free radicals.[48,120] In addition, hypothermia has been shown to have anti-epileptic effects.[121] The beneficial effect of therapeutic hypothermia is most pronounced when applied before the onset of the secondary energy failure, which is approximately within 6 hours of birth, preferably as soon as possible after injury.[122] Applying hypothermia within 6 hours of birth to maintain the core body temperature at 33.5 ± 0.5°C for 72 hours followed by slow rewarming at a rate of 0.5°C/h to normothermia, has been shown in numerous randomized controlled trials to improve outcomes in neonates with moderate to severe HIE.[36,123–125]

Mild cooling has an effect on all organ systems, and physiologic changes are commonly seen after the initiation of therapeutic hypothermia (Table 55.4).[34,36,38–40,123,124,126,127] Alteration in pharmacokinetics, particularly enzyme-dependent metabolism (e.g., cytochrome P450—phenobarbital, fentanyl, midazolam, phenytoin, corticosteroids, and vecuronium) and enzyme facilitated conjugation (e.g., hepatic glucuronidation—morphine), is affected by hypothermia.[126] Therefore, particular attention needs to be given to pharmacotherapy during therapeutic hypothermia.

In animal studies, decreased blood flow to the intestines was described during hypothermia, in human neonates; however,

TABLE 55.4 Effects of Hypothermia

	Effect	Mechanism
Metabolic rate	↓ 5–8% per 1°C below 37.0°C	Decreased—primary energy conserving mechanism
Cerebral blood flow	↓ 5% per 1°C below 37.0°C	Decreased cardiac output and metabolic rate
Heart rate	↓ 10 bpm per 1°C below 37.0°C	Slowing of conduction through the sinoatrial node
Cardiac output	↓ 7% per 1°C below 37.0°C	Decreased metabolic rate
Myocardial contractility	↑	Increased myofibrillar sensitivity to calcium
Peripheral vascular resistance	↑	Peripheral vasoconstriction
Pco_2	↓ 2 mmHg per 1°C below 37.0°C	Increased solubility of gases (Henry's law with Siggaard Andersen correction)
Po_2	↓ 5 mmHg per 1°C below 37.0°C	
pH	↑ 0.015 per 1°C below 37.0°C	
Alteration of platelet function	Mildly thrombophilic	↑ Platelet aggregation
Medication clearance	Delayed clearance at 34°C: • Morphine 25% • Phenytoin 67% • Phenobarbital 99%	Altered CYP450 enzymes and glucuronidation

therapeutic hypothermia has not been associated with an increased risk of necrotizing enterocolitis either with hypothermia alone or in conjunction with initiation of enteral feedings during cooling therapy.[128–130]

Subcutaneous fat necrosis has been described in neonates undergoing therapeutic hypothermia, particularly on the back and shoulders, with an incidence of 2.8% in the Swiss cooling registry.[131] Strict nursing care protocols and awareness can decrease the frequency.[132]

Studies evaluating a different duration (120 hours vs. 72 hours) and depth (32.0°C vs. 33.5°C) of therapeutic hypothermia[133] or later initiation between 6 and 24 hours of life[134] have not shown benefit but did show potential harm.

Since therapeutic hypothermia is most effective when implemented within 6 hours after delivery, timely referral to a center with a therapeutic hypothermia program is crucial. Passive cooling can be done before active cooling with close monitoring of core temperature. However active cooling during transport is preferred as patients reach the target temperature earlier and are less likely to be over or undercooled.[135–137]

Centers offering therapeutic hypothermia should have access to cEEG or aEEG, a trained pediatric neurologist, MRI with neuroradiology interpretation, and access to ancillary services such as physical therapy, speech therapy, and high-risk infant follow-up programs as recommended by the American Academy of Pediatrics[138] and the state of California.[139]

The use of medication to provide sedation and prevent shivering in cooled newborns with HIE is controversial and needs to be studied in more detail. The current use of morphine during therapeutic hypothermia is not evidence-based and may not be ideal due to its side effect profile (e.g., respiratory depression, urinary retention, constipation) and because it does not specifically prevent shivering. Dexmedetomidine and clonidine both α_2-adrenergic receptor agonists, are promising alternative sedatives because they specifically prevent shivering without suppressing respirations. Dexmedetomidine also reduces inflammation,[140,141] without producing abnormal brain histology,[142] and provides neuroprotection in animal models of HIE.[143–145]

Outcomes

Prior to therapeutic hypothermia, mortality for infants with moderate to severe HIE surpassed 60% and the disability rate has been described to be as high as 100% in neonates with severe HIE. With the introduction of therapeutic hypothermia, the death and disability rates have improved significantly. A Cochrane metaanalysis included 1505 term and late preterm infants with moderate to severe HIE from 11 randomized controlled trials and concluded that therapeutic hypothermia results in fewer deaths (25% compared to 34%, risk ratio [RR] of 0.75; 95% CI 0.65–0.88 and better neurodevelopmental outcomes in survivors.[125] For infants with moderate to severe HIE, outcomes at 18 to 24 months (assessed in seven and eight studies in a Cochrane review[125]) and at 6 to 7 years (assessed in two major studies, the NICHD[146] and the TOBY-Xe trial[147]) are summarized in Table 55.5. The number needed to treat (NNT) to prevent death or moderate to severe neurodevelopmental disability in one newborn is 7 (95% CI 5–10). Based on the proven benefits, therapeutic hypothermia is now standard of care for term and near-term newborns with moderate to severe HIE in high-resource environments.

Of course, outcomes vary by severity of presenting HIE. Mild HIE has now been recognized as causing significant brain injury in 18–38% of affected infants on MRI.[148–151] There remains controversy as to whether these infants should be routinely cooled. Common practice trend leans clearly towards cooling,[149] however, the benefit of therapeutic hypothermia for mild HIE remains under investigation.

In general, neonates who have a quick clinical recovery, a normal EEG background pattern, a normal MRI, and a normal neurological examination at 7 to 10 days of age often have a favorable long-term outcome. An early abnormal neurologic examination has limited long-term predictability, in contrast to an abnormal examination noted at the time of discharge which correlates significantly with adverse outcomes.[152] Excellent prediction of outcomes at 18 to 24 months can be made by combining early laboratory data, the need for anticonvulsant and pressor support with MRI evaluation of cortex, basal ganglia/thalami, white matter, and posterior limb of the internal capsule.[153]

Electroencephalogram Findings and Seizures

Seizures are common in neonates with HIE and are independently associated with worse outcomes. Seizures occur more frequently in neonates with severe compared to moderate HIE; the total seizure burden is about 2.5× higher in severe HIE compared to

TABLE 55.5 Outcomes of Neonates With Moderate to Severe Hypoxic-Ischemic Encephalopathy at 18–24 Months and 6–7 Years

	18–24 MONTHS			6–7 YEARS		
Outcome	**Hypothermia**	**Control**	**RR (95% CI)**	**Hypothermia**	**Control**	**RR (95% CI)**
Death overall (moderate and severe HIE)	27%	37%	0.73 (0.61–0.89)	29%	35%	0.81 (0.63–1.04)
Death (moderate HIE only)	14%	23%	0.60 (0.41–0.88)	NA	NA	NA
Death (severe HIE only)	53%	68%	0.77 (0.64–0.93)	NA	NA	NA
Major neurodevelopmental disability overall (moderate and severe HIE)	26%	39%	0.67 (0.67–0.80)	29%	49%	0.60 (0.44–0.81)
Major neurodevelopmental disability (moderate HIE only)	27%	39%	0.67 (0.5–0.90)	NA	NA	NA
Major neurodevelopmental disability (severe HIE only)	37%	51%	0.75 (0.50–1.12)	NA	NA	NA
Cerebral palsy	23%	35%	0.66 (0.54–0.82)	20%	33%	0.59 (0.40–0.87)

HIE, Hypoxic-ischemic encephalopathy; *NA*, not available.

moderate HIE and the duration of individual seizures is longer. The onset of seizures in moderate to severe HIE occurs around 13 hours of life,[64] and in ~10% of patients, seizures are seen for the first time during rewarming.[116] Maximum seizure burden is commonly observed approximately 4 hours after onset of seizures. Kharoshankaya et al. calculated that for every minute of seizures, the odds of an abnormal outcome increased by 2.2%.[64]

In general, the seizures in this population are acute symptomatic and prolonged treatment with antiepileptic medications after seizure resolution is not necessary.[2] However, 4–16% of newborns with HIE and acute symptomatic seizures will go on to develop childhood epilepsy or infantile spasms.[67,154] EEG background activity and the evolution over the first few days of life have been shown to have prognostic value regarding the long-term neurodevelopmental outcome, as it reflects the severity of the injury. The assessment at or after 48 hours of life has been particularly helpful in prognostication.[155] For example, background pattern with persistently low voltage (around or below 5 μV), flat tracing (very low voltage below 5 μV), and burst suppression (discontinuous background pattern with periods of inactivity intermixed with higher amplitude bursts) after 48 hours is highly predictive of an abnormal outcome.[156,157] In contrast, normalization of background pattern, particularly within the first 24 hours is associated with a favorable outcome.[2,157] and return of sleep-wake cycling is seen as a favorable prognostic indicator.[155,156,158] A normal EEG pattern by 6 hours of age that remains normal has a 100% positive predictive value for a normal outcome at 2 years.[159]

Predictive Value of Magnetic Resonance Imaging and Spectroscopy

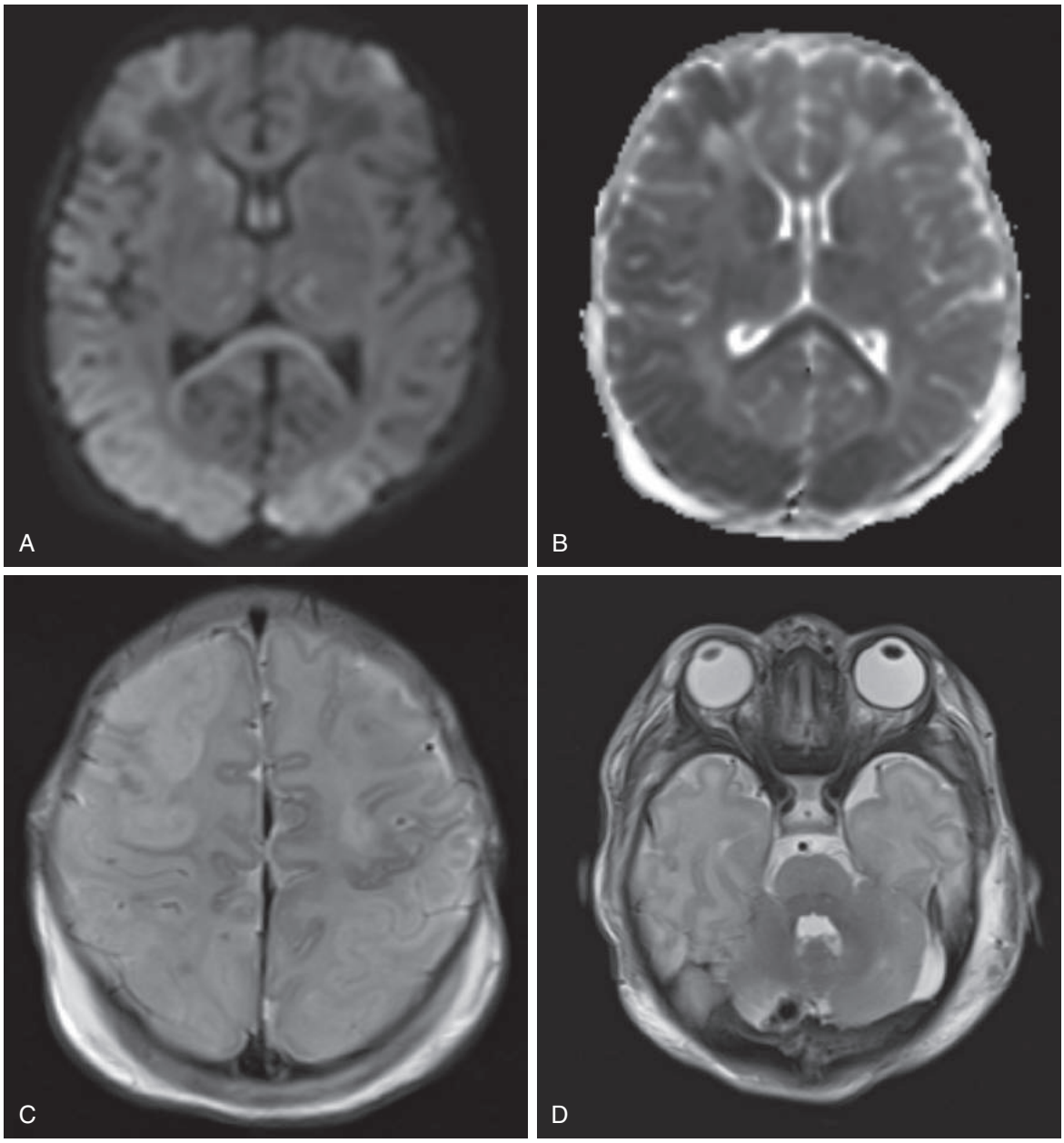
A normal MRI in the first week of life is associated with favorable outcomes, while outcomes with watershed or basal ganglia injury on MRI are more uncertain.[160–162] Watershed patterns are associated with less severe outcomes, whereas deep gray matter involving the thalamus and basal ganglia and abnormalities of the posterior limb of the internal capsule are associated with both motor and neurocognitive impairment at 2 to 3 years of age (Fig. 55.2).[163–167] MRS can also be helpful in prognosticating, as a low NAA peak and high lactate peaks correspond to severe injury and neurodevelopmental outcomes at ≥12 months of age when measured in the basal ganglia.[108,168]

Adjunctive Neuroprotective Treatments Plus Therapeutic Hypothermia

While therapeutic hypothermia has clear and important therapeutic benefits, the risk of poor outcomes from HIE remains significant. Investigators are therefore pursuing adjunctive treatments with the goal of further improving outcomes. In countries where therapeutic hypothermia is available, these therapies are being evaluated to be used in conjunction with hypothermia. In countries where therapeutic hypothermia is not available, these therapies may in some cases be considered as monotherapies (see Fig. 55.1).

Erythropoietin

Erythropoietin (Epo) is a glycoprotein originally identified for its role in erythropoiesis but also has remarkable neuroprotective and reparative effects in the CNS.[169,170] In the setting of hypoxia-ischemia, Epo receptor expression is rapidly upregulated.[171,172] If Epo is available to bind to the upregulated receptor, cell survival is promoted. However, in the absence of Epo, the pathway of programmed cell death predominates.[172,173] Epo has both early and late beneficial effects. Early benefits include anti-apoptotic and anti-inflammatory effects,[174–178] while later effects include increased neurogenesis, plasticity, and tissue remodeling after hypoxia-ischemia.[179–183] This creates an important rationale for exogenous Epo administration.



• **Fig. 55.2** A Term Male Neonate With Severe Hypoxic-Ischemic Encephalopathy. (A) Axial diffusion-weighted image and corresponding (B) apparent diffusion coefficient map shows widespread, patchy cytotoxic edema involving the cerebral cortex, deep nuclei, and corpus callosum. (C) Axial T2-weighted image shows patchy cortical T2-weighted signal abnormality as well. (D) Dark signal in the lower medial right cerebellar hemisphere seen on axial T2-weighted image indicates intraparenchymal cerebellar hemorrhage. (Images courtesy of Drs. Teresa Chapman and Francisco Perez, Seattle Children's Hospital, Seattle, WA.)

Pre-clinical studies of Epo neuroprotection following hypoxic-ischemic brain injury show robust histologic and functional evidence for benefit.[169,170,184–191] In nonhuman primates, Epo reduces the combined outcome of death or cerebral palsy and improves neurologic function in animals undergoing therapeutic hypothermia for HIE.[192] Phase I and II clinical trials have demonstrated safety and feasibility,[193] and suggest that infants with HIE treated with multiple doses of Epo during the first week of life have better neurologic outcomes as measured by early MRI, biomarkers, 6 months, and 12 to 24-month outcomes, even among infants with significant brain injury seen on MRI.[193–196] Phase III trials are now in the final stages of execution internationally, including the Preventing Adverse Outcomes of Neonatal Hypoxic Ischaemic Encephalopathy with Erythropoietin trial (PAEAN trial, Australia—clinicaltrials.gov: NCT03079167) and High-dose Epo for Asphyxia and Encephalopathy (HEAL trial, USA—clinicaltrials.gov: NCT02811263).

Xenon

Xenon, a noble gas that crosses the placenta and the blood-brain barrier, binds to *N*-methyl-D-aspartate glutamate receptors to inhibit function, thus decreasing neuronal apoptosis.[197–199]

Significant benefit from xenon was demonstrated in preclinical studies of HIE.[200] A small multicenter trial adding xenon as an adjunct therapy to therapeutic hypothermia was conducted in the United Kingdom.[201] Although no serious adverse events were recorded, no significant differences in magnetic resonance imaging were detected between groups. Based on the results, 30% xenon for 24 hours begun after 6 hours after birth combined with therapeutic hypothermia is not likely to improve clinical outcomes compared to TH alone for newborns with HIE. The inhibitory effect of xenon on glutamate receptors has anti-epileptic function and has shown to be clinical effective in a small subgroup of five neonates with seizures secondary to moderate to severe HIE in the TOBY-Xe trial.[202] Further studies on animals showed dose-dependent effect of xenon as anti-epileptic therapy after hypoxic injury and thus secondary neuroprotective benefits.[203] Multiple factors such as timing, dose, and duration of treatment may impact inhaled xenon treatment outcomes and need further investigation. Study results of the CoolXenon3 Study (clinicaltrials.gov: NCT02071394) combining TH with 18 hours of 50% xenon inhalation in cooled infants with HIE are pending.

Argon

Argon is a noble gas that has shown promise as an adjunct to hypothermia in preclinical models including in vitro and in vivo models of neonatal encephalopathy.[204,205] Argon is more abundant than xenon, cheaper, and more practical to use clinically as it does not require the complex rebreathing/scavenger setup needed for xenon. As of yet, no clinical trials using argon to treat any neurological injury are reported.

Melatonin

Melatonin exerts its neuroprotective function through anti-apoptotic and anti-oxidative properties.[206] Neuroprotective benefits have been demonstrated in an HIE piglet model, in which intravenous melatonin plus hypothermia significantly improved cerebral energy metabolism based on proton MRS studies, reduced apoptosis in deep brain structures, and decreased microglial activation in the cortex at 48 hours post-injury.[207] In uncooled full-term human newborns with HIE ($n = 10$), oral administration of melatonin (8 doses of 10 mg each separated by 2-hour intervals) started within the first 6 hours after birth reduced serum malondialdehyde, a lipid peroxidation product, and nitrite/nitrate levels at 12 and 24 hours compared to untreated, uncooled controls ($n = 10$), suggesting a role for melatonin in reducing oxidative damage.[208]

In a prospective trial of term newborns with HIE, melatonin (10 mg/kg daily × 5 enteral doses) plus TH ($n = 15$) compared to TH alone ($n = 15$), was associated with decreased seizures on EEG and fewer white matter abnormalities on MRI after 2 weeks of age as well as improved survival without neurological or developmental abnormalities at 6 months of age.[209] In a recent metaanalysis, a trend towards decreased mortality was observed when melatonin was given as an adjunct therapy to therapeutic hypothermia, compared to hypothermia alone.[210] No larger randomized controlled trials have been published yet, but two clinical randomized trials are currently in progress (clinicaltrials.gov: NCT03806816, NCT02621944).

Stem Cells

For several decades stem cells have promised to revolutionize the treatment of diseases from Alzheimer's to cerebral palsy.[211–213] Stem cells have several properties that provide neuroprotection (e.g., anti-apoptotic, anti-inflammatory, paracrine-neurotrophic) and regeneration (e.g., cell differentiation and replacement or regeneration of injured cells).[214] The therapeutic window for stem cell therapy has the potential to be much wider because the beneficial effects target mechanisms that extend well into the secondary and tertiary phases of HIE. Multiple types and sources of human stem cells have been studied in the context of brain injury. The umbilical cord blood contains several stem cell populations: mesenchymal stem cells (see below), endothelial progenitor cells, and umbilical cord blood mononuclear cells.

Neuroprotective mechanisms of mesenchymal stem cells include anti-inflammatory and immunosuppressive properties which mediate anti-apoptotic properties as well as neuro-regenerative functions.[215,216] Preclinical studies have shown promising results toward improved neurologic function.[217] A small clinical trial using autologous umbilical cord blood cells demonstrated feasibility and safety.[218] A phase II study showed that autologous umbilical cord blood-derived stem cells are a safe feasible adjunct therapy to therapeutic hypothermia and trends toward decreased death (11% vs. 24%) and improved neurodevelopmental outcomes (cognitive, language, and motor domain of the Bayley III >85) at 1 year (72% vs. 41%) were observed.[219] A larger phase II clinical trial was planned but stopped early due to poor enrollment. The results showed no difference in mortality between groups (5.9% in the intervention vs. 5.6% in the placebo group), but an improved neurodevelopmental outcome at 22 to 26 months, with 72% of participants having a Bayley III score of greater than 85 in the cognitive, language, and motor domain compared to 40% in the placebo group, was present (clinicaltrials.gov: NCT02612155).

Other stem cell types such as embryonic stem cells, neural stem cells, induced pluripotent stem cells, bone marrow-derived mesenchymal stem cells, and amniotic fluid-derived stem cells all have a potential benefit but ethical problems in obtaining those cells (e.g., embryonic or fetal tissue), time constraints in finding, preparing, and administering matching cells, as well as immunologic concerns as the source is most often not autologous, have made these cell types less feasible for neonates with HIE. However, neural stem cells derived from reprogrammed induced pluripotent stem cells could offer autologous use. The timing of administration of stem cells will need to be studied, as hypothermia may diminish their effect.

Cannabinoids

The activation of the endocannabinoid system decreases glutamate excitotoxicity, attenuates microglia activation, and reduces cell death.[220] Systemic cannabinoid administration in piglets with HIE improved oxygenation and EEG features.[221] At this time, however, no clinical studies in humans are ongoing.

Allopurinol

Allopurinol is a xanthine oxidase inhibitor, which decreases free radical and superoxide formation. Neuroprotective effects of allopurinol have been shown when given shortly after the ischemic insult in an HIE rat model.[222] In small clinical trials, the effect of allopurinol given within 4 hours of birth to neonates with moderate to severe HIE was equivocal, but trends towards improvement, particularly in neonates with moderate HIE, were seen.[223] Allopurinol might be more effective when given prior to reperfusion injury,[224] which is currently being evaluated in a phase III clinical trial (clinicaltrials.gov: NCT03162653).

Azithromycin

Azithromycin has anti-inflammatory neuroprotective effects through its immune-modulatory properties.[225] Pre-clinical trials in a rat model of HIE showed a dose-dependent reduction in brain injury and improvement in sensorimotor function.[226] Clinical trials to study the neuroprotective effects of azithromycin in neonates with moderate to severe HIE alone or in conjunction with therapeutic hypothermia are in the early phases.

Further ongoing research is targeting the inflammatory response, autophagy, and mitochondrial function. Optimal neuroprotection will likely include multiple targeted approaches at different times.

Other Causes of Neonatal Encephalopathy

Neurovascular disorders including perinatal stroke and cerebral sinus thrombosis are discussed in detail elsewhere (see Chapter 56).

Metabolic Causes of Neonatal Encephalopathy

Neonatal Hypoglycemia

Symptomatic hypoglycemia is a well-known cause of neurologic injury, but the exact glucose concentration or duration of hypoglycemia that will result in injury remains unclear. Blood glucose values in the fetus are 70% of maternal levels and rapidly fall in the first hour after birth to as low as 25 mg/dL, with a gradual increase over the next hours and days.[227] Glucose concentration in the brain is approximately 30% of the systemic blood concentration, and this level is tightly controlled via glucose transporter type 1 (GLUT1) since the intact blood-brain barrier prevents the free diffusion of glucose.[228]

Hypoglycemia in the newborn can be transient and physiologic or secondary to an underlying metabolic or endocrine disorder. Risk factors for prolonged and/or symptomatic hypoglycemia include small or large for gestational age, maternal diabetes, perinatal asphyxia, respiratory distress, sepsis, metabolic disorders, and congenital abnormalities, particularly midline defects. Hypoglycemia may be asymptomatic or can manifest as cyanosis, tremors, apnea, seizures, change in consciousness, irritability, high-pitched cry, altered muscle tone, and feeding problems.

The proposed mechanism of hypoglycemia-induced injury is hypoglycemia-induced neuronal depolarization and subsequent increase in presynaptic glutamate, which leads to excessive NMDA receptor activation. This activation induces increased intracellular sodium and calcium concentrations. Increased calcium influx into cells alters mitochondrial function and generates free radicals. ATP production is hampered, which leads to apoptosis and neuronal necrosis.[229] Hypoglycemia may result in brain swelling, necrosis, and white matter demyelination, especially in areas rich with NMDA receptors. On MRI, brain regions affected include the cerebral cortex, dominantly in the parieto-occipital region, corpus callosum, basal ganglia, thalamus, and posterior limb of the internal capsule.[230,231] The degree of injury is likely directly related to the depth and duration of hypoglycemia and the presence of any comorbidities, especially HIE.

Long-term sequelae associated with hypoglycemia include visual impairment, epilepsy, and cognitive deficits. While neurocognitive outcomes at 2 years of age between hypoglycemic and non-hypoglycemic neonates remain similar,[232,233] differences become more apparent during mid-childhood with odds of 3.62 for an abnormal neurodevelopmental outcome in hypoglycemic neonates.[232]

Inborn Errors of Metabolism

Most inborn errors of metabolism that manifest in the immediate neonatal period are accompanied by systemic symptoms including neurologic findings. Encephalopathy is commonly seen in affected infants due to the primary or secondary toxic effects of the involved metabolites (e.g., ammonia) or as a symptom of ongoing energy depletion in organs with high energy demand such as the brain and the heart in the case of mitochondrial disorders, respiratory chain disorders, or pyruvate dehydrogenase deficiency.[234]

Metabolic Encephalopathies due to Toxic Metabolite Accumulation

This extensive category includes a variety of metabolic disorders and can affect an array of metabolic pathways. Fetal development is rarely affected since the placenta clears most of the toxic substrates. Thus, malformations are uncommon, and the pregnancy appears uncomplicated. The newborn often appears well at birth, only to deteriorate over the initial days to weeks. Symptoms can be triggered by catabolic states, initiation of protein intake, or acute illness, depending on the underlying defect. Clinical symptoms are often rapidly progressive in the newborn period and are related to the accumulation of toxic metabolites. While many of the metabolic disorders in this category are now somewhat treatable, massive metabolite accumulation in the presenting stage can impact survival and can impair neurodevelopmental outcomes in survivors. Therefore, rapid removal of the toxic product before it causes permanent damage is crucial. Evaluation of plasma and urine amino acids, urine organic acid profile, and assessment of acylcarnitines can be diagnostic. Some of the more common disorders presenting with neonatal encephalopathy are described below.

Urea Cycle Disorders

Urea cycle disorders (UCDs) are loss of function defects of any of the urea cycle enzymes. The dominant source for ammonia detoxification is via the urea cycle, which converts excess ammonia into excretable urea and produces arginine. Urea cycle disorders are autosomal recessive disorders with the exception of ornithine transcarbamylase (OTC) deficiency, which is X-linked. An estimated 1:35,000 newborns are affected by UCD[235] and 27% become symptomatic in the neonatal period.[236] Neonates with absent urea cycle enzyme activity typically present after the first 24 hours of life with feeding difficulties and progressive lethargy or even coma, which is caused by the rapid accumulation of ammonia and subsequent development of cytotoxic edema and seizure.

Hyperammonemia leads to metabolic acidosis, which initially is often attempted to compensate for by the newborn clinically visible as tachypnea, and on blood gas as hyperventilation. Blood glucose levels are often normal. Diagnosis is made by obtaining plasma amino acids which often show an increase in glutamine and alanine and a decrease in citrulline and arginine. Subsequent targeted genetic testing allows to identify the individual enzyme defect. Outcomes are strongly related to the duration and extent of hyperammonemia. Therefore, the initial treatment has to focus on quickly and effectively decreasing ammonia levels (pharmacological and/or dialysis) and the prevention of further accumulation

(cessation of an exogenous protein supply, and provide a high energy supply to avoid endogenous protein catabolism). Seizures can also be present as ammonia has an epileptogenic effect, and therefore, EEG monitoring is recommended. On MRI, cerebral edema is the most common acute finding but changes in the white matter involving the deep sulci of the insular and peri-rolandic watershed territories can be seen. MR spectroscopy allows direct measurement of metabolites. The mortality of UCD is approximately 24% and neurocognitive morbidities vary among the defects. Global developmental delay and abnormal gross motor function are not uncommon.[236]

Methylmalonic Acidemia

Methylmalonic acidemia (MMA) is a deficiency of the mitochondrial enzyme methyl-malonyl–coenzyme A mutase (MCM), a deficiency of its cofactor adenosyl-cobalamin (*cblA* or *cblB*-MMA), or deficiency of the enzyme methylmalonyl-coenzyme A epimerase. The absence of one of the enzymes results in the accumulation of methylmalonic acid. MMA can present in the newborn who was healthy for the first day to weeks of life, with a presenting history of poor feeding, vomiting, progressive lethargy, and decreased muscle tone. The incidence is about 2:100,000 live births, and approximately 50% of patients become symptomatic in the neonatal period. Typical laboratory findings include significant metabolic acidosis, hyperammonemia, and plasma and urine ketones with or without hypoglycemia. Abnormal acyl-carnitines (C3-carnitine) and unspecific amino acid elevations, most commonly glycine and alanine, can be found in blood specimens. Urine organic acids demonstrate large amounts of methylmalonic acid, 2-methylcitrate, propionic acid, 3-hydroxy propionic acid, and triglycine. Definite diagnosis is made by mutation testing for the five genes associated with MMA: *MMUT (*encodes MCM*)*, *MMAA (*encodes cobalamin A—cblA*)*, *MMAB (*encodes cblB). MRI scans typically demonstrate bilateral involvement of basal ganglia and white matter lesions, with the globus pallidus being selectively affected.[236a] Therapy focuses on the elimination of ammonia, establishing a catabolic state, restricting dietary precursor amino acids, and promoting urinary excretion of MMA by providing adequate hydration. Mortality during early infancy is ~30% and survivors often show neurocognitive disabilities. A liver transplant can significantly reduce episodes of hyperammonemia[237] and thereby improve outcome.

Molybdenum Cofactor Deficiency

Molybdenum cofactor deficiency (MCOD) is a disorder of the sulfur amino acid metabolism that occurs in 0.5 to 1:100,000 live births.[238] There are three types described: MCOD type A results from *molybdenum cofactor synthesis (MOCS) 1* gene mutation, MCOD type B is the result of *MOCS 2* mutations, and type C is associated with *gephyrin (GPHN)* mutations. The absence of molybdenum cofactor results in functional deficiencies of molybdenum cofactor-dependent enzymes (sulfite oxidase, xanthine dehydrogenase, aldehyde oxidase, and mitochondrial amidoxime reducing component) which leads to an accumulation of their metabolites sulfite, taurine, *S*-sulfocysteine, and thiosulfate, which produces the severe neurologic symptoms seen in the affected patient. Newborns become symptomatic soon after birth and present with intractable seizures and an encephalopathic picture often so fast and profound that their clinical appearance is indistinguishable from a newborn with HIE. On MRI, diffusion restriction in the cortex and subcortical necrosis can be seen and in later stages, multicystic white matter lesions and atrophy are seen (Fig. 55.3). Diagnosis is made by targeted genetic testing of the affected genes. Serum uric acid is commonly elevated, and urine studies reveal an elevated uric acid, *S*-sulfocysteine, xanthine, and hypoxanthine. While therapy for MOCD type B and C is supportive and death occurs typically in early infancy,[238] a treatment for MOCD type A has recently become available. Cyclic pyranopterin monophosphate (cPMP), when applied shortly after birth, has shown significant improvement in an otherwise fatal disease.[239]

Nonketotic Hyperglycinemia

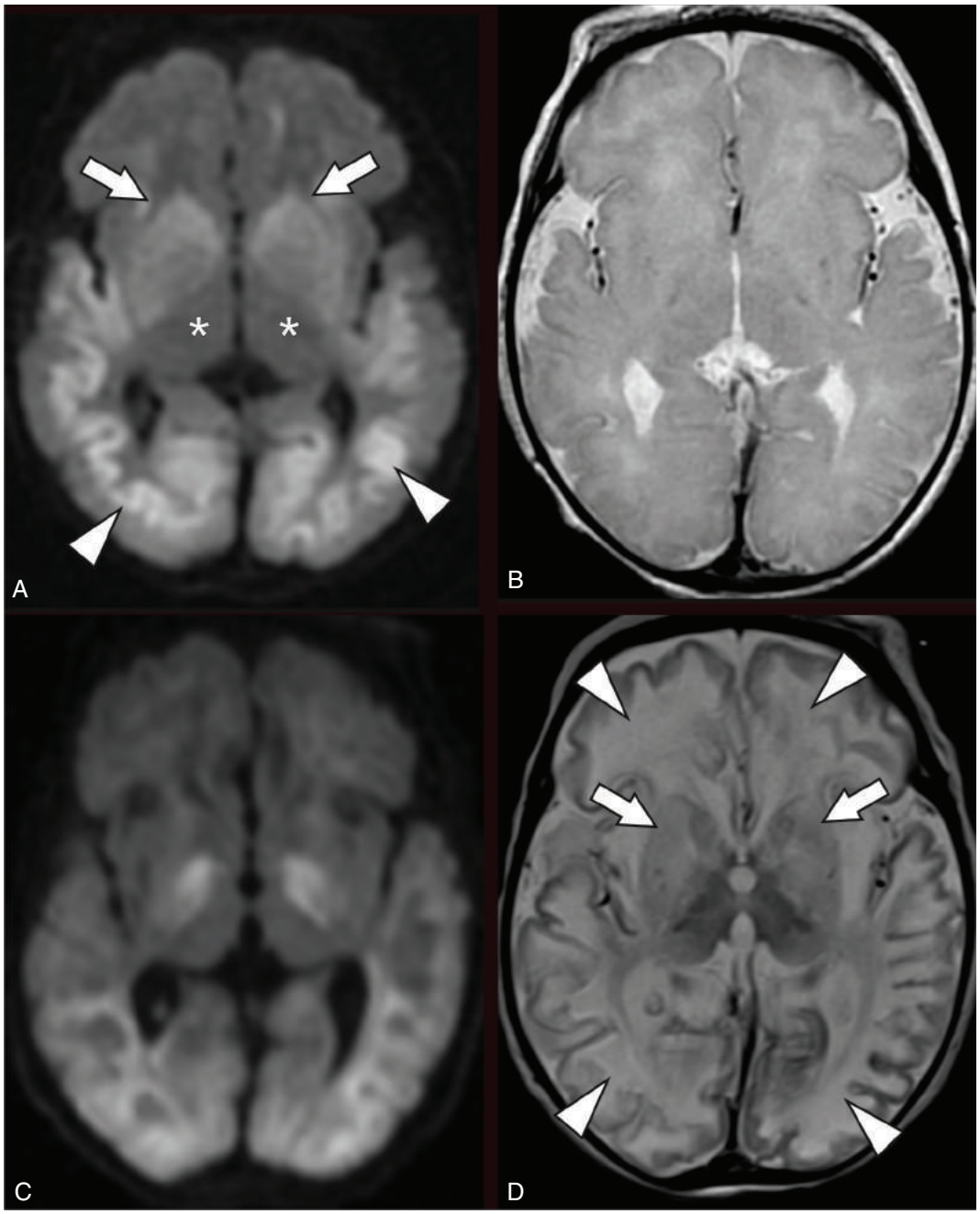
Classic nonketotic hyperglycinemia (NKH) occurs in 1:76,000 live births and is caused by a mutation in the *GLDC* and/or *AMT* gene, which encode protein components of the glycine cleavage enzyme system and results in absent or significantly decreased activity.[240] Glycine accumulates in the body, particularly in the brain and causes overstimulation of the NMDA receptors. Patients usually present in the immediate neonatal period with progressive encephalopathy and intractable seizures. Frequent hiccupping is common and is often present prenatally. Diagnostic testing includes amino acid profiles, which show elevation of glycine in plasma, CSF, and urine samples. CSF glycine is highly suggestive of NKH. Confirmatory testing is done via sequencing of the *GLDC* (affected in 80% of patients) and *AMT* genes. MRS can show high glycine peaks, and on occasion, nonspecific brain anomalies such as abnormal corpus callosum, hydrocephalus, and cerebellar hypoplasia are present. The outcome is universally poor for patients with classic NKH, with up to 30% mortality in the neonatal period and significant developmental delay and intractable seizures in survivors. The treatment is largely symptomatic and supportive and focuses on the elimination of glycine and NMDA-receptor blockage.

Energy Deficiency Disorders

This group of metabolic disorders is characterized by insufficient energy supply, either caused by defects in production or transportation. In contrast to metabolic disorders accumulating toxic metabolites, metabolic disorders affecting the energy metabolism can become symptomatic during fetal development and affected organs are those of high energy demand such as the brain, liver, and heart. Therefore, abnormal development of the brain and cardiovascular system are the most common prenatal findings. Neonates with energy deficiency disorders often do not experience a symptom-free period and can present with encephalopathy at the time of birth. Since the neonatal brain consumes about 30% of the body's energy, it is not surprising that disorders affecting the energy supply frequently present with neurologic symptoms, in particular hypotonia and seizures. Brain imaging can reveal abnormal development of various structures, such as cerebral dysgenesis, thinning of the corpus callosum, and cerebral and/or cerebellar heterotopia.[241] Other common presenting clinical features include cardiomyopathy, liver failure, and adrenal insufficiency.

Mitochondrial Disorders

Mitochondrial disorders are the most severe forms of energy deficiency disorders. This group consists of defects in aerobic glucose oxidation, mitochondrial respiratory chain disorders (including the respiratory chain, mitochondrial energy transporter molecules, or coenzyme Q10 biosynthesis), and fatty oxidation defects.[242] They are caused by mutations in mitochondrial protein-encoding genes, found on either mitochondrial DNA or nuclear DNA, and lead to defects in the mitochondrial electron transport chain and/



• **Fig. 55.3** A Term Female Neonate With Molybdenum Cofactor Deficiency. (A) Axial diffusion-weighted image (DWI) obtained on day of life 2 shows widespread diffusion restriction predominantly in the subcortical white matter *(arrowheads)* and basal ganglia *(arrows)* with relative sparing of the thalami *(asterisks)*. (B) The corresponding axial T2-weighted image shows normal white matter and basal ganglia signal at day of life 2. (C) Axial DWI image obtained on day of life 12 shows persistent and new areas of DWI signal abnormality. (D) The corresponding axial-T2 weighted image at day of life 12 demonstrates evolution of the injury with new abnormal signal hyperintensity in the basal ganglia *(arrows)* and subcortical white matter *(arrowheads)* indicating early cystic changes. (Images courtesy of Dr. Francisco Perez, Seattle Children's Hospital, Seattle, WA.)

or oxidative phosphorylation. The dysfunctional mitochondria are unable to produce and supply enough energy to maintain adequate organ function. Brain, muscle, liver, heart, and adrenal glands are often significantly affected. Therefore, the most common presenting findings are symptoms of encephalopathy, including hypotonia, feeding difficulties, seizures, cardiomyopathy, liver dysfunction, and adrenal insufficiency. Laboratory evaluation commonly reveals lactic acidosis and hypoglycemia, ketones can be normal or elevated, and liver dysfunction and secondary hyperammonemia might be present. Therapy remains symptomatic, and to date only very few defects can be improved with pharmacological intervention. Prognosis is in general poor, and survivors of the neonatal period often experience life-altering disabilities and epilepsy.

Genetic Causes of Neonatal Encephalopathy

Genetic syndromes and disorders often also affect brain development. Genetic epilepsies (covered in Chapter 58) present within the first few days to weeks of life with symptoms resembling neonatal encephalopathy. Brain malformation presenting in the immediate neonatal period is often suspected during pregnancy, but alterations might go unrecognized until the newborn presents with neurologic symptoms after birth.

Holoprosencephaly

Holoprosencephaly is a divergence in brain development that results from an abnormal cleavage of the prosencephalon into the two hemispheres. The incidence is 1:16,000 live births. The three forms of holoprosencephaly are alobar, semilobar, and lobar holoprosencephaly. Alobar holoprosencephaly is the most severe form with a single common central ventricle and complete absence of hemispheric separation. The cortical structure is frequently malformed which results in intractable seizures. Semilobar holoprosencephaly is characterized by partial separation of the frontal and parietal lobes and partial separation of the deep gray matter nuclei. Lobar holoprosencephaly is the least severe form with incomplete separation of the frontal lobe and complete separation of the deep gray matter nuclei.[243]

Chromosomal abnormalities account for up to 50% of cases, and the prevalence is with 70% highest in trisomy 13 but is also relatively common seen in trisomy 18 and triploidy.[243] Copy number variants include multiple described deletion and duplication syndromes often involving genes associated with holoprosencephaly and account for approximately 25% of cases. Mutations in the *SHH* (sonic hedgehog) gene and *ZIC2* (encodes zinc finger protein 2) gene are the most common single-gene mutations described and account for approximately 10% of cases with holoprosencephaly,[244] but multiple other genes have been associated with holoprosencephaly, and with the ability of advances in genetic testing, the list is constantly growing. Neonates with holoprosencephaly commonly present with distinguishing facial features such as cyclopia, single nares, and cleft palate. The severity of facial deformity correlates with the degree of holoprosencephaly.

Heterotopias and abnormal cortical development often cause intractable seizures and other neurologic symptoms such as hypotonia and irritability. Furthermore, the development of the pituitary gland can be absent or incomplete, and structural defects of the thalamus can be seen, both of which may result in significant endocrinopathies, such as panhypopituitarism.[243] The prognosis depends on the severity and form of holoprosencephaly. While newborns with isolated alobar holoprosencephaly often do not survive the first year of life, patients with milder forms can reach early adulthood. In cases of association with cytogenetic abnormalities, as few as 2% survive the first year of life.[245]

Neuronal Proliferation Defects

Cortical dysplasia spectrum, including focal cortical dysplasia and hemimegalencephaly. Patients with significant involvement can present during the neonatal period, most commonly with intractable seizures and feeding difficulties. Since seizures are often refractory to pharmacological treatment, surgical options, including hemispherectomy can be offered.[246]

Neuronal Migration Defects—Lissencephaly

Lissencephaly is the description of a smooth brain appearance on MRI as a result of a simplified gyration pattern (pachygyria). Classic lissencephaly is commonly related to abnormalities in the *LIS1* gene, which affects microtubular functioning and intracellular transport, but a variety of copy number variants and mutations in other genes (*TUBA1A, TUBB2B, ACTB, ACTG1, DCX* among others) have also been associated with classic lissencephaly.[246] Depending on the degree of abnormal structures, patients may present in the neonatal period with seizures, hypotonia, and feeding difficulties.

Postmigrational Development Defects—Polymicrogyria

Polymicrogyria occurs at the end of neuronal migration and during cortical development and results in abnormal cortical folding and cortical disorganization which predisposes affected patients to seizures. The occurrence can be associated with congenital infections (cytomegalovirus), vascular anomalies, genetic syndromes (e.g., Zellweger syndrome, 22q11.2, or 1p36 deletion syndromes), and single-gene mutations.[247] Clinical presentation depends on the extent and location, and affected neonates commonly present with seizures, abnormal tone, and feeding difficulties.

1p36 Deletion Syndrome

This deletion syndrome is one of the most common deletion syndromes and affects 1:5000 newborns.[248] This syndrome is characterized by terminal and interstitial deletions throughout the 30 Mb of DNA constituting the 1p36 region. The phenotype varies widely depending on the size and location of the deletion and involved genes. Multiple of the involved genes have a role in brain development, seizures, and congenital heart defects. Commonly seen clinical features include seizures, fetal akinesia, hypotonia, neurodevelopmental impairment, neuropsychiatric anomalies, brain anomalies (cortical development, hippocampal development, delayed myelination), ventriculomegaly, microcephaly, intellectual disability, developmental delay, vision problems, hearing loss, congenital heart defects, noncompaction cardiomyopathy, orofacial clefting, retrognathia, renal anomalies, and short stature.[248–250] The majority of fetuses affected by this deletion syndrome have signs of perinatal distress, 59% of term-born infants need some form of resuscitation, and 18% present with cardiac arrest.[251] The clinical presentation is consistent with neonatal encephalopathy in many cases and can even mimic HIE.

Hypophosphatasia

Hypophosphatasia is a rare disease of defective mineralization, caused by mutations in the *APLP* gene, which encodes the enzyme tissue-nonspecific alkaline phosphatase (TNSALP). Inheritance, particularly in severe forms, is most commonly autosomal recessive but dominant forms have also been described.[252] Two forms are present in the prenatal or perinatal period: the severe form and the benign form. The severe form is characterized by minimal to no bone mineralization, resulting skeletal deformities, lung hypoplasia, and seizures. The benign form is characterized by poor feeding, hypotonia, irritability, and seizures. Skeletal deformations

are not consistently present in the benign form. The incidence of the severe form is estimated to be 0.2 to 1:100,000 live births and 1 to 5:10,000 live births for milder forms.[252] Affected patients can present in the immediate neonatal period with symptoms of neonatal encephalopathy, resembling HIE.[253]

Seizures in affected patients occur secondary to disruption of pyridoxal-5′-phosphate (PLP) conversion to pyridoxal (PL) by TNSALP in neuronal cells. PL is able to cross the cell membrane and is intracellularly rephosphorylated to PLP, a cofactor in inhibiting excitatory neurotransmitter activity. Therefore, decreased central nervous system (CNS) PLP results in an increase in excitatory neurotransmitter activity and decreased seizure threshold. The seizures are commonly pyridoxine responsive, but the exact mechanism by which pyridoxine mitigates the intracellular PLP deficiency is incompletely understood. Diagnosis can be suspected when plasma PLP levels are elevated, alkaline phosphatase levels are decreased, and hypocalcemia is observed. Definitive diagnosis is made via molecular genetic testing. While the severe form was historically lethal in the neonatal period, enzyme replacement therapy with asfotase alfa is now available which can restore TNSALP levels and improve survival from 42–95% at 1 year and decrease ventilator dependence to 25% among survivors.[254]

Central Nervous System Infections and Neonatal Encephalopathy

Bacterial Meningitis

Bacterial meningitis is a serious infection of the CNS affecting the meninges surrounding the brain and spinal cord. Neonates are at greater risk of meningitis than other age groups because of the inefficiency of the alternative complement pathway, deficient migration and phagocytosis of neutrophils, and decreased T-cell and B-cell activity, leaving them at risk for infections with encapsulated bacteria.[255] *Streptococcus agalactiae*, group B streptococcus (GBS), is responsible for 50% of meningoencephalitis in the term newborn period, followed by *Escherichia coli* (30–40%) and *Listeria monocytogenes* (5–7%).[256]

The incidence of bacterial meningitis is 0.3:1000 live births.[257,258] Neurologic injury can result primarily from the direct insult of the pathogen or its toxin, or secondary to the inflammatory reaction associated with the acute infection, leading to impaired cerebral autoregulation, vasculitis including microthrombi, and oxidative injury. The blood-brain barrier becomes permeable which contributes further to the development of cytotoxic edema and compromise of cerebral perfusion.[258]

Clinical symptoms include temperature instability, apnea or bradycardia, hypotension, feeding difficulty, hepatic dysfunction, irritability alternating with lethargy, and seizures. Any neonate with signs of sepsis or unexplained neurologic symptoms should have a lumbar puncture to examine the CSF. Up to one-third of infants with negative blood cultures have positive CSF cultures, suggesting that cases of meningitis may be missed if lumbar punctures are not performed.[259,260] No single CSF parameter can reliably exclude the presence of meningitis in a neonate.[261] Real-time polymerase chain reaction (RT-PCR) technique allows for increased diagnostic accuracy compared to conventional culture,[262,263] particularly after antibiotic treatment has already been initiated by identifying the DNA of bacterial components.

Seizures occur in up to 40% of newborns with meningitis and therefore, monitoring with EEG is indicated.[256] Cerebral abscesses develop in 13% of neonates with meningitis and should be considered with new seizures, signs of elevated intracranial pressure, or new focal neurologic signs, and brain imaging with contrast is essential for making the definitive diagnosis.[264] Ventriculitis occurs in as many as 20% of neonates with meningitis and results in sequestration of infection to areas that are poorly accessible to systemic antimicrobial drugs.[265] Inflammation of the ependymal lining of ventricles often obstructs CSF flow and can lead to hydrocephalus in up to 24% of infants. Imaging can give information about complications of meningitis. The choice of an antibiotic regimen should be based on the likely pathogen, ability to penetrate the blood-brain barrier, and the local patterns of antimicrobial drug sensitivities. Treatment duration is usually 14 to 21 days but depends on the identified organism and extent of infection (e.g., abscess formation). Most experts suggest a repeat lumbar puncture 2 to 3 days into treatment. Survivors of neonatal meningitis are at significant risk for white matter injury and neurodevelopmental sequelae. The most common sequelae of neonatal meningitis are motor deficits, including cerebral palsy, epilepsy, deafness, and neurodevelopmental impairment. In a prospective sample of more than 1500 neonates surviving to the age of 5 years, 55% had a normal outcome, 29% had mild neurodevelopmental impairment, and 16% had moderate to severe neurodevelopmental impairment. Among survivors of meningitis, motor disabilities (including cerebral palsy) were present in 8.1%, learning disability in 7.5%, epilepsy in 7.3%, speech and language problems in 15.6%, behavioral problems in 11.9%, vision problems in 13.7%, and hearing problems in 25.8%.[266]

Human Parechovirus

In recent decades with the increased diagnostic ability of PCR techniques, human parechovirus (HPeV) meningoencephalitis, particularly type 3, has emerged as a newer virus identified in neonates presenting with seizures, poor feeding, irritability, and sepsis-like symptoms within the first weeks of life. CSF studies often show no to mild pleocytosis. HPeV RNA induces the release of inflammatory substances which compromise preoligodendrocytes and axons.[109] In addition, inflammatory changes particularly in the periventricular white matter are characteristic findings.[267]

On MRI, diffuse abnormalities in the supratentorial white matter tracts with thalamic involvement[268] and later evolution into cystic encephalomalacia have been described.[269] Affected patients are at high-risk for impaired neurodevelopmental outcomes, including cerebral palsy, vision deficits, and developmental delay.[269]

Cytomegalovirus

Cytomegalovirus (CMV) is the most common congenital viral infection, with an incidence of 6 to 7.5:1000 live births in the United States.[270] Primary infection of the mother or reactivation of a latent infection at any gestational age can result in transmission of the virus to the fetus. Congenital infections may result in intrauterine growth restriction, thrombocytopenia, hydrops, jaundice, hepatosplenomegaly, microcephaly, periventricular calcification, seizures, and sensorineural hearing loss. About 40–58% of newborns who are symptomatic at birth go on to develop sequelae, including sensorineural hearing loss, intellectual disability, seizure disorder, cerebral palsy, visual deficits, or developmental delay.[271,272]

The diagnosis of CMV in the neonate can be made by PCR of urine, blood, or saliva.[273] Antibody titers cannot reliably

indicate the diagnosis, as maternal CMV immunoglobulin G crosses the placenta, and neonates mount weak immunoglobulin M responses. Audiologic assessment should be performed on all infants with congenital CMV infection, as sensorineural hearing loss (SNHL) affects greater than 70% of symptomatic newborns; 80% of those show SNHL early on but may be absent at birth and evolve over time.[274] Therefore, frequent assessments throughout childhood are necessary to detect later onset hearing deterioration.[275] SNHL can be ameliorated by early treatment with ganciclovir. One randomized study indicated that 84% of ganciclovir recipients either had improved hearing or maintained normal hearing between baseline and 6 months. In contrast, only 59% of control patients had improved or stable hearing.[276] Results were even more encouraging when the study and control groups were compared for subsequent maintenance of normal hearing, as none of the ganciclovir recipients had a worsening in hearing between baseline and 6-month follow-up, compared with 41% of control patients. Furthermore, neonates treated with ganciclovir show fewer developmental delays at 6 and 12 months compared with untreated infants.[277]

Zika Virus

The World Health Organization declared the rapidly spreading epidemic of Zika virus (ZIKV), an arbovirus (mosquito-borne) member of the Flaviviridae family, a "Public Health Emergency of International Concern" on February 1, 2016, based on emerging evidence that the virus might cause severe fetal brain injury,[278] specifically severe microcephaly much more pronounced than in other congenital viral infections. While many different cell lines can be infected with ZIKV, neural progenitor cells (NPCs) are a direct target of ZIKV.[279] Normal brain development is highly dependent on NPC differentiation, migration, and maturation. ZIKV-infected NPCs had increased cell death, downregulated proliferation, and altered neurosphere production.[279–281] leading to microcephaly and congenital contractures.[282] Children born with congenital Zika syndrome have significant long-term morbidities, including epilepsy in ~50%, hearing loss, blindness, hypotonia, and significant global neurodevelopmental delay.[282]

Toxoplasmosis

The incidence of congenital toxoplasmosis is 0.1 to 1:1000 live births and is caused by *Toxoplasma gondii*.[273] Human infection occurs via ingestion of contaminated meat or soil and can disseminate via the placenta to the fetus. Pregnant women are cautioned to avoid exposure to uncooked meat and cat feces. The immune response resulting from placental and fetal infection as well as direct impact of the parasite causes leptomeningeal and cerebral necrosis, which can lead to dystrophic calcifications in the basal ganglia and periventricular region, white matter lesions, and subsequent development of hydrocephalus.[283] Neonates can present with neurologic symptoms, including microcephaly, seizures, and feeding difficulties, in addition to clinical findings of systemic involvement, such as jaundice, hepatosplenomegaly, chorioretinitis, petechiae or purpura, and intrauterine growth restriction.[284] Congenital toxoplasmosis treatment consists of pyrimethamine, sulfadiazine, and leucovorin for up to 1 year[285] and is most effective in reducing CNS involvement and serious neurologic sequelae when initiated prenatally. Vision impairment due to macular involvement is the most common long-term consequence in addition to cognitive and motor impairment.

Suggested Readings

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56

Neonatal Neurovascular Disorders

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KEY POINTS

- Perinatal stroke is a vascular event causing focal interruption of blood supply and can be categorized based on the vascular distribution of stroke (arterial or venous), age at the time of stroke, and age at presentation.
- When a stroke is suspected in a neonate, neuroimaging is required for confirmation of diagnosis, followed by risk factor assessment and creation of a specific treatment plan.
- In infants and neonates, cerebral sinus venous thrombosis usually presents with seizures and/or encephalopathy, and treatment varies from conservative neuromonitoring to anticoagulation.
- Subdural and subarachnoid hemorrhages are both associated with vacuum/forceps-assisted deliveries and coagulopathy. Evaluation includes neuroimaging and monitoring, and outcomes vary based on location and size.
- Vein of Galen malformation is the most common arteriovenous malformation of the newborn, often presenting with cardiac and/or neurologic complications. The clinical picture depends on the age at presentation.

Perinatal Stroke

Epidemiology

Ischemic perinatal strokes (IPS) are focal or multifocal arterial or venous infarctions occurring between 20 weeks' gestation and 28 days' postnatal life and are confirmed by neuroimaging or neuropathologic studies.[1,2] The reported incidence varies between 1 in 1600 and 1 in 5000 live births,[3,4] with likely higher incidence given that most of the studies were retrospective and magnetic resonance imaging (MRI) was not routinely used. The IPS is responsible for one-third of term and late-preterm children affected with hemiplegic cerebral palsy (CP).[5] IPS is slightly more common in males and non-Hispanic black ethnicity when compared to whites and occurs most often in the left middle cerebral artery (MCA) distribution, with the most affected region being the left cerebral hemisphere.

Risk factors for perinatal stroke include maternal primiparity, preeclampsia, prolonged rupture of membranes, chorioamnionitis, and cord anomalies.[6] Presence of more than one of these risk factors can increase the probability of perinatal stroke to 1 in 200.[3] Complicated deliveries involving emergency cesarean section or instrumentation have also been associated with IPS. Table 56.1 includes multiple proposed risk factors for perinatal stroke, mostly from studies presenting associations rather than causation. Most cases lack definitive causes.

Pathophysiology

Ischemic perinatal stroke is pathological or neuroradiological evidence of focal arterial or venous infarction that occurred in the perinatal period. The pathogenesis of IPS is not well understood. Physiologic changes in the mother during pregnancy may cause a hypercoagulable and prothrombotic state. Fetuses are also at increased risk for developing clots as physiologic polycythemia leads to hyperviscosity, and there is a depressing anticoagulant activity present. These factors, coupled with the placenta having areas of reduced blood flow, increase the proclivity for thrombotic generation on the fetal side of the placenta. These thrombi will travel via the umbilical vein and are poised to pass through the patent foramen ovale to enter the systemic and, most importantly, the cerebral arteries. Other fetal conditions leading to increased risk of perinatal stroke include twin pregnancies, twin-to-twin transfusion, arteriovenous malformations, prolonged neck traction, and cardiac defects.[7,8] Perinatal arterial stroke (PAS) lesions are usually singular (70%), involving the anterior circulation (71%), posterior circulation (7%), or both (20%).[9] Strokes are most commonly left-sided (51% of all strokes, 73% of all anterior strokes), with 9% occurring on the right and 20% showing bilateral distribution.[9]

Classification of perinatal strokes can be categorized based on the vascular distribution of stroke (arterial or venous), age at the time of stroke, and age at presentation, with multiple authors using different terms to describe the IPS (Table 56.2).[3,10–17] Because the timing of the vascular event leading to IPS is almost always unknown, it has been suggested that the classification of IPS be based on the gestational or postnatal age at diagnosis.

Clinical Presentation

Diagnosing an infant with perinatal stroke is challenging in the newborn period. Most infants with PAS are asymptomatic at birth, and signs of acute illness are only seen in 25% of cases.[9] Diffuse neurologic signs and symptoms are more common than focal signs, with the abnormal tone, apnea, and depressed level of consciousness more common than hemiparesis, which is usually absent or subtle in the neonate.[18] Nonspecific symptoms include breathing and feeding difficulty. In the week following

TABLE 56.1 Risk Factors for Perinatal Stroke

Maternal/Placental	Fetal/Neonatal
History of infertility	Growth restriction
Primiparity	Multiple gestation
Pre-eclampsia	Twin to twin transfusion syndrome
Maternal diabetes	Trauma to great arteries at birth
Autoimmune disorders (e.g., systemic lupus erythematous)	Intrapartum asphyxia
Maternal prothrombotic disorders	Congenital heart disease
Maternal antiphospholipid antibodies	Vascular anomalies
Coagulation disorders	Col4a1 and 2 mutations
Anticardiolipin antibodies	Infection (e.g., central nervous system, systemic)
Drug use (cocaine, smoking)	Thrombophilia
Maternal infection (chorioamnionitis)	Antiphospholipid antibodies
Maternal fever	Hypoglycemia
Trauma	Extracorporeal membrane oxygenation (ECMO)
Placental abnormalities (e.g., thrombotic vasculopathy, emboli, inflammatory mediators)	Other central catheterization

(From Dr. Catherine Amlie-Lefond and Dr. Nina Natarajan, Seattle Children's Hospital, Seattle.)

birth, most newborns with perinatal arterial ischemic stroke (PAIS) become symptomatic, with the most prevalent symptom being seizures (large range, up to 70% to 90%). Approximately 12% of infants with PAS present with recurrent focal seizures with typical onset at 12 to 48 hours of age.[4,13,19–21] Typical presentation for different forms of perinatal stroke is presented in Table 56.2.

Evaluation

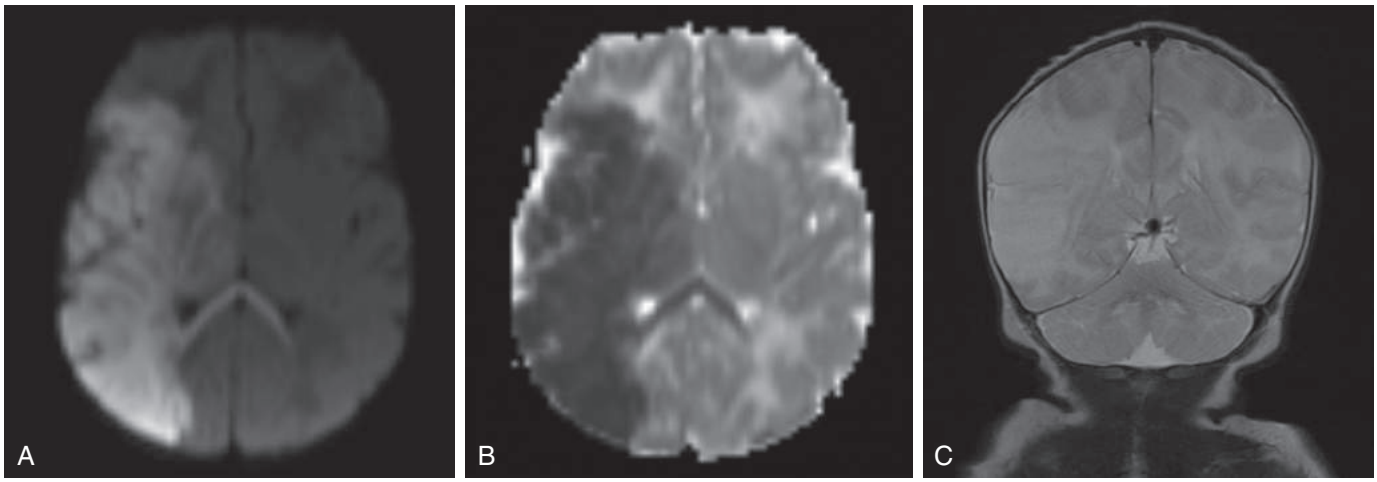
Assessment of the neonate with perinatal stroke includes neuroimaging (cranial ultrasound [CUS], head computed tomography [CT], brain MRI), electroencephalography, and echocardiogram to evaluate for congenital heart disease or intracardiac thrombus. Risk factors include a maternal history of autoimmune disorders, recurrent pregnancy loss, or thrombosis, and placental pathological examination and toxicology screens may also provide helpful information. Given that the most common presenting symptom is seizures, the work-up should begin with ruling out other etiologies of seizure such as hypoglycemia, hypocalcemia, electrolyte disorders, infection, and metabolic syndromes. In nonhemorrhagic stroke, MRI is considerably more sensitive than CUS or CT,

TABLE 56.2 Ischemic Perinatal Stroke Classification

Age at Diagnosis	Terminology	Description	Typical Presentation
ARTERIAL DISTRIBUTION			
Fetal	Fetal Arterial Stroke	Arterial ischemic stroke found on prenatal imaging.	Incidental finding of diffusion restriction on fetal MRI.
Preterm	Perinatal/Neonatal Arterial Ischemic Stroke (P/NAIS)	Arterial ischemic stroke in infants <35 weeks' gestational age.	Incidental finding on cranial ultrasound; less likely to present with seizures.
Term	Perinatal/Neonatal Arterial Ischemic Stroke (P/NAIS) "Early PAS"	Acute symptomatic neonatal arterial ischemic or fresh arterial strokes presenting in the first week of life.	Typically presents with seizures, apnea, or encephalopathy.
Term, 7–28 days	Perinatal/Neonatal Arterial Ischemic Stroke (P/NAIS) "Late PAS"	Acute symptomatic neonatal arterial ischemic or fresh arterial stroke presenting during weeks 2–4 of life.	Typically presents with seizures or encephalopathy.
Infancy and/or later	Presumed Perinatal Ischemic Stroke (PPIS)	Arterial distribution stroke that presumably occurred between 20 weeks' gestation and 28 days of life.	Normal neonatal course; present in infancy with hemiplegia, early handedness, or seizures.
VENOUS DISTRIBUTION			
Fetal	Fetal Venous Stroke	Venous distribution stroke is found in prenatal imaging, typically in the distribution of periventricular hemorrhagic infarction.	Incidental findings on fetal MRI or cranial ultrasound.
Preterm	Periventricular Hemorrhagic Infarction (PVHI)	Ischemic perinatal stroke in preterm infants often involves the periventricular region resulting in presumed venous infarction in infants born before 32 weeks.	Found on routine cranial ultrasound with associated intraventricular hemorrhage. Presentation ranges from subclinical to catastrophic.
Term	Cerebrosinovenous Thrombosis (CSVT)	Venous strokes in the term infant are secondary to partial or complete occlusion of a cranial venous sinus, deep cerebral vein, or smaller cortical veins. These may have a hemorrhagic component. Additionally, medullary vein thrombosis in term neonates may result in a periventricular infarct.	Can present with seizures; any term infant with intraventricular hemorrhage or temporal lobe hemorrhage should be evaluated further for CSVT.

TABLE 56.2 Ischemic Perinatal Stroke Classification—cont'd

ARTERIAL DISTRIBUTION			
Age at Diagnosis	**Terminology**	**Description**	**Typical Presentation**
Infancy and/or later	Presumed Perinatal Ischemic Stroke (PPIS)	"Fetal" stroke not diagnosed prenatally or asymptomatic perinatal stroke. PPIS often involves the periventricular region resulting in presumed venous infarction (PVI) in utero or in infants born prematurely. Criteria suggestive of PVI unilateral injury, focal periventricular encephalomalacia, internal capsule T2 prolongation, relative cortical sparing relative basal ganglia sparing, and remote hemorrhage. Medullary vein thrombosis is an additional venous etiology.	Typically present in infancy with hemiplegia/early handedness or seizures.



• **Fig. 56.1** A 4-Day-Old Term Male Neonate With Right Middle Cerebral Artery Stroke. (A) Axial diffusion-weighted image shows abnormal bright signal throughout the right cerebral hemisphere in the middle cerebral artery territory. The corpus callosum is also involved. (B) Apparent diffusion coefficient map shows a dark signal, confirming this is true cytotoxic edema. (C) Coronal T2-weighted image shows abnormal loss of gray-white matter differentiation, consistent with a subacute time frame (at least 6 hours) for the injury. A head magnetic resonance angiogram showed no definite stenotic lesion (not shown here). (Images courtesy of Dr. Randolph Otto and Dr. Teresa Chapman, Seattle Children's Hospital, Seattle.)

making MRI the preferred imaging modality.[22] MRI is the gold standard test for the detection of PAS.[22] MRI sequences to be used include T1- and T2-weighted images, diffusion-weighted imaging (DWI), and MRI angiography, a technique that is helpful in detecting vascular stenosis, occlusion, or arteriopathy.[23] Fig. 56.1 demonstrates an MRI of a term male with a PAS involving the right MCA. Of these sequences, the most sensitive is DWI performed in the first week of life. The area of infarction appears as a zone of high intensity on DWI and low intensity on the apparent diffusion coefficient (ADC) map. After the first week, the ischemic tissue appears to normalize, even though the area continues to be ischemic, a process known as pseudonormalization. Reduced contrast between cortex and white matter can be seen in the first 48 hours on T2-weighted imaging. Lower signal intensity will be seen on T1-weighted images. As time passes from the injury, cortical highlighting develops.

When a perinatal stroke is associated with a seizure, electroencephalography (EEG) could help localize the origin of the seizures and aid in the management of seizures. EEG is usually abnormal in infants with PAS, and the findings correlate with the location of infarction.[24,25] Cranial US is widely available in most centers that do not have MRI capability. The sensitivity of CUS improves from 68% in the first few days to 87% in the first week.[15] Because strokes may occur in the posterior circulation, posterior fontanelle imaging is necessary. Unlike MRI, CUS is operator dependent, and thus detection rates vary between centers. The use of CT is limited to situations when CUS and MRI are not available. CT is discouraged in neonates because of poor sensitivity and high radiation exposure.

The optimal thrombosis evaluation is currently debated, and laboratory results are usually inconclusive in neonates.[26] Rates of possible thrombophilia as an underlying cause of PAIS vary in

the literature up to as high as 68%. However, the International Pediatric Stroke Study found that only 19% of PAIS infants were diagnosed as having possible increased lipoprotein(a) level, methylene tetrahydrofolate reductase mutations, elevated β_2-glycoprotein level, factor V Leiden, prothrombin gene 20210A, low antithrombin III level, antiphospholipid antibodies, plasminogen activator inhibitor, or low protein S level.[9] Routine thrombophilia testing of the neonate is not indicated without clinical suspicion.

Management

Therapy for PAS is largely supportive and includes neuroprotective measures with avoidance of dehydration, hypoglycemia or hyponatremia, and maintenance of normothermia. Anticonvulsant therapy has the best evidence for use, but most infants will not require long-term therapy, and many experts increasingly suggest early discontinuation.[9] Anticoagulation is currently debated in the literature, and the type of antithrombotic medication used varies from country to country. There is most consensus for the use of anticoagulant therapy in infants with congenital heart disease (CHD) and PAIS, as they are at increased risk of ongoing clot formation.[27] Anticoagulation treatment should be considered in proven cardiac embolism, recurrent AIS, or prothrombotic state.[27–29] Referral to physical, occupational, and speech therapy should be considered, as well as close follow-up after discharge.

Outcomes

In infants with acute PAS, EEG is a useful tool for prognostication. Specifically, abnormal background activity between 2 and 4 days of life can predict abnormal motor outcomes.[30] Abnormal physical exams at discharge and seizures during the neonatal period are also predictive of long-term disability.[31] Neurodevelopmental outcomes depend on the size and location of the stroke. The risk of recurrence and mortality in PAIS is very low.[32,33] PAIS occurring in the MCA distribution may result in a hemiplegia rate of 50%.[10] While most infants will be discharged seizure-free, up to 50% will later develop epilepsy or infantile spasms.[34,35] Occurrence of more than ten neonatal seizures increases the risk of subsequent epilepsy 30-fold.[36,37] Cognitive and language deficits are more common in children with hemiplegia or epilepsy following PAS.[38] Neuromotor impairment and hemiplegia are associated with PAS affecting basal ganglia, thalamus, or posterior limb of the internal capsule.[39,40] In acute settings, motor outcomes may be predicted by the diffusion restriction within the corticospinal tract, while their asymmetry on MRI predicts the development of spastic hemiplegia.[1,41–43] Language is affected, with about half of the cases having delays at 7 years.[44] Visual function may be altered by PAS, and behavioral problems may also be seen.[13]

Sinus Venous Thrombosis

Epidemiology

Cerebral sinus venous thrombosis (CSVT) has a reported incidence of 0.6 to 12 per 100,000 live births.[45–49] The wide range of reported incidence is likely due to variable awareness among clinicians and, therefore, variable use of neuroimaging to detect CSVT. The most commonly affected sinuses in neonates are the superior sagittal and the transverse sinuses. The transverse sinuses are more frequently involved in children older than 2 years of age (60% vs. 39%).[45,50]

Pathophysiology

Impaired venous drainage from CSVT causes increased venous pressure that can result in increased capillary hydrostatic pressure. This elevated pressure leads to vasogenic edema and hemorrhagic infarction in the distribution of the cerebral sinus venous. When venous pressure is higher than arterial pressure, arterial flow decreases, and arterial ischemia and hemorrhagic infarction may occur.[51] Maternal risk factors for CSVT include preeclampsia, chorioamnionitis, and gestational diabetes. Complicated delivery, meconium aspiration, and the need to be intubated have also been associated with CSVT. Similar to PAS, CHD is a major risk factor. Postnatal conditions such as meningitis, sepsis, dehydration, and ECLS are associated with CSVT.

Clinical Presentation

Infants usually present with seizures and/or encephalopathy. Risk factors for sinovenous thrombosis include hypoxic-ischemic encephalopathy, complicated delivery, complicated pregnancy, dehydration, prematurity, congenital heart disease, sepsis, and prothrombotic abnormalities.[52] Presenting signs and symptoms are subtle and nonspecific: seizure is the most common symptom, but respiratory distress, lethargy, apnea, and poor feeding can also be present with CSVT.

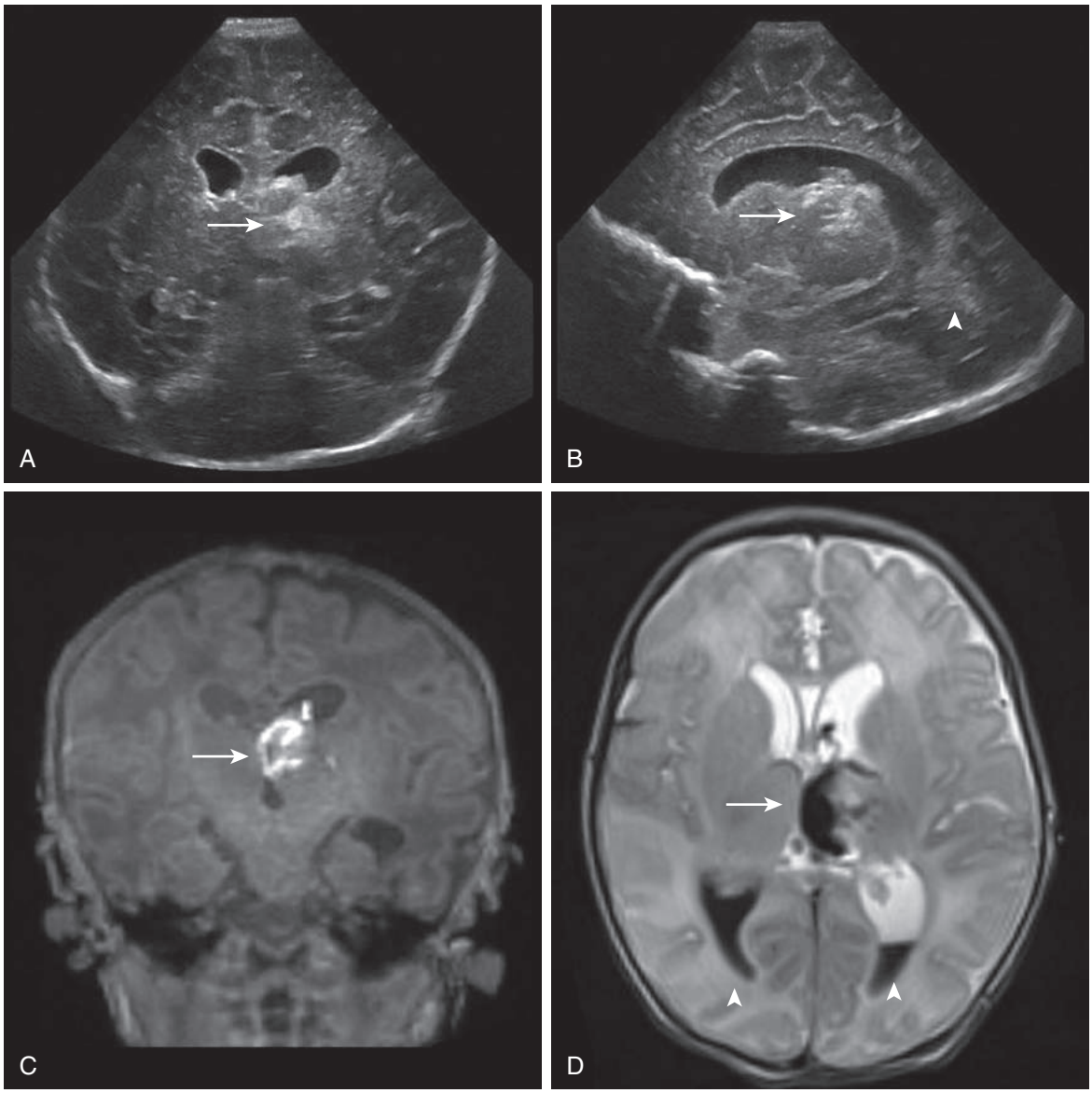
Evaluation

The nonspecific clinical presentation in neonates and children makes the diagnosis of CVST particularly difficult as frequently the symptoms can be attributed to more common diseases such as infections or dehydration. Neuroimaging is necessary for detecting sinovenous thrombosis as well as following patient response to therapy. The superior sagittal sinus has the highest rate of thrombus, followed by straight and transverse sinuses. Most neonates have multiple sinuses involved.

From an imaging standpoint, sinovenous thrombosis is often initially detected by CUS or MRI as IVH with or without associated thalamic hemorrhage (Fig. 56.2).

MRI is the gold standard, especially when the MRI venography (MRV) sequence is performed (Fig. 56.3). MRI serves two purposes: (1) to document the sinus involved and (2) to identify associated lesions. CSVT can sometimes be detected by T1- and T2-weighted images. Restricted diffusion on DWI will be present in areas affected by CSVT. MRV is very helpful for delineating thrombosis, as the involvement of multiple sinuses and veins is relatively common, and this allows the clinician to see the venous system without the use of contrast.

CUS can be used to diagnose CSVT; however, it has a high false-negative rate and requires operator experience in using Doppler to measure venous sinus flow.[53] CUS can detect intraventricular and thalamic hemorrhages with ease, and CSVT should be ruled out in term neonates with unexplained intraventricular hemorrhage.[54] CT has a high false-negative rate and, given the high radiation, is generally not preferred.[55] As with PAS, thrombophilia evaluation should be performed and should include protein-based assays as well as genetic testing. While genetic testing can be done acutely, protein-based studies are most frequently performed at around



• **Fig. 56.2** Images From a Term Newborn With Sinus Thrombosis. (A) A coronal cranial ultrasonography (CUS) image with a bright-appearing thalamic hemorrhage (*arrow*). (B) A parasagittal view of the left lateral ventricle. Note the thalamic hemorrhage (*arrow*) and blood in the occipital horn of the ventricle (*arrowhead*). (C) The coronal T_1-weighted magnetic resonance (MR) image corresponds to the CUS image in A. The thalamic hemorrhage appears bright (*arrow*). (D) An axial T_2-weighted MR image in which hemorrhage appears dark. Note the thalamic hemorrhage (*arrow*) and blood in the lateral ventricles (*arrowheads*).

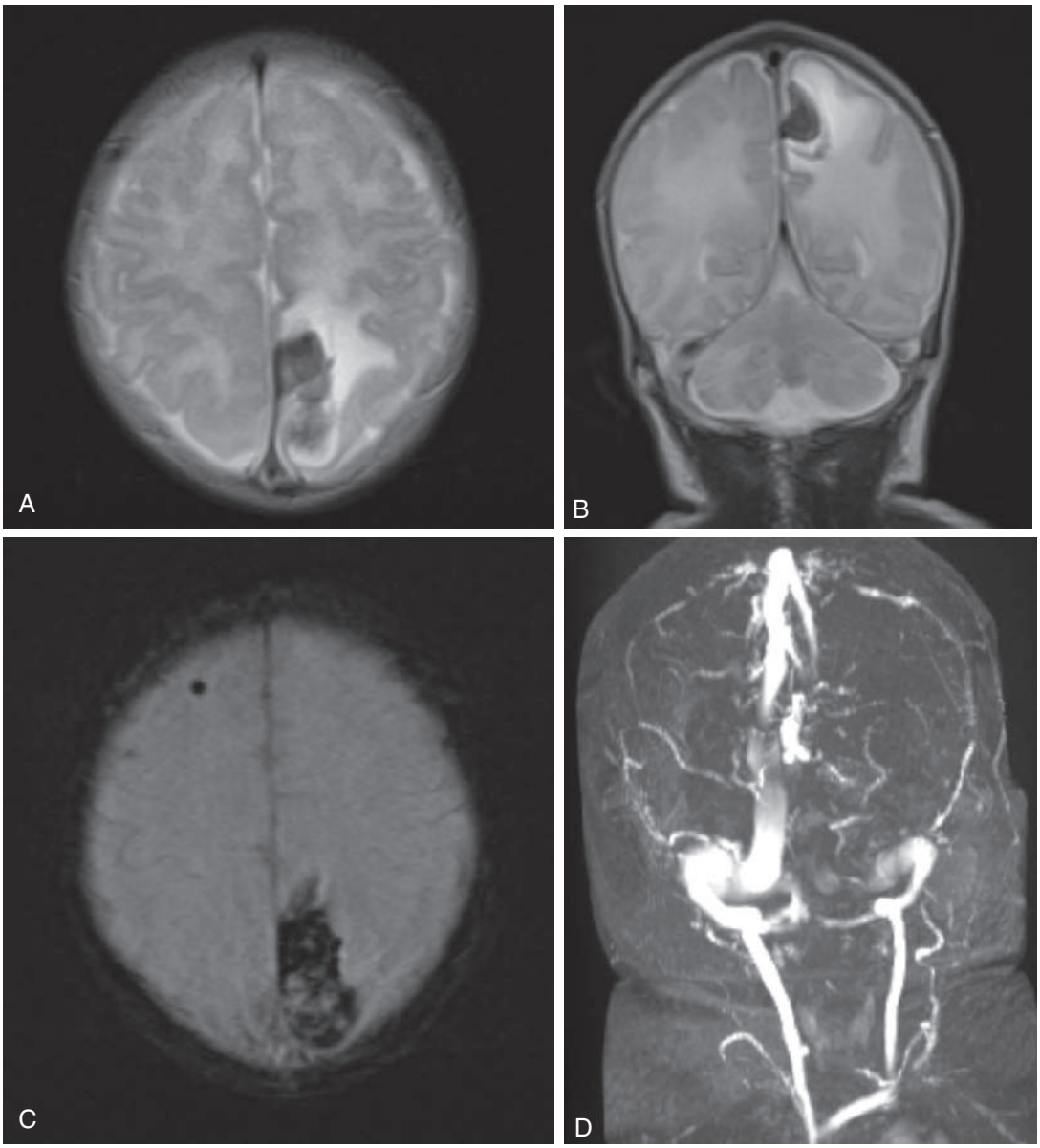
6 months of age because of the large volume of blood required for testing and because consumption of factors during acute thrombosis may lead to inaccurate results.

Management

There is no consensus on the acute phase management of CSVT in neonates, and both anticoagulation and a conservative approach should be considered in treating concomitant illnesses.

Treatment of CSVT should target the inciting factor: for instance, treating dehydration, meningitis, or CHD. Identifying the extent of thrombosis is important because it is a treatable condition, and it is common to use anticoagulant therapy (cautiously, in the presence of significant hemorrhagic injury). MR venography provides a means of evaluating the response to anticoagulant therapy, and follow-up imaging is typically obtained some weeks following the initiation of therapy.

Thrombus progression is seen in 25% of neonates, and no complications have been seen with anticoagulation for infants with associated intracranial hemorrhages.[56] Therefore, most practitioners will treat CVST with unfractionated heparin or low-molecular-weight heparin in neonates without hemorrhage. Those with hemorrhage that show a progression of the CVST at 5 to 7 days should have anticoagulation therapy started. The treatment duration is between 6 and 12 weeks.[57] An emerging treatment using direct oral anticoagulants (factor Xa inhibitors [rivaroxaban, apixaban, edoxaban] and a direct thrombin inhibitor [dabigatran]) represent a fascinating option for the treatment of CVST, taking into consideration their safety profile and the lack of laboratory monitoring required.[58,59]



• **Fig. 56.3** A 15-Day-Old, Former 34 Weeks' Gestation, Preterm Male Neonate With Hypoxic–Ischemic Encephalopathy and Dural Venous Sinus Thrombus. (A) Axial and (B) coronal T2-weighted images show abnormal dark signal in the medial left parietal lobe parenchyma, with surrounding high signal. (C) Axial susceptibility weighted imaging shows a dark signal and blooming artifact consistent with blood products. (D) Coronal maximum intensity projection image from a magnetic resonance venogram shows diminished flow in the lower sagittal sinus and left transverse sinus consistent with thrombus. Findings are consistent with hemorrhaging venous infarct and surrounding vasogenic edema. (Images courtesy of Dr. Jason Wright and Dr. Teresa Chapman, Seattle Children's Hospital, Seattle.)

Outcomes

Few studies of CVST have been performed, and follow-up data are limited. Early mortality figures showed death rates between 2% and 5%; however, more recent studies with improved detection rates of CVST show mortality rates between 19% and 25%.[60] Recanalization rate in neonates and children varies, with a recent study reporting a rate of 85% at 3 months in neonates compared with 56% in children.[56]

Increased recurrence risk (4- to 11-fold) has been associated with the lack of recanalization, the presence of the G20210A prothrombin gene mutation, and in patients not treated with anticoagulant therapy.[61,62] There is very limited follow-up data, including neurodevelopmental outcomes of neonates with CVST. Of the survivors, 60% to 80% develop motor impairment, including CP, cognitive delay, and/or epilepsy.

Subdural and Subarachnoid Hemorrhages

Epidemiology

Aside from the intraventricular hemorrhage seen in preterm infants, which is described in Chapter 54: Brain Injury in the Preterm Infant, the other major, clinically important types of neonatal intracranial hemorrhage (ICH) include: (1) epidural hemorrhage, (2) subdural hemorrhage, including posterior fossa subdural

hemorrhages, (3) primary subarachnoid hemorrhage, and (4) other forms of intraparenchymal hemorrhages (other than cerebellar). Here we will discuss the subdural and subarachnoid bleeds in detail. The approximate incidence, anatomic site of blood, relative frequency in preterm versus term-born neonates, and the usual clinical gravity of these hemorrhages are noted in Table 56.3.

The incidence of ICH has been challenging to define, as most studies have focused on symptomatic newborns, and some hemorrhages are asymptomatic. Hence the incidence of ICH is likely higher than reported.[63] In one small study of symptomatic newborns, the estimated incidence was 4.9 per 10,000 live births.[64] The largest epidemiologic data related to the California Perinatal Database, which includes maternal and neonatal hospital discharge records on 600,000 infants (2500 to 4000 g) born to nulliparous women. In this study, the incidence of symptomatic intracranial hemorrhage associated with spontaneous delivery was 1 per 1900 births, vacuum extraction delivery was 1 per 860 births, and forceps delivery was 1 per 664 births.[65] In contrast, more recent studies utilizing MRI in asymptomatic newborns in the first month of life have revealed a much higher frequency of ICH. A large prospective study found an 8% prevalence of subdural hemorrhage in this population.[66,67]

Table 56.3 provides a summary of the location, incidence, and usual clinical outcomes of the main types of hemorrhage. Table 56.4

TABLE 56.3 Intracranial Hemorrhage

Hemorrhage	Incidence (%)	Site of Blood	Full Term or Preterm	Usual Clinical Outcome
Extradural (Epidural)	Very rare	Between skull and outside of dura	FT > PT	Variable
Subdural	5–25	Between dura and arachnoid	FT > PT	Benign
Subarachnoid	1–2 FT 10 PT	Between arachnoid and pia	PT > FT	Benign
Cerebellar	0.1 FT 0.2 5 PT	Cerebellar hemispheres and/or vermis	PT > FT	Serious
Intraventricular	0.2 FT 15 PT	Within ventricles or including periventricular hemorrhagic infarction	PT > FT	Serious
Parenchymal	0.1 FT 2–4 PT	Cerebral parenchyma	FT > PT	Variable

FT, Full term; *PT*, preterm.

TABLE 56.4 Characteristics of Subdural and Subarachnoid Hemorrhages in Newborns

	Subdural Hemorrhage	Subarachnoid Hemorrhage
Epidemiology	25% (8%–45%) of all intracranial bleeds.[68,69] Rate: between 2.9/10,000 for spontaneous deliveries to 21.3/10,000 when both vacuum and forceps are used in delivery[65]	Rate: 1.3 per 10,000 spontaneous vaginal deliveries, with a higher prevalence in vacuum and/or forceps-assisted deliveries[65]
Location	Below the dura mater and superior to the subarachnoid villi	Below the arachnoid mater, in the subarachnoid space
Pathophysiology	Trauma/tearing of veins and venous sinuses	Trauma to the veins of the subarachnoid villi
Risk factors	Vacuum- or forceps-assisted delivery; coagulopathy	
Clinical presentation	Posterior fossa (infratentorial): severe hemorrhage with acute signs: stupor, lateral eye deviation, unequal pupils, nuchal rigidity, opisthotonos, bradycardia, respiratory compromise, apnea, or death. Insidious onset: may be clinically silent for days, followed by lethargy, full fontanel, irritability, respiratory abnormalities, apnea, bradycardia, and eye deviation. Hemorrhage over convexities: may have minimal or no symptoms; severe hemorrhage with acute signs: seizures, lateral eye deviation, nonreactive dilated pupil on the side of the hematoma, hemiparesis; insidious onset: may be clinically silent for months with initial presentation of increased head circumference (may occur if chronic subdural effusion)	Rarely of clinical significance and often asymptomatic May have early onset refractory seizures (usually on the second postnatal day) due to meningeal and cortical irritation or secondary hydrocephalus[69]
Outcomes	Less severe hemorrhages have variable prognoses: • ~80%–90% will have normal outcomes • ~10%–15% may have serious sequelae, including hydrocephalus requiring shunt placement • ~5% mortality Severe infratentorial hemorrhage has an extremely poor prognosis.	Very good prognosis in general. Frontal lobe or multiple hemorrhages are associated with higher rates of disability.[63]

compares subdural and subarachnoid hemorrhages. Incidence, location, risk factors, presentation, and outcomes are presented side by side. Note that subdural hemorrhage is more frequent, and risk factors for both types are related to delivery mode, instrumentation, and the presence of coagulopathy.

Pathophysiology

Subdural hemorrhages occur when bridging veins that carry blood through the dura mater to the arachnoid mater of the meninges are torn. This bleeding results in blood collecting below the dura and superior to the subarachnoid villi. Subarachnoid hemorrhage occurs when the veins of the subarachnoid villi are torn, resulting in a collection of blood in the subarachnoid space.

Clinical Presentation

Subdural and subarachnoid hemorrhages often occur with no injury to the scalp or head to suggest intracranial injury. Thus, the hemorrhages may go unrecognized. Most neonates with subdural hemorrhage remain asymptomatic, and the lesion resolves without consequence. Clinical signs of subdural hemorrhage arise when there is a large-volume hemorrhage or if bleeding slowly continues over hours or even days, as in cases of bleeding disorders. Symptomatic subdural hemorrhages often present 24 to 48 hours after birth with nonspecific signs, including apnea, respiratory distress, altered neurologic state, or seizures.

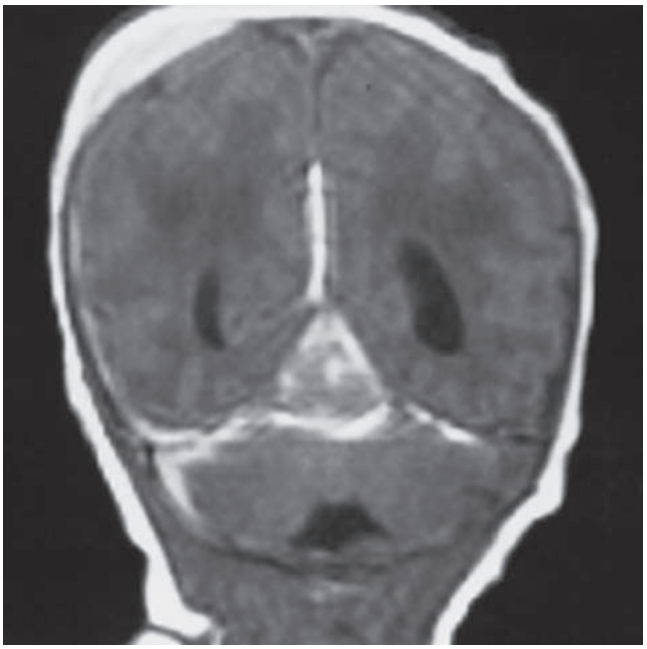
The most common clinical presentation of subarachnoid hemorrhage is seizures, as the blood from the hemorrhage can irritate the meninges and adjacent cortex. In some cases, a large subarachnoid hemorrhage irritates the meninges and causes a secondary impairment of cerebrospinal fluid (CSF) resorption resulting in hydrocephalus.

Evaluation

The three primary brain imaging modalities—CUS, CT, and MRI—have different sensitivities for detecting hemorrhage. CUS is not the modality of choice for all forms of hemorrhage: it lacks the sensitivity of MRI and CT for identifying intracranial injury and hemorrhage (other than intraventricular) and is particularly limited for the detection of extra-axial hemorrhage (subdural, subarachnoid, and extradural).[70,71] CUS also lacks sensitivity in detecting subarachnoid hemorrhage because of the normal increase in echogenicity around the periphery of the brain.[72]

CT was recommended in the 2002 American Academy of Neurology practice parameters for neonates with birth trauma and a low hematocrit or coagulopathy[73] based on data from two small studies reporting on CT diagnoses of ICH leading to interventions.[74,75] However, given the risks of radiation exposure associated with CT imaging, we suggest using MRI, when available, as the preferred method of evaluation. The use of MRI has the added benefit of better sensitivity for detecting parenchymal injury than CT. The development of more rapid MRI sequences to allow for shorter studies to detect cerebral hemorrhage should enhance physician comfort with this as a first-line technique.

MRI is more effective than CT in the delineation of posterior fossa subdural hemorrhage. Detection of subdural hematoma by ultrasound scanning, although reported, generally is difficult and requires imaging through the mastoid fontanelle in addition to the anterior fontanelle. Moreover, even when these hematomas are detected, the extent and distribution of supratentorial lesions are usually demonstrated far better by MRI or CT, and infratentorial lesions are detected better by MRI. In addition, the vast majority of subdural hematomas are infratentorial, where ultrasound has even greater challenges in accurate diagnosis (Fig. 56.4).



• **Fig. 56.4** Tentorial Subdural Hemorrhage With Blood Layering Along Both Leaves of the Tentorium and Posterior Falx. (Adapted from Castillo M, Fordham LA. MR of neurologically symptomatic newborns after vacuum extraction delivery. *AJNR Am J Neuroradiol*. 1995;16:816–818.)

Similarly, the diagnosis of primary subarachnoid hemorrhage is usually made by MRI or CT and, on rare occasions, by ultrasound.[71] On CT, the distinction between the normal, slightly increased attenuation in the regions of the falx and major venous sinuses and the increased attenuation caused by subarachnoid hemorrhage may be difficult. Sometimes, the possibility of primary subarachnoid hemorrhage is raised initially by the findings of an elevated number of red blood cells and an elevated protein content in the CSF, usually obtained for another purpose (e.g., to rule out meningitis). Exclusion of the relatively common (e.g., extension from subdural, cerebellar, or IVH) and uncommon (e.g., tumor, vascular lesions) causes of blood in the subarachnoid space is best done by MRI.

Management

Most neonates with subdural hemorrhage can be managed symptomatically. Serial hematocrits and vital signs should be monitored frequently. In most cases, the blood collection will gradually resorb over weeks to months. In rare cases of large subdural hemorrhage that cause increased intracranial pressure or mass effect, neurosurgical drainage may be required. Seizures are treated with antiseizure medications. Neonates with subarachnoid hemorrhage should receive serial head circumference measurements and serial head ultrasounds to screen for hydrocephalus.

Outcomes

The outcomes of neonates with subdural and subarachnoid hemorrhage are generally good. An estimated 80% of infants with

subdural hemorrhages will have no disability. The location and extent of the subarachnoid hemorrhage can impact outcomes. Hemorrhages in the frontal lobe or in multiple areas of the brain are associated with higher rates of disability.

Vascular Malformations

Arteriovenous malformations (AVM) are fast-flow vascular defects consisting of connections between the arterial and venous vessels through a fistula or a nidus.[76] Intracranial AVM is the most common type of AVM, affecting 1/10,000 people.[77] They represent 27% to 44% of the vascular malformations causing ICH.[78,79] Up to 80% hemorrhagic presentation in children with AVMs has been reported.[80] There is a wide range of vascular malformations; some are only found in children, and the lesion included here for discussion is the vein of Galen malformation.

Vein of Galen Malformation

Epidemiology

The vein of Galen aneurysmal malformation (VGAM) is a rare congenital vascular malformation that constitutes about one-third of the pediatric vascular and about 1% of all pediatric congenital anomalies.[80–83] It is the most common arteriovenous malformation of the newborn, and the majority (approximately 60% of all pediatric cases of VGAM) are identified during the neonatal period.[84] The overall incidence of VGAM is estimated to be 1 in 10,000 to 1 in 25,000 births.[85]

Pathophysiology

The main feature of VGAM is the dilation of the vein of Galen (Fig. 56.5). Vein of Galen malformations arise because of direct arteriovenous communication between the arterial network and the median prosencephalic vein. During neurovascular development in fetal life, between 6 and 10 weeks of gestation, the choroid plexus is responsible for fluid circulation. During this period, the median prosencephalic vein of Markowski develops and is responsible for venous drainage. After the 10th week, the venous drainage from the choroid plexus is the role of the newly developed paired internal cerebral veins. They terminate in the posterior portion of the Markowski vein, which normally disappears by the 11th week, and remnants of it form the vein of Galen.[86–88] The formation of the VGAM is then promoted by the enlargement of the median prosencephalic vein of Markowski, and this is consistent with the variation in drainage through either a normal sinus or a persistent falcine sinus, a normally transient fetal vessel.[89]

The arterial supply to the dilated vein of Galen is the posterior choroidal artery, the anterior cerebral (pericallosal) artery, the middle cerebral artery, the anterior choroidal artery, and the posterior cerebral artery.[84,87,90]

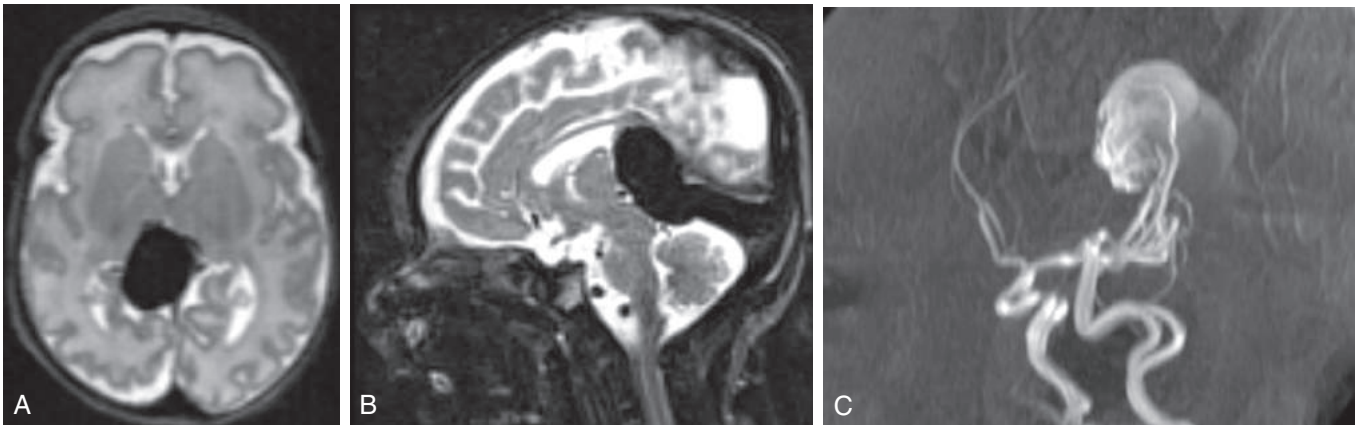
There are two classification systems that are used in clinical practice, proposed by Lasjaunias and Yasargil (Table 56.5). The Lasjaunias system includes two types of aneurysmal malformations: a primary (true) vein of Galen malformation that could be mural or choroidal form, and a secondary type resulting from a deep AVM that drains into the vein of Galen.[91]

A different classification proposed by Yasargil et al. is based on the arterial feeder patterns of drainage into the vein of Galen and is divided into four types.[92] Type I includes direct fistulas between the pericallosal and posterior cerebral arteries and the vein of Galen, type II is made up of numerous fistulas between the thalamoperforators and the vein of Galen, type III consists of multiple fistulous connections from different vessels having characteristics of type I and II malformations, and type IV has adjacent AVMs that drain into the vein of Galen and cause a secondary aneurysmal venous dilatation. Distinguishing between true VGAM (where the vein of Markowski is the pathological vessel) and AVMs that can cause aneurysmal dilatation of the vein of Galen is extremely important in order to describe the features, natural history, and treatment options of VGAMs.[93] Based on the two classifications used in clinical practice, true VGAMs are represented by the primary malformation (Lasjaunias classification) and types I to III malformations (Yasargil classification). In contrast, the secondary vein of Galen malformations (Lasjaunias classification) and type IV malformations (Yasargil classification) are parenchymal AVMs that generate secondary dilatation of the vein of Galen (Table 56.5).

The pathological findings observed with VGAM consist of a variety of ischemic, hemorrhagic, and mass effects of the malformation.[86,87,94,95]

Cardiovascular Findings

The arteriovenous connection present in the VGAM is a high-flow, low resistance system that causes an increase in cardiac output and high-output heart failure. Heart failure is usually present shortly after birth after the loss of the low-resistance



• **Fig. 56.5** Images From a Term Newborn With Vein of Galen Malformation. (A, B) Note large flow void on the T2-weighted images. (C) Corresponding angiogram. (Images courtesy Dr. Bob McKinstry.)

TABLE 56.5 Classification of Vein of Galen Aneurysmal Malformation

Classification	True VGAM	Secondary Aneurysmal Dilation of Vein of Galen Due to Parenchymal Arteriovenous Malformations (AVM)
Lasjaunias[91]	Primary malformation Mural type: • Fistulae in the subarachnoid space in the wall of the median prosencephalic vein • Presents later (infant) with hydrocephalus Choroidal type: • Multiple feeders, including: thalamoperforating, choroidal and pericallosal arteries are located in the subarachnoid space in the choroidal fissure • Presents earlier (neonate) with more severe shunts	Secondary malformation: deep AVM that drains into the vein of Galen
Yasargil[92]	Type I: fistulas between the anterior and posterior pericallosal and posterior cerebral arteries and the vein of Galen Type II: multiple fistulas between the thalamoperforator network that lies between the arterial feeders and the vein of Galen Type III: high-flow type I or II malformations with multiple fistulous connections from different vessels	Type IV: adjacent AVMs of the mesencephalon that drain into the vein of Galen and cause a secondary aneurysmal venous dilatation

VGAM, Vein of Galen aneurysmal malformation

placental circulation. After birth, blood flow increases significantly through the VGAM.[96] As much as 80% of the left ventricular output may be supplied to the brain in severe cases.[97] The high cardiac output, low diastolic pressure, increased intraventricular pressure, and impaired coronary blood flow contribute to myocardial ischemia, making the cardiac failure multifactorial and challenging to manage. The intracranial "steal" caused by the absent or reversed diastolic cerebral blood flow and congestive heart failure result in cerebral ischemia.[97–99] The increased venous return and left to right shunts through the patent foramen ovale and the patent ductus arteriosus can lead to and worsen pulmonary hypertension.[100,101]

Neurologic Findings

The neuropathological findings in the VGAM include impaired cortical development, cerebral atrophy, hemorrhagic lesions (thrombosis of the dilated vein of Galen with hemorrhagic infarction and or intracerebral hemorrhage, vascular rupture, and massive hemorrhage), or hydrocephalus (from compression and obstruction of the cerebral aqueduct or from the high venous pressure in the medullary veins that prevents reabsorption of cerebrospinal fluid due to venous hypertension).[90,95,99,101–105]

Clinical Presentation

The clinical picture of patients with VGAM depends on the age at presentation and is commonly characterized by cardiac and neurologic complications. About 44% of the cases are detected in the neonatal period, and the presentation varies with the size of the malformation. The vast majority of the neonates with VGAM present with high-output cardiac failure, pulmonary hypertension, and, in more severe cases, multiorgan system failure.[82,84,88,100,106–110] The timing of presentation and symptoms are dependent on the size of the aneurysm (the greater the size, the larger the degree of shunting through the lesion, and the earlier the presentation). Neonates tend to present clinical signs and symptoms in the first few hours of age that may worsen over the first 3 days of life. Some of the features present with the VGAM are the bounding carotid pulses with or without prominent peripheral pulses and the continuous cranial bruit over the posterior fontanelle and cranium. Cyanosis may be a presenting sign seen in these patients, and a diagnosis of congenital cyanotic heart disease is often in the differential. Due to congestive heart failure and diastolic flow reversal in the descending aorta, some infants may present with hepatic or renal insufficiency and prerenal azotemia.[96,99]

The clinical presentation differs in infants and older children. Infants present most commonly with hydrocephalus (about 15% of the overall presentation for VGAM), and children present most commonly with neurologic signs and symptoms like headaches, focal neurologic deficits, and syncope.

Evaluation

The antepartum diagnosis for VGAM can be made during the routine prenatal screening ultrasound or with the fetal MRI, which is increasingly used for more detailed characterization prenatally. When a dilated structure is visualized posterior to the third ventricle, pulsatile flow within it helps differentiate VGAMs from other midline cystic lesions.[111–114] However, based on prenatal imaging, the clinical course in neonates with VGAM has been difficult to predict. Every neonate with prenatally suspected or diagnosed VGAM should be admitted to the neonatal intensive care unit for complete evaluation and management, including weight and head circumference. Cardiac evaluation, including echocardiography, renal and liver function tests, and head imaging, should be part of the initial evaluation. When there is no prenatal data or suspicion, the diagnosis of VGAM should be considered in any neonates with high-output congestive heart failure, unexplained intracranial hemorrhage, or hydrocephalus. In this scenario, the initial evaluation is performed using cranial ultrasonography with a Doppler.[115,116] The use of ultrasound adds important value to the follow-up of patients after treatment to assess the status of the shunt and the presence of thrombosis.

Another useful imaging method to detect a mass, mostly in older infants, is CT.[117] When used in combination with angiography, a better understanding of the vascular structures can be mapped. CT angiography can be part of the planning process for intervention. MRI can be used to demonstrate the location, and the vascular components, including the status of venous drainage. The location and detection of major arterial vessels feeding the fistula are better identified on MRI than CT. Angiography remains the gold standard imaging modality to evaluate and define the architecture of the VGAM, including size, location, arterial feeders, and the dynamic aspect of venous drainage, which helps with decisions regarding intervention.

Management

The management of the patient with significant VGAM manifesting in the neonatal period remains a big challenge. Timing and approach to treatment depend on the patient's age, the severity of congestive heart failure, and the architecture of the lesion at the time of diagnosis.[118] Overall, the management approach, when indicated, is divided into medical, endovascular, or neurosurgical interventions. The main therapy goal is to minimize congestive heart failure using different therapeutic approaches that may include combinations between systemic vasodilators and low-dose dopamine.[119]

Embolization (transvenous or transarterial approach) results in better survival compared to surgical techniques and are thus the preferred approach for intervention.[120] For many years, to evaluate the risks and benefits of interventions, clinicians used the Bicêtre neonatal evaluation score.[93] A Bicêtre score of less than 8 out of 21 is historically associated with a near-fatal prognosis. Hence, these neonates are not considered good candidates for emergent embolization. A score between 8 and 12 characterizes neonates who are most likely to benefit from emergent embolization. A score greater than 12 suggests the infant can be managed medically and does not require embolization. In recent years, many centers have moved away from using Bicêtre score cutoffs as it is possible for some neonates with scores less than 8 to have good outcomes from embolization.

Embolization is considered the main approach to treatment and can be performed by an arterial transarterial approach using liquid adhesive agents or micro coils or by a transvenous approach typically using the umbilical or femoral veins.[89,93,96,102,120,121]

Even though microsurgery is no longer a primary treatment strategy, neurosurgical intervention plays an important role in persistent hydrocephalus, intracranial bleeds, hematomas, or when embolization fails.[90,122,123]

Outcomes

The development and implementation of endovascular interventions have been critical in improving outcomes in patients with VGAM, and despite therapeutic techniques, morbidity and mortality remain high.[90,106,108,124,125] As the treatment evolves and becomes more centralized, the spectrum of impairments for survivors with poor outcomes has to be better characterized. One of the largest, most recent cohorts from the UK ($n = 85$) reported that more than one-third of newborns with a vein of Galen malformation did not survive and that outcome was good in about half of the survivors.[124] Two other meta-analyses described poor clinical outcomes or death in almost one-half of neonates with VGAM.[126,127] Prognosis depends on the size of the malformation, age at diagnosis, the severity of congestive heart failure, the degree of brain injury, and the success of embolization. Neonates with untreated VGAM that survive the neonatal period with medically managed congestive cardiac failure are at increased risk for developmental delays. Later presentations include failure to thrive, seizures, focal neurological deficits, intracranial bleeds, and progressive hydrocephalus.[90,99,101,102,128,129]

Acknowledgment

The authors would like to acknowledge Dr. Ryan McAdams, Christopher Traudt, Jeffery Neil, and Terrie Inder for their work on this topic in the previous edition of this textbook. We would also like to acknowledge Dr. Catherine Amlie-Lefond and Dr. Nina Natarajan for their assistance with this chapter.

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Neonatal Neuromuscular Disorders

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KEY POINTS

- Evaluation of neonatal hypotonia includes neuromuscular conditions, and the diagnostic work-up should be approached in a stepwise manner.
- A normal creatine phosphokinase does not completely rule out muscle disease.
- Electromyography is useful in the diagnostic evaluation of hypotonia and weakness.
- Spinal muscular atrophy (SMA) can present in the neonatal period and has time-sensitive treatments. Genetic testing for the commonly found gene deletion in SMA is increasingly available as newborn screen testing in the United States.
- The search for a genetic diagnosis is crucial in patients with neuromuscular disease.

Neonatal Neuromuscular Disorders

Neuromuscular disorders comprise diseases of the muscle (congenital myopathies and muscular dystrophies), neuromuscular junction (myasthenia gravis and congenital myasthenic syndromes), nerves (neuropathies), and anterior horn motor neurons (spinal muscular atrophies). They present in the neonatal period as floppy infant syndrome with or without contractures. Respiratory insufficiency and swallowing difficulties can be in the forefront of the clinical picture and are frequently associated with significant hypotonia and weakness.

This chapter reviews our current knowledge of neuromuscular disorders with neonatal onset and their clinical details alongside pathologic, genetic, and radiologic aspects as applicable. Finally, an approach to the diagnostic evaluation of neonates when a neuromuscular disorder is suspected is discussed.

Primary Muscle Disorders

Historically, neonatal muscle disorders were divided based on histopathologic criteria into (1) congenital muscular dystrophies (CMDs), (2) congenital myopathies (CMs), (3) congenital myotonic dystrophy, and (4) metabolic myopathies. CMDs demonstrate dystrophic changes on muscle biopsy, with disruption of the muscle fiber and its architecture. Congenital myopathies have more subtle changes with preservation of the muscle fiber architecture. While histopathologically and genetically distinct, their phenotypes are often indistinguishable and characterized by congenital onset of muscle weakness and hypotonia. Some distinguishing features of various disorders are apparent at birth, while others become apparent later. Muscle weakness tends to be progressive with CMDs and relatively static in CMs. Improvement in strength has been reported with some congenital myopathies. Involvement of the central nervous system is seen more often with CMDs and congenital myotonic dystrophy and less so with CMs. Creatine phosphokinase (CPK) tends to be elevated with CMDs and normal or mildly elevated with CMs. Many entities are sporadic or inherited in autosomal recessive fashion with few notable exceptions. Congenital myotonic dystrophy type 1 is inherited in autosomal dominant pattern and shows anticipation. Collagen VI- and *RYR1*-related disorders can have both autosomal-dominant and autosomal-recessive inheritance. The genetic advancements of the last decade promised a better understanding of this heterogeneous group of disorders. Instead, it became clear that a pure genetic classification remains impractical for the practicing physician. There are numerous situations where one gene leads to multiple phenotypes and the same phenotype is caused by numerous genes. Recent attempts to classification are based on genetic but also pathologic and clinical data. In the end, a classification that follows a mechanistic approach will likely prove to be most helpful.

Congenital Muscular Dystrophies

CMDs comprise a heterogeneous group of disorders characterized by a dystrophic process on muscle biopsy. Classification schemas alongside diagnostic approaches have been proposed that take into account the recent expansion of knowledge.[1–3] Given the numerous genes discovered, CMDs have most recently been separated into seven subtypes of disorders: merosin-deficient congenital muscular dystrophy, α-dystroglycanopathies, collagen VI-related disorders, *LMNA*-related congenital muscular dystrophy, *SEPN1*-related myopathy, *RYR1*-related myopathies, and CMD without a genetic diagnosis.[2] *SEPN1* and *RYR1*, which are typically considered myopathies, are in this classification scheme secondary to the varying phenotype at presentation.

LAMA2-Related Congenital Muscular Dystrophy (Merosin-Deficient Congenital Muscular Dystrophies; MDC1A)

LAMA2-related CMD is due to autosomal recessive mutations of *LAMA2* gene known to encode the α_2 subunit of merosin. Merosin is an essential component of the extracellular matrix. In the UK, *MDC1A* was the most common form of congenital

muscular dystrophy, followed, as a group, by dystroglycanopathies and collagen VI myopathies.[4] Clinically, *MDC1A* often presents at birth or early infancy with severe hypotonia and diffuse weakness. A weak cry, poor suck and swallow, and respiratory failure are common. Contractures may be present at birth in the more severe cases or develop in time. As opposed to dystroglycanopathies, neonates with *MDC1A* typically have no encephalopathy, and cognitive as well as speech development is normal. On brain MRI, white matter T2 and FLAIR signal abnormalities become apparent in the second half of the first year[5,6] and approximately 20% of patients develop seizures. A mild demyelinating neuropathy is often present, generally not at the forefront of clinical picture. CPK levels in neonatal period and infancy are elevated four to five times above normal limits.[7] Muscle biopsy demonstrates decreased or absent laminin α_2 immunostaining aside from dystrophic features. Care for patients with *LAMA2* mutations is supportive in nature.

Dystroglycanopathies

Dystroglycan complex includes α- and β-dystroglycan and represents one of the transmembrane complexes that link cytoskeleton with extracellular matrix as part of the larger dystrophin-glycoprotein complex. α-Dystroglycan, the extracellular component of the complex, through its heavily glycosylated segment, interacts with several extracellular matrix proteins such as laminin α_2 and agrin. As a receptor for several extracellular matrix proteins, dystroglycan plays a major role in the maintenance of muscle cell structural integrity and synaptogenesis. In the central nervous system, dystroglycan plays an important role in forebrain development, specifically neuronal migration as well as synaptic plasticity and blood-brain barrier integrity.[8,9] Dystroglycan plays important roles in other tissues such as eye and secreting tissues.

Dystroglycanopathies are a phenotypically heterogenous group of disorders that share a common pathophysiologic theme: abnormal interaction of dystroglycan complex with extracellular matrix proteins because of defective α-dystroglycan O-glycosylation. Their phenotype ranges from severe neonatal muscle weakness with early lethality as well as abnormal brain and eye development to asymptomatic hyperCKemia discovered in adult years.

The modern classification of these disorders is based on their genotype and pathophysiology instead of severity. In this classification, dystroglycanopathies are subdivided into primary (due to mutations in *DAG1* gene which encodes the two dystroglycans), secondary (due to mutations in genes known to encode enzymes involved in O-glycosylation of the α-dystroglycan), and tertiary (due to mutations in genes known to encode enzymes and other factors implicated in production of the oligosaccharide building blocks). Primary dystroglycanopathies are the most recent addition to the group and includes a handful of cases, all found in consanguineous families. Their phenotype parallels the more common secondary dystroglycanopathies and includes severe as well as mild forms.[10] Secondary dystroglycanopathies are due to malfunction of various enzymes involved in α-dystroglycan O-glycosylation at the endoplasmic reticulum and Golgi apparatus levels. The number of enzymes involved and their encoding genes have seen significant expansion over the last two decades.

Severe forms, classically labeled as Walker Warburg syndrome" or "Muscle Eye Brain disease", present at birth with severe muscle weakness and hypotonia, as well as often severe brain and eye malformations. Although hypotonia and muscle weakness are severe, the clinical picture is dominated by encephalopathy, brain and eye malformation, and sometimes seizures. CPK is generally elevated. Brain involvement includes one or a combination of the following findings: agyria, lissencephaly (type 2, "cobblestone"), focal pachygyria or polymicrogyria, heterotopia, complete or partial agenesis of corpus callosum, cerebellum abnormalities, brainstem abnormalities including a "kinked" appearance, posterior fossa cyst, occipital encephalocele microcephaly, hydrocephalus, and white matter changes.[11] Eye abnormalities are quite variable as well and can include cataracts, abnormalities of the anterior chamber, abnormalities of the posterior chamber, microphthalmia, microcornea, small lens, retinal abnormalities, optic nerve hypoplasia, coloboma, and glaucoma. Many of these patients have significantly shortened life span and show little psychomotor developmental progress. Milder phenotypes present at birth or soon after with hypotonia and muscle weakness. MRI might show white matter abnormalities starting in the second half of the first year and cognitive disability which first becomes obvious as various degrees of global developmental delay. Yet, milder forms exist with onset as late as adult years. Tertiary dystroglycanopathies is another emerging group which phenotypically is indistinguishable from other dystroglycanopathies.

Collagen VI-Related Disorders

Collagen VI-related disorders are caused by mutations in the genes that encode one of the three subunits of collagen VI (*COL6A1*, *COL6A2*, *COL6A3*) and are classically divided into Ullrich CMD and Bethlem myopathy. Overlaps between the two phenotypes are common though, and the reader is encouraged to think about this group of disorders as a continuum between the two entities.[12] In collagen VI-related disorders there is often a combination of joint laxity and joint contractures in addition to hypotonia and weakness. While Ullrich CMD has a more severe phenotype and onset in utero, often with congenital contractures, Bethlem myopathy tends to be milder and has more variable onset starting in utero and extending into adult life. Ullrich CMD presents at birth with severe muscle weakness, hypotonia, and a combination of marked joint laxity (involving the distal joints) and joint contractures (involving the proximal joints, kyphoscoliosis, and torticollis). Weakness is slowly progressive and respiratory insufficiency is either present at birth or develops later. When it presents in utero or at birth, Bethlem myopathy tends to have a milder phenotype and behaves more like a congenital myopathy. Other useful distinguishing features for collagen VI-related disorders include a prominent calcaneus, hyperkeratosis pilaris on the extensor surfaces, keloid formation, and sometimes congenital hip dislocation. CPK is normal or moderately elevated and muscle biopsy can show both myopathic and dystrophic features. Diagnosis is generally suspected based on clinical grounds and confirmed by targeted genetic testing.

LMNA-Related Congenital Muscular Dystrophy

The *LMNA* gene, which is associated with autosomal dominant form of Emery–Dreifuss syndrome in older children or adults, has been found mutated in neonates and children with CMD.[13–16] *LMNA* encodes for lamin A/C, which is a nuclear envelope protein. The syndrome is classically described as reduced fetal movements, severe hypotonia, and weakness with a "dropped head" appearance because of involvement of the neck muscles.[15]

SEPN1-Related Myopathies

SEPN1-related myopathies straddle the demarcation line between CMDs and CMs, and encodes for selenoprotein N. *SEPN1* is an endoplasmic reticulum glycoprotein preferentially expressed early in the development, and with roles in redox signaling and Ca homeostasis.[17] Most patients with *SEPN1*-related myopathies present at birth or within the first 2 years of life with predominantly axial hypotonia, poor head control, and feeding difficulties. The distinguishing features of *SEPN1*-related myopathies, including spine rigidity, amyotrophy, and respiratory impairment, became apparent in childhood.[17] Despite a relatively well-defined phenotype, the pathologic findings are variable and include dystrophic features, multi-minicore lesions, fiber size disproportion, and desmin inclusions.

Congenital Myopathies

The term congenital myopathies (CMs) refers to muscle disorders that present in neonatal period or early infancy and lack dystrophic changes on muscle biopsy. CMs tend to have a slowly progressive or nonprogressive course. The severity spectrum is wide, starting with severe illnesses often fatal in the first years of life (*MTM1* and severe *ACTA1* disorders), to mild muscle weakness leading to mild gross motor developmental delay. Muscle biopsy shows structural changes at the myofiber level in absence of dystrophic features. The type of structural abnormalities defines the various types of congenital myopathies. Electron microscopy is often very helpful and should always be included when CM is suspected. As with CMDs, genetic advances expanded our understanding of congenital myopathies. We now know that certain genotype might lead to several different histopathologic and clinical phenotypes.[18] The best recognized congenital myopathies are core myopathy, nemaline myopathy, centronuclear myopathy, fiber-type disproportion myopathy, and myosin storage myopathy. In a population study, core myopathies were the most common, representing approximately half of all CMs cases, followed by nemaline and centronuclear myopathies each representing approximately 15% of all CMs cases.[19] Typical neonates present with hypotonia, and weakness. More severe cases have respiratory insufficiency and swallowing difficulties. Elongated and weak face, high-arched palate, and mild ptosis are seen in some of the CMs (nemaline and centronuclear myopathies). Skeletal abnormalities such as hip dislocation, club feet, and pectus excavatum are common. As opposed to congenital muscular dystrophies, CPK may be normal or mildly elevated. Typically, electromyography (EMG) shows myopathic change. EMG can also be normal and even show neurogenic features. Aside from nonspecific myopathic features, muscle biopsy often reveals specific structural abnormalities that define each group of disorders. Genetic testing is often employed first nowadays.

Core Myopathies

Central core and multi-minicore disease together comprise the "core myopathies" and are the most common form of congenital myopathies.[20] Histopathologically, focal myofibrillar disruption with absence of mitochondria leads to formation of single or multiple cores visible on oxidative stains such as Gomori trichrome. The majority of cases are due to autosomal recessive or autosomal dominant mutations in the *RYR1* gene. Clinically, patients with *RYR1*-related central core or multicore myopathies tend to have a milder phenotype with hypotonia and muscle weakness, and often lack facial involvement.[20] The more severe cases, often autosomal recessive, present at birth with contractures, arthrogryposis, and respiratory insufficiency.[21] Serum CPK is often normal or mildly elevated. The *RYR1* gene encodes the ryanodine receptor, a sarcoplasmic reticulum calcium channel with role in excitation-contraction coupling.[22] Mutations in *RYR1* can lead to various phenotypes (nemaline myopathy, congenital myasthenic syndrome),[23] as well as malignant hyperthermia. Malignant hyperthermia precautions are needed every time *RYR1* mutations are a possibility.

Multi-minicore disease is rare in the neonatal period and, when present, is notable for marked axial weakness, myopathic facies, and respiratory failure. Patients may present with arthrogryposis. The two genes most often associated with multi-minicore myopathy are *SEPN1* and *RYR1*. *SEPN1*, discussed previously, accounts for the majority of patients.[24] There is significant phenotypic overlap with rigid spine syndrome.

Nemaline Myopathy

Nemaline myopathy derives its name from "nema," the Greek word for thread. The muscle biopsy shows threadlike rods. The rods stain red on Gomori trichrome, giving its characteristic appearance. Newborns may present with hypotonia with weakness including bulbar involvement. The facial and axial muscles are often involved. Neonates may require respiratory support because of weak respiratory muscles, frequent suctioning, and nutritional support often via gastrostomy tube due to swallowing difficulties. In more severe forms, reduced fetal movements and polyhydramnios occur, and the neonate has severe respiratory failure and feeding difficulties in addition to arthrogryposis.[25]

The most severe cases of nemaline myopathy, often caused by *ACTA1* mutations, have poor prognosis with rare survival past the first year of life. Those with the milder presentation may show improvement and, some achieve independent ambulation. However, many may still require respiratory assistance because of nocturnal hypoventilation and may have failure to thrive or scoliosis. More than 10 genes are associated with nemaline myopathy with *NEB* causing most autosomal recessive cases, and *ACTA1* most autosomal dominant cases.[26]

Centronuclear Myopathy

Centronuclear myopathy is a rare cause of neonatal weakness. The name comes from the histopathologic appearance of centrally located nuclei.[27] Both X-linked as well as autosomal dominant and recessive forms exist as mutations in more than 10 genes, which are associated with this phenotype. The X-linked form caused by mutations in *MTM1* gene is the most common form and often associated with a severe phenotype. Presentation in neonates is notable for severe hypotonia and weakness, associated with bulbar and extraocular muscle involvement, and myopathic facies. Respiratory compromise and need for ventilation are common.[27] Neonates are often macrocephalic, with long, narrow face and may have undescended testes. The *MTM1* gene, encoding for myotubularin, is located on the X chromosome at Xq28, thus resulting in the male predilection for this disease. However, secondary to random X-inactivation, females may be affected.[28]

Autosomal dominant *DNM2* mutations, as well as autosomal recessive *RYR1*, *TTN* mutations, may also lead to neonatal disease.[23]

Congenital Fiber-Type Size Disproportion Myopathy

Congenital fiber-type size disproportion myopathies are characterized by type 1 fibers that are significantly and uniformly smaller than the type 2 fibers. So far, over 10 genes are associated with this pathologic phenotype inherited in both autosomal dominant and recessive fashion. Clinically they share often neonatal onset, with hypotonia, muscle weakness, and variable facial, bulbar, extraocular, and respiratory muscle involvement.

Myosin Storage Myopathy

In addition to several muscle disorders and cardiomyopathy, *MYH7* mutations are also responsible for myosin storage myopathy, also known as hyaline body myopathy. Onset is neonatal with hypotonia and weakness. On muscle biopsy, hyaline bodies are noted predominantly in type 1 muscle fibers on H&E and myosin ATPase stains, and defined as granular material on electron microscopy.

Congenital Myotonic Dystrophy

Myotonic dystrophy is a multisystem, triple repeats disease with wide phenotypic variation dependent, in great part, on the number of cytosine–thymine–guanine (CTG) repeats in the *DMPK* gene. The transcribed CUG RNA repeat has negative impact on expression of *DMPK* as well as other genes such as *SIX5* and splicing of mRNA of the *ClC1* gene. The congenital form is the most severe and is generally associated with CTG repeats higher than 1000.[29] In these cases, pregnancy is usually remarkable for polyhydramnios and reduced fetal movements. Newborns with congenital myotonic dystrophy are often delivered prematurely. At birth, there is marked hypotonia and paucity of movements. Breathing, sucking, and swallowing difficulties are often present and persistent, often leading to gastrostomy tube placement and tracheostomy. Talipes equinovarus is often present. Facial features include facial diplegia with a "carp" mouth appearance and bilateral ptosis. Clinical examination demonstrates hyporeflexia or areflexia and diffuse weakness more severe in distal muscles than proximal ones. Grip and percussion myotonia are not present at this age and develop in childhood. There may be pulmonary hypoplasia. These factors lead to high morbidity in the neonatal period and infancy. The duration and severity of the respiratory muscle weakness and pulmonary hypoplasia are key determinants of outcome. Prolonged mechanical ventilation, defined as greater than 4 weeks in duration, is a negative prognostic factor in these neonates.[30] Some neonates with severe myotonic dystrophy require tracheostomy placement; however, it is not uncommon for older infants or children to be decannulated.

The diagnosis of congenital myotonic dystrophy should be considered when there is a positive family history. The disease is transmitted by mother and shows anticipation. A detailed history and examination of the mother often, but not always, reveals characteristic facial features associated with classical myotonic dystrophy, including frontal balding, ptosis, facial diplegia, temporal wasting, or cataracts. In addition, examination of the mother typically reveals grip myotonia. These symptoms are often subtle, such that the mother is unaware of her diagnosis.

Laboratory evaluation reveals normal to mildly elevated CPK. Muscle biopsy is often remarkable for markedly increased number of internal nuclei. Electrographic or clinical myotonia is absent in the neonatal period. The diagnosis is often suspected on the clinical basis and confirmed by genetic studies. The *DMPK* gene, located at chromosome 19q13.3, contains a CTG trinucleotide repeat in the 3′ noncoding region.[31] Unaffected individuals have between 5 and 27 repeats, while patients with a classical (not congenital) presentation have 50 to 1000 repeats. Just like other triple repeats diseases, myotonic dystrophy exhibits anticipation, resulting in earlier and more severe presentation.

Neonates who survive the neonatal period typically require ongoing respiratory support and can survive into adulthood with close respiratory and cardiac monitoring and with therapy; however, cognitive impairment is frequent and can be severe.[32]

Metabolic Myopathies

Neonatal presentation is not typical for metabolic myopathies except acid maltase deficiency. Other glycogen storage disorders rarely present at this age and when they do, myopathy is associated with other systemic features such as cardiomyopathy and liver involvement. Infantile form of acid maltase disease, also known as Pompe disease, can present at birth with severe and progressive muscle weakness and hypotonia. The majority of patients have cardiac disease, respiratory insufficiency, and a fatal course unless enzyme replacement therapy is initiated early. CPK is usually elevated, and EMG is myopathic with frequent myotonic and complex repetitive discharges. Muscle biopsy shows vacuoles which stain positive with acid phosphatase.

Motor Neuron Disorders

Motor neuron disorders comprise a group of genetic disorders that share involvement of the anterior horn motor neurons. This group is dominated by 5q spinal muscular atrophy (SMA), the most common inherited motor neuron disorder. A substantial number of other rare forms of motor neuron disease are characterized; the more common ones will be mentioned below.

5q Spinal Muscular Atrophy

5q SMA is an autosomal recessive disorder with an incidence of approximately 1 in 10,000 live births, and is the most common form of SMA.[33] Onset and severity fall along a wide spectrum, from very severe cases with intrauterine onset, to mild cases with onset in adult years and mild disability.[34] Given the wide variability, SMA categorized by age of onset and anticipated motor outcome, classically as types 1 to 4, though some recognize "type 0", reserved for those cases with in utero onset.[35] The majority of patients have a homozygous deletion of the *SMN1* gene, survivor motor neuron 1. This gene is expressed in all cell types, and severity of disease is modified by the *SMN2* gene, survivor motor neuron gene 2. The *SMN2* gene is nearly identical to *SMN1* and produces principally a truncated, nonfunctional protein as well as

a small amount of functional protein. Increased copies of *SMN2* modify the severity of disease, with greater copies correlating with the milder phenotype.[36]

Historically, patients undergo a course that includes a decline phase followed by a plateau phase. The decline phase occurs in utero for SMA type 0 newborns affected at birth. For infants whose onset is after birth, a period of normal development is followed by a decline phase that usually lasts for weeks to months.

SMA type 0 neonates have onset of weakness in utero and present with arthrogryposis at birth. Respiratory distress and facial weakness can be present in addition to profound hypotonia and limb muscle weakness. Most of these patients die in the first weeks of life. SMA type 1 or Werdnig–Hoffmann disease presents between birth and 6 months of age. Some of these neonates become symptomatic soon after birth, while others come to medical attention at several months of age in the setting of respiratory or feeding difficulties. Typical patients have profound hypotonia and severe weakness affecting legs more than arms, and proximal more than distal muscles. Usually there are no antigravity movements of the more proximal limb muscles with some movements distally at the level of ankles/wrists or fingers/toes. Deep tendon reflexes are absent, and facial muscles are unaffected. In fact, these infants tend to have a very bright facial expression, however bulbar weakness with dysphagia and poor feeding are often present, as are tongue fasciculations. Various degrees of respiratory insufficiency are present at the time of diagnosis. Because of the disproportionate involvement of the intercostal muscles and relative sparing of the diaphragm, a "bell-shaped" chest conformation is noted. Contractures are not part of the typical initial presentation of SMA, although they can develop after prolonged immobilization. Typically, respiratory function declines over time, with historic cohorts requiring respiratory support by BiPAP or invasive ventilation in the first year of life.[34]

Diagnostic evaluation is often broad initially, beginning with CPK testing, which can be normal to mildly elevated (up to 500 IU/L), however genetic testing is imperative, with testing of *SMN1* and *SMN2* copy number variants. Most cases of 5q SMA are secondary to a homozygous deletion of the *SMN1* gene, and as noted, the copy number of *SMN2* modifies phenotype. In cases where genetic testing is unrevealing, EMG and/or muscle biopsy can be considered to aid in localization and subsequent targeted genetic testing of less common causes of SMA.

Until 2016, treatment for SMA was supportive, without therapeutic options; now, there are three Food and Drug Administration (FDA) approved drugs for the most common cause of SMA, which has altered the landscape for clinical degree of concern, testing, treatment, and outcomes.[37–39]

With the advent of therapeutic options as discussed below, there are moves toward universal newborn screening, given the recognition that early treatment augments outcome. In the United States, SMA is one of the disorders nationally recommended to be on the newborn screen, however implementation is at the state level, with nearly three-fourths of states screening as of May 2021.[40]

Any infant with positive newborn screen for SMA should undergo confirmatory genetic testing of *SMN1* and *SMN2* copy numbers, alongside consultation of neurology. Involvement of the neurologist and pulmonologist can aid in next steps on potential treatment. Consensus guidelines are available for management of the neonate with positive newborn screen findings for SMA.[41,42]

There are currently three FDA-approved medications for SMA: nusinersen (brand name Spinraza), onasemnogene abeparvovec (brand name Zolgensma), and risdiplam (brand name Evrysdi). Nusinersen is an mRNA antisense oligonucleotide, while risdiplam is an mRNA splicing modifier, both acting to increase survival motor neuron protein via the *SMN2* gene. Nusinersen is administered intrathecally, while risdiplam is administered orally. In the sentinel Phase 3 clinical trial, 37 of 73 SMA type I patients who received intrathecal nusinersen gained motor milestones compared to 0 of 37 patients who received sham injections.[43] Providing more hope for presymptomatic treatment was a Phase 2 trial of 25 asymptomatic newborns predicted to have type I or II SMA phenotype based on absence of SMN1 and copy numbers of SMN2, where 22 of 25 patients achieved independent ambulation during the 2.9 years of follow up.[44]

Onasemnogene abeparvovec (AVXS-101) utilizes adeno-associated virus, serotype 9 (AAV9) vector to delivery SMN1 gene. In the sentinel study, 15 patients with SMA type I treated with onasemnogene abeparvovec survived, were event free, defined as not requiring respiratory support for greater than 16 hours continuously for 14 days in the absence of an acute reversible illness or perioperative state at 20 months of life, and gained motor milestones after a single intravenous dose.[45] Onasemnogene abeparvovec is currently approved for those under 2 years of age.[38]

Risdiplam is the most recently approved medication (August 2020) for SMA, and is the only approved oral medication.[39] Like nusinersen, risdiplam is a small molecule, however, it is noted to affect expression of SMN protein in peripheral tissues in addition to the CNS/motor neuron. Based on two open-label studies (ClinicalTrials ID NCT02913482, NCT02908685) FDA approval was given due to clinical improvement. Currently, part 1 outcomes are available, which demonstrated increased SMN protein concentration in the blood.[46]

Non-5q Spinal Muscular Atrophies

Non-5q SMAs comprise a genetically and phenotypically heterogeneous group of disorders that share motor neuron involvement. Different classifications are used, including mode of inheritance and pattern of muscle involvement. Some of these disorders are important entities for neonatologists, while others are not seen in newborns. Although rare, two etiologies that may present in the neonatal period are addressed below.

Spinal Muscular Atrophy With Respiratory Distress

Spinal muscular atrophy with respiratory distress (SMARD) represents a group of motor neuron disorders that present at birth but are notable by a rather sudden and severe respiratory insufficiency that leads to a requirement for ventilatory support as well as predominantly distal weakness and distal contractures. Respiratory insufficiency is less common at birth, but more often noted between 6 weeks and 6 months of life. Diaphragmatic weakness leading to diaphragmatic eventration is characteristic. Clinically, SMARD can be distinguished from 5q SMA by diaphragmatic weakness and eventration with resultant normal thoracic appearance and lack of the "bell-shaped" chest. Distal weakness with contractures can be present, versus the proximal predilection

of 5q SMA. Several genes have been identified, most notably *IDHMBP2* (immunoglobulin μ-binding protein 2), which results in SMARD, type 1.[47]

Current management for SMARD is supportive. Restrictive lung disease is the main cause of morbidity and mortality. Swallowing difficulties, aspirations, and poor caloric intake are also common, leading to gastrostomy tube placement. Cognitive development is believed to be normal. Palliative care is often offered for the more severe cases, while milder cases may benefit from aggressive respiratory and gastrointestinal management. Late-onset forms with a milder phenotype have also been described.

Pontocerebellar Hypoplasia Plus Spinal Muscular Atrophy

Pontocerebellar hypoplasia is a heterogeneous group of inherited disorders that share hypoplasia or atrophy of the cerebellum and pons, with or without other brain or eye abnormalities.[48] Pontocerebellar hypoplasia type 1 can present with features of SMA, with pathologic studies demonstrating anterior horn cell degeneration. Several genes have been implicated, most commonly *EXOSC3*, with increasing knowledge of the genotypic-phenotypic variation in presentation.[49–52] All are inherited in an autosomal recessive pattern. Although the severity and age of onset varies, neonatal presentation includes hypotonia and weakness, contractures, and respiratory distress as well as encephalopathy given pontocerebellar findings. Management is supportive.

Neonatal Neuromuscular Junction Disorders

Transient Neonatal Myasthenia Gravis

Maternal myasthenia gravis is an autoimmune disorder caused by antibodies to acetylcholine receptor (AChR) or to the muscle-specific tyrosine kinase (MuSK). In a subset of mothers with myasthenia gravis, neonates develop transient weakness, often initially presenting as feeding difficulty or bulbar weakness, but can progress to respiratory failure requiring ventilatory support. Most commonly, this is in the setting of AChR receptor antibodies, however there are case reports of involvement in the setting of MuSK antibodies, which can present more severely.[53,54] Interestingly, neonatal disease does not appear to be related to antibody level in the mother, thus requiring all neonates born to mothers with disease to be monitored closely. While neonatal symptoms can occur immediately after birth, they may not develop until a few days after birth. Therefore, newborns of mothers with myasthenia gravis should be observed between 2 and 4 days post birth.[55,56] Affected newborns should receive supportive care such as nasogastric feeds or ventilatory support. Pyridostigmine is indicated in those with maternal AChR antibodies, and intravenous immunoglobulin can be considered in severe cases.[56]

Congenital Myasthenic Syndromes

Congenital myasthenic syndromes (CMS) include a growing number of heterogeneous disorders that are all characterized by the failure of neuromuscular transmission secondary to a genetic defect. A significant proportion of CMS cases present in the neonatal period or early infancy; however, presentation can be subtle, and diagnosis may be delayed by years. The general clinical characteristics include fatigable muscle weakness involving the extraocular, bulbar, respiratory, and limb muscle systems in different combinations.[57]

Certain patterns are unusual enough to deserve special mention. *Dok7* CMS patients can present in the neonatal period with stridor due to bilateral vocal cord paralysis, respiratory distress, and feeding difficulties. Intubation and ventilator support are necessary for some patients.[58] Choline acetyltransferase mutations lead to hypotonia with marked bulbar symptoms and respiratory insufficiency in the neonatal period followed by life-threatening episodes of apnea later in infancy.[59] In a retrospective review of CMS cases presenting in early infancy, 8 out of 11 patients presented at birth in general with severe respiratory distress in addition to hypotonia, weakness, and contractures.[60]

Laboratory evaluation for CMS is usually unremarkable, with normal CPK. Muscle biopsy is either unremarkable or shows mild nonspecific findings. Guidance to diagnosis comes from EMG with repetitive nerve stimulation, which historically has been paramount in the establishment of a neuromuscular junction defect. Increased availability of genetic testing has reduced the necessity of electrophysiologic testing and should be considered in the setting of phenotypic variability in presentation.

Pyridostigmine is the most commonly used medical treatment for CMS. Although a good number of CMS patients respond partially to pyridostigmine, patients with certain types of CMS may worsen. Close observation is needed when pyridostigmine is administered, especially if the exact type of CMS is not known. Other medical treatments may be available, depending on the specific CMS identified; for example, patients with *Dok7* mutations may respond to oral albuterol.[61] Respiratory support remains important though. Noninvasive ventilation is preferred as some patients improve with age. Nutritional support with a gastrostomy tube should be considered.

Peripheral Neuropathies

Hereditary peripheral neuropathies are a rare cause of floppy infant syndrome in the neonatal period. The presentations in the neonatal period can vary from severe hypotonia and weakness with respiratory difficulties, to milder with feet deformities.[62] Electrophysiologic studies will confirm the neuropathy and orient the genetic testing by subdividing them into axonal versus demyelinating. Management is supportive.

Approach to the Hypotonic Newborn

The field of pediatric neuromuscular disorders has exploded following the genetic advances of the last two decades. While the old clinicopathologic classification remains useful, advances in genetics have elucidated the degree of genotypic-phenotypic variability and have implications in the approach to diagnostic evaluation. Hypotonia in the newborn can occur from many reasons, including neurologic, systemic (such as sepsis), and genetic such as trisomy 21 or Prader-Willi syndrome. Neurologic etiologies can originate across the neuroaxis, from central to peripheral (neuromuscular) etiologies. Central causes constitute the majority of cases, accounting for between 60% and 80% of hypotonic newborn.[63,64]

Initial efforts to evaluate hypotonia start with history and physical examination, including a neurologic examination. Historical features include quality of reported fetal movements and complications such as polyhydramnios or previous pregnancy losses. Polyhydramnios suggests poor swallowing ability in utero. Three generations of family history can identify other affected family members, recognizing limitations given intrafamilial variability of neuromuscular conditions. Examination of the neonate's mother for grip myotonia and querying maternal history of easy tripping or muscle stiffness such as hand cramping will help point the clinician toward congenital myotonic dystrophy. This is something that should be done each time a newborn is evaluated for hypotonia; grip myotonia can be tested by asking the mother to squeeze her hand tight and quickly let go, or by testing for percussion myotonia at the thenar eminence or brachioradialis.

Examination of the floppy newborn should include delineation of hypotonia (axial vs. proximal vs. distal vs. diffuse) as well as degree of weakness. Hypotonia is when the tone of the muscle is decreased, whereas weakness refers to decreased muscle strength. Weakness equal to or greater than hypotonia is suggestive of a peripheral etiology and can be further classified as primarily proximal or distal, while weakness that is minor as compared to hypotonia is more suggestive of a central etiology. Weakness can be noted by a newborn's ability to generate strength, even if hypotonic. The presence or absence of deep tendon reflexes, the resting position, and the frequency of spontaneous movements are important.

Central hypotonia is more common than neuromuscular causes, with 60% to 80% of neonatal hypotonia in this category.[63,64] Signs and symptoms suggestive of CNS involvement include, but are not limited to, microcephaly or macrocephaly, hyperreflexia, encephalopathy, seizures, dysmorphic features, history suggestive of hypoxic-ischemic injury, severe hypotonia in a setting of mild weakness, and metabolic derangements. A history of hypoxic-ischemic injury is not mutually exclusive for a neuromuscular condition, as many of these conditions place the newborn at risk for hypoxic-ischemic injury in and of themselves, and thus detailed prenatal and perinatal history obtained in those presenting with presumed hypoxic-ischemic injury should still assess for signs of hypotonia prior to delivery. MRI of the brain should be obtained in newborns with hypotonia. In most neuromuscular conditions, these studies are normal. Pontine and cerebellar hypoplasia will point in the direction of SMA with PCH. Some forms of CMD can often present with significant brain malformations, as mentioned above. The white matter changes described in merosin-deficient CMD are not apparent in the neonatal period.

In addition to the history and physical examination, the tools available to the clinician include the following:

1. CPK
2. EMG with nerve conduction studies
3. Muscle biopsy
4. Genetic testing

Few gestalt diagnoses exist. Congenital myotonic dystrophy presents with typical facial features. If this is combined with maternal myopathic facial appearance and grip myotonia, one may go straight to genetic confirmation. The presentation of SMA in the neonatal period is another situation when gestalt diagnosis is possible for the experienced neonatologist and is increasingly available on newborn screen.

Creatine Phosphokinase

CPK is a rapid test that should be performed when a neuromuscular condition is first suspected. Significantly elevated values (more than five times normal) will point toward a muscle disorder, more likely a CMD. Normal or mildly elevated values can be seen in congenital myopathies and SMA.

Electromyography

Seen as a difficult test to perform in newborns, when performed by experienced electrophysiologists, EMG can be of immense help. The main advantage of EMG is a rapid, on the spot, diagnosis of a neurogenic process versus myopathic process versus neuromuscular junction defect. Given increasing availability of genetic testing, pragmatically, EMG is now often reserved when genetic testing options are limited, or targeted genetic testing is required.

Muscle Biopsy

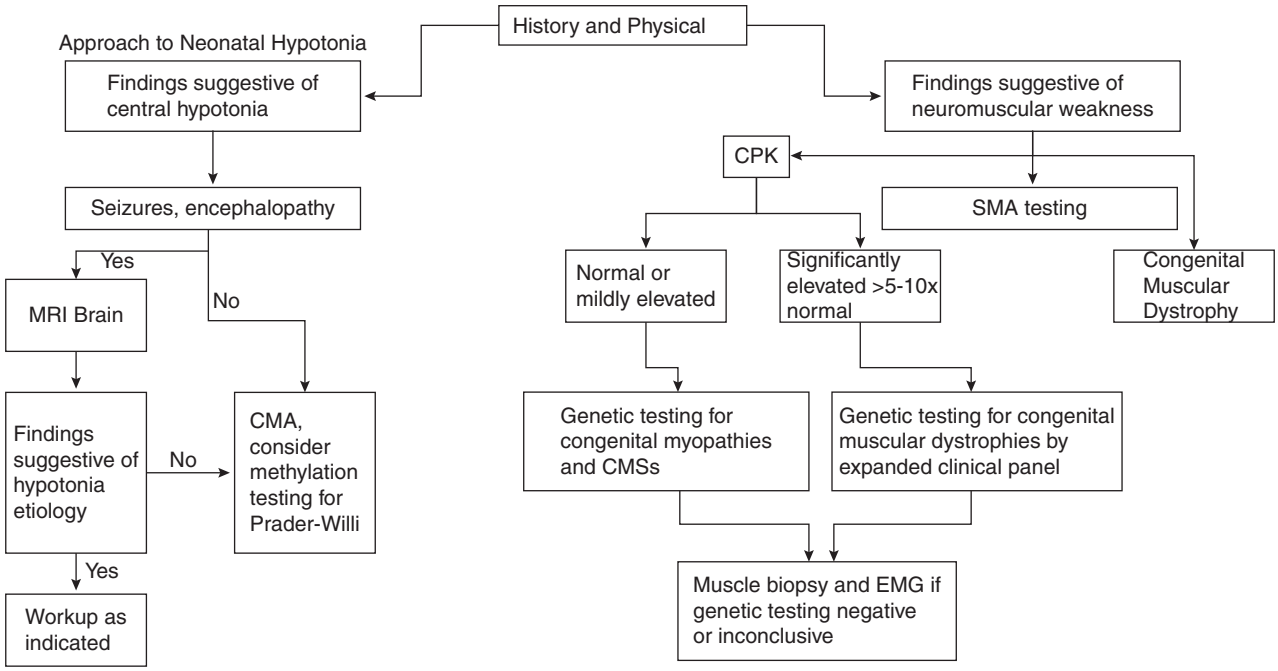
Despite advances in genetic diagnosis, muscle biopsy remains an important tool in the diagnosis of neuromuscular disorders. Its main utility consists in identification of particular types of congenital myopathy or CMD and, as a consequence, directing the genetic testing toward smaller panels of genes. As the pricing of genetic testing is decreasing, it is becoming feasible to start the work-up with genetic testing and employ muscle biopsy only if the first round of genetic tests fails to reveal a genetic abnormality.

Genetic Testing

The availability of genetic testing has increased exponentially in the last decade. A clinicopathologic diagnosis is no longer sufficient, and every effort should be made for genetic confirmation. Single genes as well as panels of genes are now commercially available from multiple commercial laboratories.

In newborns with multiple congenital abnormalities in addition to hypotonia, genetic testing should begin with karyotype and chromosomal microarray (CMA). Microarray may also detect Prader-Willi syndrome, as can methylation testing. Increasingly, whole exome sequencing is available, and is noted to provide diagnoses in 25% to 49% of cases in concerns for pediatric neuromuscular disorders or neurologic disorders.[65–69] Notably, whole exome sequencing has limitations: secondary to technical limitations, it cannot assess for trinucleotide repeats, and thus will not detect disorders with anticipation, such as congenital myotonic dystrophy. Additionally, it may not detect copy number variants, or certain single gene deletions secondary to probe size. Concomitant chromosomal microarray may aid in this detection.

There is increasing interest and availability of whole genome sequencing as well, which has been used to evaluate critically ill neonates using trio technique (e.g., testing the patient, as well as biologic mother and father).[70,71] Limitations currently include availability of this testing, and challenges in interpretations of variants in noncoding regions. A suggested approach to evaluating neonatal hypotonia is presented in Fig. 57.1.



• **Fig. 57.1** Diagnostic approach to neonatal hypotonia. *CMA*, Chromosomal microarray; *CMS*, congenital myasthenic syndrome; *CPK*, Creatine phosphokinase; *EMG*, electromyography; *SMA*, spinal muscular atrophy.

Suggested Readings

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Neonatal Seizures

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KEY POINTS

- Neonatal seizures are common.
- Clinical assessment alone is insufficient for diagnosis, and EEG evaluation is necessary.
- Seizures are often symptomatic of an underlying cause requiring investigation.
- Confirmed seizures should be treated with antiseizure medications.

Neonatal Seizures

Seizures in the neonate occur in 2 to 4 per 1000 live births and are a cause of neonatal morbidity and mortality.[1–3] Frequently, this onset is a neurologic emergency, requiring prompt and thorough diagnostic investigations and therapeutic interventions. Seizures in the newborn may be transient due to electrolyte abnormalities, the harbinger of underlying brain injury or developmental abnormalities, or the initial presentation of an underlying epilepsy. The clinical appearance of seizures in the neonate differs from that seen in older infants and children. Seizures themselves may be subtle, or without clinical manifestations, and challenging to differentiate from other involuntary movements in the neonate. There are ongoing efforts to determine how aggressively to treat seizures, which medications to use, how long to treat, and the impact of neonatal seizures on neurodevelopmental outcomes.

This chapter discusses the diagnosis, neurophysiologic criteria, etiologic considerations, treatment, and prognosis of neonatal seizures. For the purposes of this chapter, the term *seizure* refers to an *epileptic event*: that is, an event with an electrographic correlate.

Classification of Neonatal Seizures

Seizures in the neonate often are often difficult to clinically differentiate from nonepileptic movements and may present differently than seizures in older infants or children. In 2021, the International League Against Epilepsy (ILAE) published a new framework for the classification of neonatal seizures.[4] The updated classification system emphasizes the need to incorporate electroencephalography (EEG) evaluation (Fig. 58.1), as fewer than 50% of paroxysmal clinical events are correctly identified as seizure versus non-seizure, with poor inter-observer agreement, regardless of the observer's specialty.[5] The ILAE classification system recognizes that approximately 50% to 80% of neonatal seizures are electrographic only and categorizes electroclinical seizures as either (1) motor seizures characterized by abnormal movements, (2) nonmotor seizures characterized by autonomic changes or behavioral arrest, or (3) sequential seizures or unclassified (Table 58.1).[6–9]

Motor Seizure

Automatisms

Neonatal seizures with automatisms are typically manifested as oral–buccal–lingual movements, often with impairment of consciousness. They may be seen in conjunction with other seizure types, such as clonic or sequential seizures. These seizures often appear voluntary and are notoriously difficult to determine clinically. Seizures may have associated vital sign changes, including otherwise unexplained fluctuations in heart rate, blood pressure, or oxygen saturation. As more benign movements may mimic the motor features of these seizures, confirmation with EEG is mandatory.

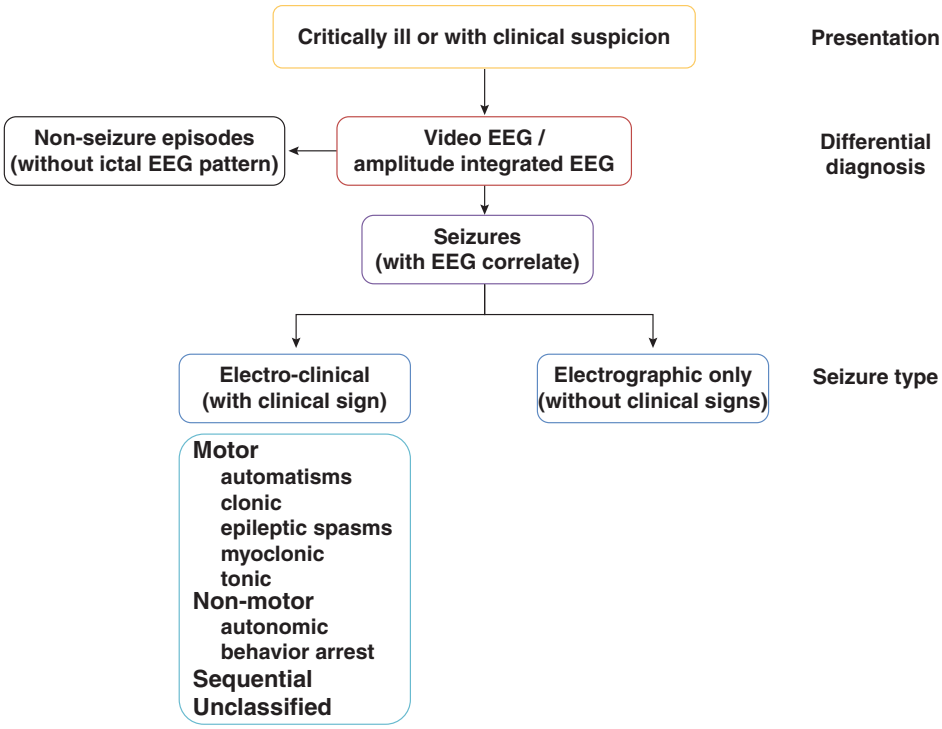
Clonic Seizures

Clonic seizures are characterized by rhythmic movements with a rapid flexor phase followed by a slower extension phase persisting despite flexion of the affected limb. Movements may be symmetric or asymmetric. Clonic seizures can be mistaken for nonepileptic phenomena such as tremor or jitteriness and may be differentiated by the rhythmicity of the event and its ability to be suppressed or altered by changes in positioning.

Focal clonic or hemiclonic seizures can be seen in neonates with injury localized to a specific site, such as a perinatal stroke or another cerebrovascular event.[10–13] Multifocal seizures—clonic seizures that arise, at times, from multiple locations—can be seen in neonates with multifocal or generalized brain abnormalities, such as hypoxic-ischemic encephalopathy.

Myoclonic Seizures

Myoclonic movements are rapid, lightning fast (<100 ms) jerks that can be focal, multifocal, or generalized in nature. Myoclonus can occur at multiple levels of the nervous system: cortical regions, brainstem, and spinal cord. Myoclonus may be epileptic (myoclonic seizures) or nonepileptic, requiring EEG with an electromyography (EMG) channel to differentiate. Myoclonic seizures are most often seen in inborn errors of metabolism and early onset genetic epilepsies.[10,14–17]



• **Fig. 58.1** Diagnostic framework of seizures in the neonatal period including classification of seizures. Neonates present with discrete events suspected to be epileptic seizures or are critically ill (often ventilated, sedated, and treated with muscle relaxants in the neonatal intensive care unit). (From Pressler RM, Cilio MR, Mizrahi EM, et al. The ILAE classification of seizures and the epilepsies: modification for seizures in the neonate. Position paper by the ILAE Task Force on Neonatal Seizures. *Epilepsia*. 2021;62:615–628.)

TABLE 58.1 Seizure Types

Seizure Type	Characteristics	Nonepileptic Differential
Electrographic only	No clear clinical manifestations	None
Motor: Automatisms	Coordinated motor activity, typically oral, often associated with impaired consciousness	Normal behavior
Motor: Clonic	Rhythmic jerking, focal symmetric or asymmetric	Tremor Jitteriness
Motor: Epileptic spasms	Sudden extension/flexion, predominantly involving proximal extremities and trunk, often in clusters	Nonepileptic myoclonus
Motor: Myoclonic	Lightning-fast jerk of a single limb or of multiple extremities	This can be epileptic or nonepileptic
Motor: Tonic	Stiffening of an extremity or hemibody	Provoked bilateral posturing "Brainstem release phenomena" Gastroesophageal reflux
Nonmotor: Autonomic	Paroxysmal alteration of cardiovascular, pupillary, vasomotor, or respiratory function with ictal EEG correlate	Nonepileptic apnea, tachycardia, or autonomic fluctuation
Nonmotor: Behavioral Arrest	Pause in normal activities with ictal EEG correlate	Normal behavioral pause
Sequential	Variable progression of clinical features with ictal EEG correlate	Behavioral movements

Epileptic Spasms

Epileptic spasms in neonates consist of sudden flexion or extension of the proximal and truncal muscles. Duration is longer than myoclonic movements but briefer than tonic seizures and can occur in clusters. Subtle forms can consist of grimacing, head nodding, or eye movements. EEG is required to differentiate from a nonepileptic phenomenon and often requires an EMG channel to differentiate from myoclonus not originating from the central nervous system. These are rare in the neonatal population and classically have been associated with inborn errors of metabolism.[14–23]

Tonic Seizures

Tonic seizures are those with sustained flexion or extension of a muscle group lasting seconds to minutes. Tonic seizures can affect both limbs, eye muscles with sustained eye deviation, or the neck with head version. Neonatal tonic seizures may be focal, unilateral, or bilateral asymmetric and are commonly seen in early infantile onset epileptic encephalopathies.[10,16,23,24]

Nonepileptic bilateral tonic extension not having a correlate on EEG[7] may represent "brainstem release" in the setting of extensive cortical dysfunction. This cortical dysfunction may allow uninhibited subcortical expression to occur. Other movements that may be misidentified as tonic seizures include dystonic posturing and may coexist in neonates with seizures, especially in the setting of brain injury.

Nonmotor Seizure

Autonomic Seizure

Autonomic seizures are defined as paroxysmal onset of alteration in cardiovascular, pupillary, gastrointestinal, vasomotor, or respiratory without alternative explanation and with EEG epileptic correlate. Neonatal seizures with isolated autonomic features are rare with less than 5% of studies obtained for isolated apnea having an ictal correlate,[25] but can be seen in intraventricular hemorrhage, temporal lobe and occipital lesions, and occasionally in early-onset epileptic encephalopathies.[7,18,26,27]

Behavioral Arrest

Ictal behavioral arrest is difficult to ascertain in neonates and is rarely seen in isolation. More commonly, a pause in ongoing activities is seen as part of a sequential seizure.[10,18] EEG is required to differentiate ictal behavioral arrest from nonepileptic behavioral pause.

Sequential Seizure

Neonatal seizures are classified as sequential when there is no single predominant ictal feature, and events instead progress through a series of features with associated EEG changes.[4] The individual features and lateralization may change within or between seizures. This type of seizure was classically associated with KCNQ2 encephalopathy but has been described with other genetic epilepsies.[24,28–31]

Nonepileptic Neonatal Movements

Newborns are prone to a variety of movements that may raise suspicion for seizures but represent other neurologic or nonneurologic entities.[5] Common nonepileptic mimics which can be differentiated from seizures with EEG[32] include tremulousness or jitteriness, nonepileptic myoclonus, dyskinesias, and hyperekplexia.

Tremulousness or Jitteriness

Tremors can be seen frequently in the neonate and can be mistaken for clonic activity. The phenomenology of this movement is flexion and extension, with equal phases and amplitude of both, distinguishing this from clonic activity. Repositioning of the affected limb may often decrease or extinguish the movement, as can flexion. Neonates may be alert or hyperalert, although this may also be present in neonates with somnolence secondary to encephalopathy. Tremors may be asymmetric in nature and of varying amplitudes. Movements can be spontaneous or induced by stimulation.

Tremors, as well as jittery movements, can occur secondary to metabolic derangements such as hypoglycemia or hypocalcemia, as well as in intracranial hemorrhage, newborns with growth restriction, and hypothermia. Exposure to maternal medications such as selective serotonin reuptake inhibitors (SSRIs) or illicit substances including cocaine and marijuana has also been associated with tremulousness.

Jitteriness is common in both healthy and ill neonates, with one study showing mild, moderate, or excessive jitteriness in 44% of healthy term infants. Movements generally decrease with increasing postconceptional age, with a normal neurologic outcome.[33]

Myoclonus Without Electrographic Correlate

Myoclonus is defined as brief shock-like movements caused by sudden involuntary contraction or relaxation of one or more muscles, often occurring as repetitive nonrhythmic movements.[34] Nonepileptic myoclonic movements may originate from multiple levels of the neuroaxis and are more frequent in preterm newborns but also occur in term neonates. It can be benign in some instances, while a harbinger of underlying abnormalities in others.

Myoclonus occurring in an infant with encephalopathy is not typically benign. In this case, myoclonus may be stimulus-induced and occur in the setting of severe brain injury. Myoclonus in the neonate with encephalopathy may also occur in the setting of encephalitis or meningitis, intraventricular hemorrhage, periventricular leukomalacia, or genetic[35] or metabolic disorders such as glycine encephalopathy.[17,20,21,36] In these latter cases, the EEG background is typically abnormal and may have epileptiform discharges, although the myoclonus itself lacks epileptiform correlate.[36]

Midazolam-induced and lorazepam-induced myoclonus are reported in preterm or very low birth weight neonates.[37–39] If medication-induced myoclonus occurs, further use of the offending drug should be limited in the neonatal period where possible. As the response is thought to be developmental, the use of benzodiazepines need not be avoided in infancy or childhood.

Benign neonatal sleep myoclonus is considered a diagnosis of exclusion.[40] Suspicion for this should arise when myoclonus occurs in an otherwise healthy newborn only during sleep, resolves with awakening, and is typically arrhythmic and of varying amplitude. It may increase with attempts of physical restraint or be induced by rocking and occurs in all stages of sleep.[41] This typically resolves by 3 months of age, although some infants may have symptoms until 6 to 12 months of age.[41]

Dyskinesias

Dystonic and dyskinetic movements are frequent and commonly mistaken for seizures. Dystonia is the involuntary sustained or intermittent co-contraction of agonist and antagonist muscles resulting in abnormal posture.[34] This group of movements is associated with dysfunction of the basal ganglia or the extrapyramidal pathways and can occur secondary to acute or chronic injury of these structures. In the neonate, it often represents intrapartum or antepartum injury with severe injury to the basal ganglia.[42] Some inborn errors of metabolism may present with hypertonicity, opisthotonic posturing, or dystonia (e.g., maple syrup urine disease, monoamine neurotransmitter disorders).[43,44] As dyskinetic and dystonic movements are frequent in encephalopathic neonates who may also have epileptic events, the use of continuous video

EEG is imperative to prevent misdiagnosis and inappropriate treatment of either diagnosis.

Hyperekplexia

Hyperekplexia is characterized by an exaggerated startle reflex that does not typically extinguish and is associated with hyperreflexia, and hypertonia. It has predominantly been described as an autosomal dominant disease associated with mutation of the inhibitory glycine receptor gene, although rare cases of autosomal recessive disease have been reported.[45] Hyperekplexia is an important differential for paroxysmal events in the neonate as it is associated with sudden death secondary to apnea and is readily treated with scheduled benzodiazepines.[46,47] The associated apneic spells can be mitigated by the use of the Vigevano maneuver, which utilizes forced flexion of the head and legs towards the trunk.[48]

Evaluation of Neonatal Seizure

Medical providers of various backgrounds have been shown to have difficulty determining whether events represent seizures or not, based on clinical features alone. In an observational study over 70% of events identified as seizures were not found to have an electrographic correlate.[49] When experienced neonatal healthcare providers were evaluated with standardized video clips of neonatal seizures and seizure mimics, seizures were correctly identified approximately 50% of the time.[5] Additionally, since up to 80% of the seizure burden identified in prospective cohorts are electrographic only, identification of seizures by clinical criteria alone underestimates the actual number of seizures.[49,50] With this in mind, it is recommended that all events concerning for seizures be confirmed electrographically.[32] Commonly utilized tools include amplitude-integrated EEG (aEEG) and continuous electroencephalography (cEEG).

Neurophysiologic Diagnosis of Seizure

A standardized definition and characterization of neonatal seizures has been published by the American Clinical Neurophysiology Society.[51] To be considered a seizure, rhythmic activity must last 10 seconds with a minimum of 2 μV peak-to-peak voltage that evolves in quality and subsequently resolves. Events with these characteristics lasting less than 10 seconds are referred to as *brief rhythmic discharges*, and while they are not consistent with seizures, they suggest an increased risk of developing seizures.

Status epilepticus, defined in older children or adults as a seizure lasting greater than 30 minutes, or more than one seizure without a return to baseline in between, is not an appropriate definition in neonates, particularly given the often comorbid encephalopathy and overall challenges in determining true return to baseline. In neonates, the definition of status epilepticus has been established as seizures constituting greater than 50% of an hour-long epoch, which is arbitrarily defined.[51]

Amplitude-Integrated Electroencephalography

Many institutions incorporate aEEG to aid in the detection of seizures. aEEG is a cerebral function monitor widely available as a bedside tool that utilizes 2 to 4 leads, typically placed over the central and parietal regions. The minimum and maximum amplitude during each time epoch are abstracted and typically used to create a single compressed tracing per hemisphere.[52] During a seizure, there is a sudden and sustained increase of the lower and upper margins on aEEG, with raw EEG signal demonstrating a monomorphic waveform consistent with seizure.[53]

Advantages of aEEG include bedside availability and interpretation, as well as reduced cost as compared with continuous EEG monitoring.[53] However, aEEG has well-demonstrated limitations, including challenges in the detection of brief or low-amplitude seizures or seizures that do not occur in the brain regions covered by the aEEG electrode montage.[54] In a concurrent evaluation of aEEG and cEEG in 125 neonates with seizures greater than 34 weeks of age, neonatologists were able to detect on aEEG between 12% and 38% of individual seizures identified on cEEG and correctly identify between 22% and 57% of neonates with seizures.[54] aEEG may also be limited by artifactual signals from movement, high-frequency oscillator ventilation, or extracorporeal membrane oxygenation. In contrasting studies of prospectively evaluated premature infants, aEEG found a seizure incidence of 48%[55] while cEEG evaluation of a similar cohort demonstrated only 5% incidence of seizures,[56] raising concerns that particularly in the preterm population, aEEG may be prone to artifact and misinterpretation. Consequently, it is recommended that aEEG be used as a screening tool where cEEG is limited. However, cEEG should be used when seizures are suspected on aEEG.[57,58]

Continuous Electroencephalography

Continuous EEG is an invaluable tool and the gold standard for neonatal seizure detection. Multiple electrodes are placed in accordance with the international 10–20 system, or with the modified neonatal montage, alongside a single electrocardiogram lead and respiratory belt. Interpretation of cEEG requires mastery of the normal and abnormal patterns of term and preterm wakefulness and sleep. Aid from the EEG technologist or bedside nurse is imperative to note potential events of concern and artifacts (such as nursing care, feeding, etc.).

The American Clinical Neurophysiology Society developed guidelines in 2011 on the use of cEEG monitoring in high-risk neonates.[32] While it is recognized that not all facilities will have the resources necessary to monitor, this guideline provides goals as to how to monitor for seizures in high-risk neonates or which neonates with suspicious events should be evaluated with this technology. Populations in which cEEG use is recommended include acute brain injury secondary to perinatal asphyxia, neonates with clinically suspected seizures, and when neonatal epilepsy is suspected. In some instances, it may be appropriate to continue monitoring after the withdrawal of antiseizure medications (ASMs). Although seizures in the setting of acquired brain injury are not likely to recur shortly after the resolution of the acute injury, those with neonatal epilepsy syndromes or cerebral malformations may deserve monitoring when ASMs are discontinued. It is recommended that neonates remain monitored for a 24-hour period once seizure-free.

Quantitative Electroencephalography

Quantitative EEG (qEEG) is the use of sophisticated algorithmic support to evaluate EEG background trends and seizures in real time. The initial development of seizure detection algorithms focused on older populations and was inaccurate in identifying neonatal seizures.[59] Randomized controlled studies evaluating neonate-specific seizure detection algorithms have

recently demonstrated decreased seizure burden when qEEG is employed in addition to clinician review.[60] Preterm-specific qEEG analysis techniques are being developed to aid in maturational analysis and prognosis, but the burden of artifacts associated with preterm neonatal care remains a significant challenge.[61]

Electroclinical Uncoupling

Administration of ASMs in the setting of electroclinical seizures can result in a phenomenon where electrographic seizures persist while the clinical manifestations resolve. This is termed *electroclinical uncoupling*. One study estimated that 25% of neonates had persistent electrographic seizures after receiving ASMs despite the resolution of clinical seizures.[62] This information suggests that neurophysiologic monitoring is needed after treatment for neonatal seizures, even with an apparent resolution of clinical events.

Interictal Abnormalities

Beyond the determination of seizures, EEG detects interictal findings that aid patient management. The presence of background abnormalities may be serially followed to determine the progression of an encephalopathy.[62] While background findings may not be pathognomonic for particular etiologies, these findings, in conjunction with the remainder of the diagnostic work-up (history, clinical examination, and findings on laboratory and imaging studies), aid in the development of the overall prognosis for a patient.

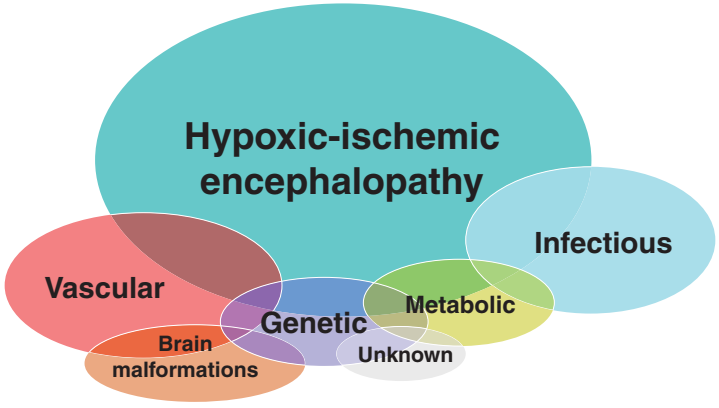
Etiologies of Neonatal Seizures

Once it is determined that a neonate is having seizures, further steps are divided into establishing the etiology of seizures, as well as the treatment of seizures. Neonatal seizures are not disease specific and can occur in a variety of conditions with onset before, during, or after birth. Seizures may occur in the setting of hypoglycemia or electrolyte disturbances such as hypocalcemia or hypomagnesemia and may respond to corrections of these disturbances. Seizures may harken underlying brain injury in the setting of hypoxic-ischemic encephalopathy, perinatal stroke, hemorrhage, trauma, or infection. Metabolic abnormalities, genetic defects, and congenital brain malformations are additional etiologic considerations (Fig. 58.2 and Table 58.2).[6]

TABLE 58.2 Differential Diagnosis of Seizure Etiology

Hypoxic-ischemic encephalopathy	Pre- and peripartum
Metabolic derangement	Hypoglycemia Hypocalcemia Hypomagnesemia Hyponatremia Hypernatremia
Cerebrovascular lesions	Perinatal arterial or embolic stroke Hemorrhage Cerebral sinus venous thrombosis Cortical vein thrombosis Hemorrhagic venous infarction
Infection	Bacterial meningitis Viral encephalitis TORCH infections
Drug exposure/ withdrawal	Includes but not limited to: Methadone Cocaine SSRIs
Congenital brain malformations	Includes but not limited to: Polymicrogyria Hemimegalencephaly
Inborn errors of metabolism	Includes but not limited to: Glycine encephalopathy Aminoacidopathies Urea cycle defects Pyridoxine-dependent epilepsy
Benign neonatal familial seizures	Caused by a mutation in one of the following genes: *KCNQ2* *KCNQ3* *SCN2A*
Progressive epilepsy syndromes	Includes but not limited to mutations in the following genes: *STXBP1* *FOXG1* *CDKL5* *KCNQ2*

SSRIs, Selective serotonin reuptake inhibitors; *TORCH*, toxoplasmosis, other agents, rubella, cytomegalovirus, herpes simplex.



• **Fig. 58.2** Relative occurrences of common etiologies of neonatal seizures in term infants. (From Pressler RM, Cilio MR, Mizrahi EM, et al. The ILAE classification of seizures and the epilepsies: modification for seizures in the neonate. Position paper by the ILAE Task Force on Neonatal Seizures. *Epilepsia*. 2021;62:615–628.)

Hypoxic–Ischemic Encephalopathy

Hypoxia–ischemia is the most common cause of neonatal seizures. Multiple randomized controlled trials published between 2005 and 2012 establish therapeutic hypothermia as the standard of care[63] for neonates with moderate to severe hypoxic-ischemic encephalopathy (HIE), which is also the population most likely to experience seizures. Further discussion of therapeutic hypothermia is included in Chapter 55 on Neonatal Encephalopathy. Therapeutic hypothermia is geared toward the prevention of secondary energy failure and additional brain injury, and studies, since therapeutic hypothermia has become standard, suggest that this therapy decreases the rate of seizures by approximately half in infants with moderate HIE.[64,65]

In newborns with HIE, it is important to recognize that injury may occur antepartum, intrapartum, or postpartum. Risk factors particularly shown to be associated with seizure include placental lesions consistent with chronic asphyxia stress,[66] intrapartum fever,[67] placental separation, preeclampsia, and cesarean section performed because of hemorrhage, meconium-stained fluid, and shoulder dystocia.[68] Additional seizure risk factors include diabetes mellitus, advanced maternal age over 40 years, and maternal infection.[69]

Cerebrovascular Lesions

Ischemic or hemorrhagic lesions of either arterial or venous origin are associated with a high risk of seizure in the newborn.[70–74] In term neonates with perinatal arterial stroke, seizure is the most common clinical presentation, accounting for between 70% and 90%, followed by hypotonia or feeding difficulties.[75,76] Neonates with cerebral infarction often are otherwise healthy in appearance, with reassuring presentation, not consistent with asphyxia. The use of neuroimaging with magnetic resonance imaging is necessary to demonstrate the focal lesion.[77,78]

In preterm infants, intraventricular hemorrhage is the most common cause of seizures[79,80] and is the etiology of seizures in as many as 45% of EEG-confirmed seizures. Seizures in preterm newborns are thought to be underestimated, as studies prospectively assessing seizure frequency in high-risk preterm neonates find a higher incidence than in those where EEG is obtained in response to a clinical event.[53,79,81]

Cerebral venous infarction may also result in neonatal seizures.[74] This may occur in the setting of systemic infection, dehydration, or poor feeding leading to cerebral venous sinus thrombosis. In preterm infants, venous thrombosis may result in periventricular hemorrhagic infarction within the deep white matter, which may be complicated by seizures.[81]

Infants requiring congenital heart defect repair, with persistent pulmonary hypertension of the newborn, or requiring extracorporeal membrane oxygenation have an increased risk of seizures caused by recurrent hypoxia hypotensive injury and embolic infarction. EEG monitoring following cardiac surgery demonstrates approximately 10% of neonates experience clinical or subclinical seizures[82–86] and in children undergoing extracorporeal membrane oxygenation up to 30% demonstrate seizures.[86,87] The anticoagulation necessary for extracorporeal membrane oxygenation circuit use may convert an ischemic injury to a hemorrhagic one, with a risk of edema or herniation. The presence of seizures is associated with increased inpatient mortality and worsened neurodevelopmental outcomes.[82–84]

Infection

Central nervous system infections antepartum or postnatally can be associated with neonatal seizures.[88] The TORCH infections (toxoplasmosis, other agents, rubella, cytomegalovirus, and herpes virus), as well as other viruses including Zika virus,[89] can produce structural brain changes resulting in a predilection for seizures and encephalopathy. These infections are discussed in detail in other chapters. Neonatal herpes encephalitis is associated with specific EEG findings, with focal or multifocal epileptiform discharges that may be periodic or quasiperiodic in nature.[90,91] Enteroviruses[92–94] and bacterial meningitis may also present with encephalopathy with seizures.[95,96] Some neonates with bacterial meningitis may suffer further complications of arterial stroke or cerebral venous sinus thrombosis, with further neurologic injury.[97] A prolonged course of seizures (>72 hours) or markedly abnormal EEG pattern is associated with poor outcome in this setting.[98,99]

Metabolic Derangements

Hypoglycemia, along with electrolyte disturbances such as hypocalcemia, hypomagnesemia, hyponatremia, or hypernatremia may result in seizures. Repletion of glucose and correction of electrolyte levels is imperative for treatment.

Hypoglycemia

Hypoglycemia is generally accepted as a glucose level less than 47 mg/dL, although the definition remains controversial.[100,101] Hypoglycemia may coexist with hypoxic-ischemic injury or with hypocalcemia, both of which may also result in seizures. Jitteriness, tremors, and abnormal tone may be present in neonates with hypoglycemia, mimicking seizures. Persistent or profound hypoglycemia may result in cerebral injury, classically described as white matter injury or occipital injury.[102,103] Seizures should first be treated by correction of hypoglycemia. Particularly if cerebral injury occurs, seizures may persist despite correction and require treatment with ASMs. Infants with hypoglycemia and cerebral injury may later develop occipital lobe epilepsy, although the severity of the epilepsy varies.[104]

Hypocalcemia

Hypocalcemia is defined as a total calcium level of less than 8.0 mg/dL (2 mmol/L) in term neonates and less than 7.0 mg/dL (1.75 mmol/dL) in preterm neonates or an ionized calcium of less than 4.8 mg/dL (1.2 mmol/L) in term infants and less than 4.0 mg/dL (1 mmol/L) in premature infants.[105] Neonates with hypocalcemia may present with seizures secondary to increased excitability of the cell membrane,[106] thus resulting in exaggerated startles, jitteriness, myoclonic jerks, or seizures.[107,108] Hypocalcemic seizures should be treated with calcium repletion.

Hyponatremia and Hypernatremia

Hyponatremia is a cause of seizures across the life span[106]; however, it is a relatively rare cause in neonates. When present, this may reflect iatrogenic causes, renal failure, a transient or constitutional defect in the mineralocorticoid pathway, or an inappropriate secretion of antidiuretic hormone.[109] Hypernatremic seizures are also rare in neonates but may be secondary to inadequate breastfeeding[110] or iatrogenic from the administration of intravenous solutions with high sodium concentrations.[111]

Drug Withdrawal and Intoxication

Newborns of mothers with prenatal substance use may be at an increased risk of seizures in the neonatal period. Prenatal exposure to opiates can result in neonatal abstinence syndrome, which, in severe cases, can result in seizures.[112] Similarly, perinatal exposure to alcohol intoxication is associated with withdrawal seizures.[113] Cocaine can produce seizures in neonates either secondary to intoxication, from withdrawal,[114,115] or from neonatal stroke, which in turn increases the risk of seizures. Exposure to other stimulants, such as methamphetamine, may be associated with a withdrawal syndrome accompanied by jitteriness, tremor, and exaggerated startle, but seizures have not been typically reported.[116]

Maternal use of SSRIs such as fluoxetine, paroxetine, and sertraline may also result in withdrawal symptoms including tremors, jitteriness, vomiting, diarrhea, and sleep disturbance. In some cases, convulsions may be present as a component of the withdrawal syndrome.[117] EEG remains imperative in diagnosis, however, as many abnormal movements noted may have an EEG correlate.

Congenital Brain Malformations

Approximately 9% of neonates presenting with seizures are found to have brain malformations.[80] These disorders are caused by alterations in stages of induction, segmentation, proliferation, migration, synaptogenesis, and myelination and are discussed in greater detail elsewhere in this text. Encephalopathy is typically present and may coexist or be mistaken for birth asphyxia. Many brain dysgenesis disorders lack specific physical examination findings, but magnetic resonance neuroimaging is appropriate to evaluate for underlying brain malformations. Neonates with brain dysgenesis and seizures in the neonatal period have an exceptionally high likelihood of subsequently developing epilepsy and requiring prolonged use of ASMs.[118]

Inborn Errors of Metabolism

Genetic biochemical abnormalities are rare causes of neonatal seizures, accounting for between 1% and 4% of cases.[119] Although uncommon, consideration of this etiology is imperative, as specific treatments may be available for some causes, based on the enzymatic defect uncovered. In cases where treatment is not available, prognostic implications remain essential. Inborn errors of metabolism causing seizures may be placed in three categories: defects in neurotransmission; disorders of energy production; and metabolic disorders resulting in brain malformation, destruction, or dysfunction.[120] Examples of each are given below, although an extensive review of inborn errors is beyond the scope of this chapter.

Signs suggestive of an inborn error of metabolism include seizures that start prenatally, refractory seizures requiring multiple ASMs, progressive clinical worsening, or deterioration of the EEG.[121] Some neonates may have an initial presentation consistent with HIE, thus a high level of clinical suspicion is necessary in neonates with refractory seizures. Specific neuroimaging may demonstrate characteristic lesions supporting a metabolic etiology.[122]

Defects in neurotransmission include glycine encephalopathy and pyridoxine-dependent epilepsy. Glycine encephalopathy, also known as nonketotic hyperglycinemia, is due to deficiencies in the ability to cleave glycine. Glycine has both inhibitory and excitatory neurotransmitter activities, and glycine encephalopathy presents with apnea, myoclonic seizures, and burst suppression on EEG. In retrospect, mothers will often note that significant hiccups were present in utero, representing fetal myoclonic seizures. Seizures may initially respond to benzodiazepines, but, long term, patients develop early myoclonic encephalopathy, or Ohtahara syndrome.[20,21]

Pyridoxine-dependent epilepsy is an uncommon but treatable cause of neonatal seizures, caused by deficiency of α-aminoadipic semialdehyde dehydrogenase, an enzyme involved in the lysine catabolic pathway. In retrospect, mothers may report paroxysmal in utero movements representing seizures, and newborns may present with seizures, encephalopathy, and hypotonia in the first few days of life.[123,124] In some patients with pyridoxine-dependent epilepsy, lactic acidosis and other biochemical abnormalities may be present, mimicking features of neonatal encephalopathy secondary to hypoxia or ischemia. It is an autosomal recessive condition, caused by a genetic mutation in *ALDH7A1*, and affected patients have elevated levels of α-aminoadipic semialdehyde (AASA) in blood and urine,[123,124] which is the standard screening laboratory evaluation. Recommended treatment and evaluation of neonates with refractory status epilepticus include an empiric trial of intravenous pyridoxine while carefully monitoring EEG for treatment response.

Examples of disorders of energy production and utilization include urea cycle defects and glucose transporter type 1 (GLUT1) deficiency. Urea cycle defects may present with encephalopathy and seizures in the setting of hyperammonemia as toxic breakdown products accumulate. Treatment includes dialysis or exchange transfusion while determining the enzymatic defect. Glucose transport to the brain is mediated by GLUT1. Reduced glucose transport through the blood-brain barrier results in hypoglycorrhachia (cerebrospinal fluid glucose levels less than 45 mg/dL or a ratio of cerebrospinal fluid glucose to serum glucose of <0.4). Patients may present in the first few months of life with significant seizures. Hypoglycorrhachia without alternate explanation should prompt further genetic testing of *SLC2A1*, the major genetic defect found in this disorder.[125] Over the past decade, the phenotype of patients has expanded significantly to include those with movement disorders or early-onset absence epilepsy.[126] Treatment of GLUT1 deficiency is the ketogenic diet, providing an alternative energy source to support brain metabolism.

Metabolic disorders resulting in brain dysgenesis and seizures include peroxisomal biogenesis disorders such as Zellweger syndrome. Infants present with characteristic facial features, hypotonia, and encephalopathy alongside seizures in the first days to weeks of life. Neuroimaging reveals neuronal migration defects such as polymicrogyria, while levels of very long chain fatty acids are elevated. Mutations in the *PEX* gene family have been associated with this group of metabolic disorders.[127]

Neonatal Epilepsy Syndromes

Seizures in the neonatal period are rarely the presentation of a chronic epileptic condition.[128] Affected neonates are more likely to present with myoclonic seizures with early myoclonic encephalopathy, tonic seizures, epileptic spasms, or sequential seizures.[4,10,14–16] These neonatal epilepsy syndromes may be termed *early infantile epileptic encephalopathy* or *Ohtahara syndrome*, and the EEG may demonstrate a suppression-burst pattern or disorganized background. Over the past decade, many genes have been associated with early-onset epileptic encephalopathies, including mutations in *ARX, CDKL5*, and *STXBP1*, among others.[129,130]

In neonates presenting with seizures for whom acute symptomatic causes have been ruled out, a genetic evaluation is

indicated.[131,132] Although there are some presentations with distinct features suggesting a targeted genetic evaluation, many neonatal epilepsy presentations have a high degree of phenotypic overlap. Combined with decreasing cost and increasing availability, this has shifted the testing paradigm towards an early, broad genetic evaluation with whole-exome[133,134] and increasingly whole-genome sequencing.[135]

Benign Familial Neonatal Seizures

A rare form of neonatal epilepsy that is inherited in an autosomal dominant pattern should be considered in newborns with a positive family history[136] or after structural, infectious, metabolic, and toxic causes have been ruled out. In a kindred with benign familial neonatal seizures, often the parents are unaware that they had a seizure in the newborn period until grandparents note this after their grandchild presents with neonatal seizures. Several genes have been implicated, including two potassium channel genes, *KCNQ2* and *KCNQ3*, as well as a sodium channel gene *SCN2A*.[137–139] *KCNQ2* mutations may also result in progressive epileptic encephalopathy; thus, caution should be exercised in counseling regarding prognosis.[140] Infants with benign familial seizures typically have a normal interictal EEG pattern and normal neuroimaging, while those who develop an epileptic encephalopathy tend to have abnormal EEGs with burst suppression, multifocal epileptiform discharges, and basal ganglia hyperintensities.[30] Infants with benign neonatal epilepsies tend to have a good response to ASMs, with reports of sodium channel agents being effective in those with *KCNQ2* mutations.[141]

Management of Neonatal Seizures

Once the diagnosis of neonatal seizures has been made, management is initiated while the determination of an etiology is under way. Rapid initial testing should detect correctable causes of neonatal seizures, such as hypoglycemia, hypocalcemia, or sodium disturbances. Hypoglycemia may be corrected with an infusion of 10% dextrose. Seizures caused by hypocalcemia should be treated with 10% calcium gluconate.[106] Magnesium levels should be checked and repeated if necessary, as hypomagnesemia may coexist with hypocalcemia. Hyponatremia or hypernatremia can be managed dependent on the etiology of sodium dysregulation. Concomitant use of ASMs is typically not needed for hypoglycemia and correctable electrolyte abnormalities.

Antiseizure Medication

Once metabolic derangements are addressed, treatment of seizures is undertaken with ASMs. As neonates may have both clinical and electrographic seizures, and treatment with ASMs may result in electroclinical dissociation, it is recommended that neonates undergoing treatment with ASMs be monitored through cEEG.[32]

Phenobarbital has remained the first-line choice for the treatment of neonatal seizures by most clinicians.[142,143] Phenobarbital is effective in the cessation of seizures in approximately 50% of neonates.[144,145] Phenobarbital is a barbiturate, acting on the γ-aminobutyric acid (GABA) receptor, enhancing GABA activity. Phenobarbital is typically given as an intravenous bolus of 20 mg/kg, with a maintenance dose of 4 to 5 mg/kg/day. The half-life of phenobarbital is quite long in the neonate, ranging between 45 and 200 hours.[146] Therapeutic levels should be assessed as levels above 40 to 60 μg/mL may not have additional therapeutic benefit.[147] Importantly, phenobarbital is known to cause respiratory depression, hypotension[148], and hemodynamic lability and has also been associated with deleterious developmental effects.[149] Due to concerns about suboptimal efficacy and side effects, newer medications are often used off-label by neurologists[150]; a commonly selected ASM is levetiracetam. Levetiracetam has been shown to have minimal adverse side effects in neonates[151–153] and retrospective studies suggested noninferiority of control of seizures relative to phenobarbital.[151] However, a recent prospective randomized trial of phenobarbital versus levetiracetam reported a greater efficacy with phenobarbital in a heterogeneous neonatal population,[154] suggesting that in most settings phenobarbital is likely to remain the first-line ASM of choice.

Treatment choices beyond phenobarbital are variable, with the most common selections being levetiracetam or fosphenytoin.[6,142,143] Fosphenytoin is a water-soluble form of phenytoin that is better tolerated, and the administered doses are expressed as phenytoin equivalents. An intravenous loading dose of fosphenytoin of approximately 20 mg phenytoin equivalents/kg is recommended followed by maintenance therapy.[155] However, phenytoin levels are often difficult to maintain, as the medication is rapidly redistributed to the tissues and follows zero-order kinetics.[156]

Benzodiazepine infusion may also be used to control refractory neonatal seizures. Intravenous midazolam has been studied in neonates for safety and efficacy[157,158] and is the most commonly utilized medication for refractory seizures in the United States.[6] Lidocaine, a sodium channel antagonist, has equal efficacy for refractory seizures when compared with midazolam.[159–161] This medication is used more commonly in Europe than in North America.[142,162]

Other ASMs that have been used in neonates include topiramate[163] and lacosamide.[164] Bumetanide, a loop diuretic, is a neuronal sodium–potassium chloride cotransporter antagonist, and in preclinical studies, it was suggested to be an effective treatment for neonatal seizures.[165] Although a randomized controlled trial was discontinued early due to lack of efficacy and concern for increased hearing loss,[166] a recent randomized controlled pilot study suggested that there may be a role for bumetanide as adjunctive therapy with phenobarbital in some circumstances.[167]

Pyridoxine

Pyridoxine-dependent epilepsy is an important etiologic consideration in the newborn presenting with seizures. Treatment should be undertaken for any infant with unexplained ASM-resistant seizure while awaiting laboratory evaluation. For infants with prolonged clinical and/or electrographic seizures, recommended treatment includes administration of 100 mg intravenous pyridoxine over 5 minutes with close evaluation of EEG response and monitoring for apnea, which can occasionally be seen in pyridoxine responders.[123,124] If a clinical or EEG response is not noted, additional 100-mg doses up to a total of 500 mg may be administered. However, as immediate intravenous responses vary, it is suggested that pyridoxine 100 mg/day (IV or enteral) should be administered until biochemical or genetic testing excludes the diagnosis.[123,168] In a newborn with frequent but relatively short ASM-resistant seizures, pyridoxine 100 mg/day (IV or enteral) should be prescribed, and seizure frequency should be monitored. Patients with pyridoxine-dependent epilepsy should show a resolution of clinical seizures within 3 to 5 days of initiating pyridoxine therapy. As above, biochemical and/or genetic confirmation of the diagnosis should be pursued.[124] If pyridoxine-dependent epilepsy is confirmed, an affected newborn should receive pyridoxine 100 mg/

day and older infants should receive 30 mg/kg/day. A lysine-lowering medical diet should be considered.[123] Some patients with medically intractable epilepsy and unresponsiveness to pyridoxine respond to the administration of pyridoxal phosphate, the active form of vitamin B6. Such a response can be seen in patients with pyridox(am)ine phosphate oxidase deficiency which may be confirmed by evaluation of the *PNPO* gene.[22,169]

Discontinuation of Antiepileptic Drugs

How long to treat a neonate with ASMs after resolution of seizures remains an area of practice variability.[170,171] Commonly used ASMs complicate assessments of arousal, tone, and feeding abilities because of their sedating qualities. Of concern, animal studies show increases in apoptosis after treatment with phenobarbital and phenytoin.[172,173] It is now generally recommended that, when possible, ASMs be discontinued while the baby is still being managed in the neonatal intensive care unit. Both retrospective and more recently prospective cohort studies have demonstrated that there is a low rate of seizure recurrence after early discontinuation of ASMs in acute symptomatic seizures and that a longer length of treatment did not prevent later development of epilepsy.[174–176] However, neonates with congenital brain malformation or genetic epilepsy syndromes have an ongoing risk for seizures and thus would be expected to continue treatment with ASMs.

Outcomes of Neonatal Seizures

Clinicians caring for a patient with neonatal seizures are commonly asked to predict how the newborn will be affected in the near and distant future. Prediction of neurodevelopmental outcomes relies first and foremost on the etiology of seizures and is often dependent on advanced neuroimaging that may elucidate congenital malformations or areas of brain injury. Neurophysiologic data also plays a role in prognostication.[177–179] Those infants with a persistently abnormal EEG pattern, such as burst suppression, are likely to have a poor outcome, especially when this pattern persists on serial EEGs.[179] Neonates with a normal EEG tend to fare better, while those with moderately abnormal EEGs have a variable outcome.[178]

Seizures themselves play an uncertain role in neurodevelopmental outcomes with increasing evidence to suggest independent impact. Animal studies have suggested variable developmental effects of neonatal seizures.[180–182] There are ethical concerns in studying the effects of neonatal seizures rigorously in the human newborn, and thus limited research findings are available. Structural studies have shown that seizure severity is independently associated with brain injury, as measured by magnetic resonance spectroscopy.[183] Research assessing the independent role of clinical seizures in the neonate with HIE has shown mixed results.[184,185] More recent studies evaluating the impact of all seizures, both electroclinical and electrographic only, have shown seizure burden associated with microcephaly, cerebral palsy, and failure to thrive[186] and status epilepticus associated with worse neurodevelopmental outcomes and increased risk of postneonatal epilepsy.[187] A randomized controlled trial prospectively studied neonates with electroclinical and electrographic seizures, treating one group for electroclinical seizures only, while treating all electrographic seizures (including those without a clinical correlate) in the other group. This study found that newborns in whom all electrographic seizures were treated had a lower seizure burden, and, although underpowered, suggested that greater seizure burden was associated with greater injury and lower Bayley Scores of Infant Development-III scores.[188] Other recent studies have similarly supported an association between increased total seizure burden and both short- and long-term negative outcomes.[189,190] Newborns who escape clear impairments in early childhood may still develop more subtle neuropsychological challenges as adolescents or in early adulthood.[191]

Post-neonatal Epilepsy

Newborns with seizures remain at risk for developing epilepsy, defined as recurrent unprovoked seizures, in later life. It is estimated that nearly a quarter of neonates with seizures symptomatic of an underlying cause go on to develop post-neonatal epilepsy.[192–195] Of neonates who later develop epilepsy, approximately two-thirds present with recurrent seizures in the first year of life,[196] although a more recent cohort found later onset and somewhat lower frequency of epilepsy.[197] Typically, there is a latency period between neonatal seizures and the onset of post-neonatal epilepsy. Neonates with refractory seizures requiring multiple ASMs, severe brain injury, or those with persistent interictal epileptiform discharges on EEG have a higher risk of developing post-neonatal epilepsy.[118,192,198]

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59 Enteral Nutrition

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KEY POINTS

- Early protein intake is associated with improved growth and neurodevelopmental outcomes in premature infants; achieving adequate intake requires a combined parenteral and enteral approach.
- Mother's own milk (MOM) is the preferred diet for preterm infants; further research is needed to develop evidence-based clinical guidelines to optimize the use of donor human milk when MOM is unavailable.
- For very low birth weight infants, unfortified human milk contains insufficient protein, energy, and many essential micronutrients, requiring supplementation with milk fortifier and/or multivitamin products.
- Postnatal growth failure remains a common complication of preterm birth and is associated with adverse outcomes; optimization of growth outcomes requires attention not only to weight gain but also to appropriate linear growth and body composition both in the neonatal intensive care unit and following hospital discharge.

Providing optimal enteral nutrition to neonates can present a difficult clinical challenge. Nutritional needs in the early neonatal period are greater than at any other time of life. Many infants are born with circumstances that place them at increased risk for nutritional deficits. These "at-risk" infants include the preterm infant, especially those born at less than 28 weeks' gestation or less than 1500 g birthweight. These infants have a high risk of feeding intolerance, necrotizing enterocolitis (NEC), infections, and postnatal growth faltering. Infants with congenital gastrointestinal disorders such as gastroschisis and intestinal atresias often have very disorganized motility and poor barrier function. Term infants with high disease acuity such as congenital heart disease (CHD),[1] disorders such as gastroschisis, congenital diaphragmatic hernia (CDH), and ECMO (extracorporeal membrane oxygenation) also have greatly increased caloric needs and unique feeding challenges. In all of these "at-risk" infants, delayed feeding causes absence of trophic factors, which enhances the complications of abnormal bacterial colonization due to antibiotics and the NICU environment, and makes provision of adequate nutrition difficult.

Preterm infants begin life with limited micro and macro nutrient deposition and often have a high degree of illness which makes the provision of adequate calories and protein immediately after birth extremely urgent. Immature gastrointestinal and endocrine function and critical illness further complicate delivery of adequate nutrition. Despite the lack of evidence, withholding or slowly advancing parenteral protein and fats and fear of NEC if feedings are advanced too quickly may also limit provision of optimal parenteral and enteral nutrition. Late preterm and term infants with CHD and CDH/ECMO quickly descend into a nitrogen and caloric deficit due to high disease acuity, fluid volume limitations, and need for pressor support. Consequently, the "at-risk" infant requires specialized nutritional support to meet their increased nutritional requirements to ensure adequate growth and meet the increased demands from critical illness.

Current recommendations for provision of enteral nutrition to premature infants are based on the elusive goal of duplicating rates of intrauterine accretion of the fetus at the same postmenstrual age. Even when guidelines for nutrient intake are meticulously followed, many preterm infants still experience postnatal growth faltering. Infants who experience one or more major morbidities such as bronchopulmonary dysplasia (BPD), severe intraventricular hemorrhage, NEC, or late-onset sepsis are at the highest risk of growth faltering. The association between suboptimal postnatal growth and poor neurodevelopmental outcomes is especially worrisome and highlights the importance of optimizing nutritional support for premature infants in the neonatal intensive care unit (NICU).

Special considerations regarding nutrient needs of preterm infants arise at birth. Because of limited body stores, increased energy expenditure, severity of illness, and/or immaturity and inability to tolerate enteral feedings, premature infants are given parenteral nutrition immediately after birth. However, to meet nutrient requirements, a combined approach with both parenteral and enteral nutrition is needed to achieve optimal growth outcomes and minimize morbidities such as NEC. This chapter describes the basis of recommendations for enteral nutrient support for high-risk neonates. In addition, evidence regarding the initiation and advancement of enteral feedings in premature infants is discussed. Finally, special considerations for enteral nutrition after discharge from the NICU are reviewed.

Macronutrient Requirements

Protein

When fetal life is interrupted by premature birth, significant protein deficits can occur and may be difficult, if not impossible, to recoup.[2,3] Several observational studies have found an association between early protein intake and postnatal growth in extremely premature infants.[4] In addition, there is increasing evidence that the amount of protein intake early in life correlates with improved neurodevelopmental outcomes. To optimize growth outcomes, particular focus must therefore be given to enteral protein intake, especially as parenteral nutrition is weaned and enteral nutrition is advanced.

The most accepted goal of provision of enteral nutrition to premature infants is to achieve growth comparable with that of the fetus at the same gestational age. Protein losses are inversely related to gestational age, providing an explanation for the higher protein requirements in extremely preterm neonates as compared to their term counterparts. Accretion of protein is dependent on protein quantity and quality, energy intake, and underlying disease states (such as sepsis or surgical stress), as well as concomitant medications (such as systemically acting steroids).

Protein requirements in preterm infants have been estimated with use of a variety of methods, including fetal animal models, the factorial approach, and estimates based on the composition of human milk. Protein and energy needs should be considered together because protein synthesis requires energy. Extremely preterm infants require a higher protein-to-energy ratio for optimal growth (Table 59.1). Protein retention, or balance, is generally a function of protein intake if energy intake is adequate. Enteral protein requirements are calculated to be higher than parenteral ones because only approximately 85% of enteral protein is absorbed. In general, published guidelines for protein intake do not take into consideration requirements for catch-up growth.

Based on currently available evidence and consensus of an expert panel, enterally fed very preterm infants should receive at least 3.5 to 4.0 g of protein/kg/day. If growth faltering is identified, additional protein (up to 4.5 g/kg/day) may be required.[5] Further studies are required to define the upper limit of enteral protein intake in premature neonates to optimize growth and neurodevelopmental outcomes.

Energy

In utero, the fetus utilizes both glucose and amino acids as a source of energy. If energy intake is not adequate, protein utilization is not efficient, resulting in lower retention of nitrogen. Coupled with the inverse relationship between protein requirements and weight, the protein-to-energy ratio required to achieve fetal growth is highest in the most premature infants. The recommended energy intake for enterally fed preterm infants ranges between 110 and 130 kcal/kg/day.[6]

The optimal ratio of enteral protein to energy intake must be defined not only in terms of optimizing weight gain but also by that which achieves optimal body composition. Consequently, attempting to duplicate the intrauterine environment may not be appropriate for extrauterine life, given differences in nutrient supply and metabolism. Changes in body composition in response to energy intake are an important consideration because excessive energy intake can contribute to excessive fat deposition, and recent studies have suggested that rapid weight gain may be associated with adverse outcomes.

The energy needs of the neonate are derived from a computation of the energy expenditure, energy storage, and energy losses. Energy expenditure consists of the energy needed to cover the resting metabolic rate, activity, thermoregulation, and the energy cost of growth. Energy storage consists of the energy (fat and lean mass) deposited for growth. Energy losses are usually due to incomplete absorption of nutrients and are greater in premature infants than in term infants or adults. The largest component of the total estimated energy requirement is that needed for the resting metabolic rate. When nourished parenterally, the preterm infant has less fecal energy loss, generally fewer episodes of cold stress, and somewhat lesser activity. When full parenterally the actual energy needs for growth are lowered to approximately 85 to 95 kcal/kg/day. In the case of chronic disease, such as BPD, the resting energy expenditure rises significantly. Total energy needs in preterm infants with BPD are increased because of greater energy expenditure, activity, and fecal energy losses. It is not surprising to find that these infants may require higher caloric intake to achieve weight gain.

Carbohydrates

The main carbohydrate in human milk is lactose, supplying nearly half of the total calories. Lactase (β-galactosidase) is an intestinal enzyme that hydrolyzes lactose to glucose and galactose in the small intestine. Despite lower levels of intestinal lactase activities in premature infants, they can efficiently digest lactose. Nonetheless, many preterm infant formulas supply glucose polymers. Glucose polymers are digested by α-glucosidases; the activity level of these enzymes approximates adult levels much sooner than does that of β-galactosidase. Theoretically, this makes glucose polymers easier for the premature infant to digest than lactose. Glucose polymers also have an advantage in that they increase caloric density without a rise in osmolality.

Fat

Fat provides a substantial source of energy for growing preterm infants. Preterm infants have low levels of pancreatic lipase, bile acids, and lingual lipase. Human milk, however, supplies a variety of lipases, including lipoprotein lipase, bile salt esterase,

TABLE 59.1 Estimated Protein and Energy Requirements to Achieve Fetal Growth

Weight (g)	Protein (g/kg/day)	Energy (kcal/kg/day)	Protein/Energy (g/100 kcal)
500–700	4.0	105	3.8
700–900	4.0	108	3.7
900–1200	4.0	119	3.4
1200–1500	3.9	125	3.1
1500–1800	3.6	128	2.8
1800–2200	3.4	131	2.6

Based on the factorial method (Ziegler EE. Meeting the nutritional needs of the low-birth-weight infant. *Ann Nutr Metab.* 2011;58[Suppl 1]:8–18).

and nonactivated lipase. The composition of dietary fat affects absorption and digestion. The absorption of fatty acids increases with decreasing chain length and with the degree of unsaturation. Consequently, medium-chain triglycerides (carbon chain length of 6 to 12) are hydrolyzed more readily than long-chain triglycerides. In contrast to formulas designed for term infants, preterm infant formulas supply medium-chain triglycerides. Human milk supplies 8% to 12% of fat as medium-chain triglycerides. The recommended intake for total enteral fat in enterally fed preterm infants ranges between 4.1 and 7.4 g/100 kcal, with medium-chain triglycerides supplying less than or equal to 40% of fat intake. [7]

Micronutrients, Vitamins, Minerals, and Trace Element Requirements

Calcium and Phosphorus

Calcium and phosphorus are primary components of the skeleton. The goal for premature infant nutrition is to achieve a bone mineralization pattern similar to that in the fetus and to avoid osteopenia and fractures. The peak fetal calcium accretion rate occurs in late gestation through active calcium influx.[8] Infants will have greater calcium and phosphorus needs when exposed to diuretics, which increase renal excretion of these minerals. Phosphorus requirements may be greater in the setting of a history of placental insufficiency, and calcium requirements may be greater for infants exposed to steroids.

Preterm human milk contains approximately 250 mg of calcium and 140 mg of phosphorus per liter. Meta-analyses of breast milk content studies reveal calcium and phosphorus levels that are similar in preterm and term milk.[9] The calcium and phosphorus contents of enteral formula products designed for premature infants in the United States are significantly greater. In human milk, calcium and phosphorus exist in ionized and complex forms that are easily absorbed. Thus, in the design of commercial formulas, greater quantities of these minerals are added to compensate for their poorer bioavailability. Therefore, additional supplementation with calcium and phosphorus is generally not necessary in term infants. However, distinct from the term infant, the preterm infant requires significantly greater quantities of calcium and phosphorus than can be provided in human milk.

For the human milk–fed preterm infant, calcium and phosphorus are deficient throughout lactation, and the levels are far below those necessary to achieve respective intrauterine accretion rates. Deficient intakes of calcium and phosphorus are associated with biochemical markers such as low serum and urine phosphorus concentrations, elevated serum alkaline phosphatase activity, and elevated serum and urine calcium concentrations. Usually, serum phosphorus concentrations are the best indicators of calcium and phosphorus status in human milk–fed premature infants, and serum phosphorus concentration below 4 mg/dL should be followed carefully. Monitoring of these laboratory markers should be undertaken during hospitalization of very low birth weight (VLBW) infants.[10] The American Academy of Pediatrics (AAP) Committee on Nutrition issued guidelines that laboratory monitoring of VLBW infants should begin at 4 to 5 weeks after birth. Prolonged deficiency of these minerals tends to stimulate bone resorption to normalize serum calcium concentrations. This bone activity is often correlated with elevated serum alkaline phosphatase activity. It has been reported that most preterm infants who had an elevated serum alkaline phosphatase activity were those fed human milk. Moreover, follow-up evaluations of the same infants at 9 and 18 months noted that linear growth was significantly lower in the group that had the higher serum activity of alkaline phosphatase in the neonatal period. A high alkaline phosphatase value in the neonatal period is a negative predictor of height in 9- to 12-year-old adolescents. Serum alkaline phosphatase levels greater than 600 international units (IU)/L or clinical concern for fractures should lead to a radiographic work-up for rickets. Dual-energy x-ray absorptiometry and quantitative ultrasonography are increasingly used as tools for studying metabolic bone disease in preterm infants but have variable clinical.[11,12] The diagnosis of rickets by neonatologists is most commonly performed by x-ray.[13]

Supplementation of human milk with both calcium and phosphorus not only improves the net retention of both minerals but also increases bone mineral content. Current management of human milk–fed preterm infants emphasizes the need for supplements of both calcium and phosphorus. A linear relationship exists between calcium (or phosphorus) intake and net retention in enterally fed premature infants. Preterm infants receiving unfortified human milk never achieve intrauterine accretion rates for calcium and phosphorus. Daily intakes of calcium at approximately 200 mg/kg/day and phosphorous at 100 mg/kg/day can be achieved with the use of specialized human milk fortifiers (HMFs) and preterm formulas, thus making it possible to meet intrauterine estimates. HMFs contain highly soluble calcium glycerophosphate, promoting calcium retention.[8] However, term infant formulas and specialized (not "preterm") formulas provide inadequate quantities of calcium and phosphorus to meet the needs of growing premature infants.[14] Several factors affect the absorption of calcium and phosphorus, including postnatal age and intake of calcium, phosphorus, lactose, fat, and vitamin D. Calcium absorption increases with a low-pH environment and high-casein, high-lactose formula. Vitamin D, however, is responsible for only a small component of calcium absorption in premature infants.

The time to supply sufficient calcium and phosphorus stores for premature infants is during the initial hospitalization before their discharge. However, because of the need for prolonged parenteral nutrition and the inability to provide "catch-up" quantities of calcium and phosphorus in milk, some infants may benefit from additional calcium and phosphorus after hospital discharge using transitional formulas. Preterm infants discharged on an exclusive breast milk diet may require continued monitoring of biochemical markers of calcium and phosphorus stores.

Magnesium

Approximately 60% of body magnesium is in bone. Magnesium has the highest fetal accretion rate in the third trimester. Preterm human milk contains approximately 30 mg of magnesium per liter. The absorption of magnesium is significantly greater from unfortified human milk than from formula. The needs of preterm infants are 8 to 15 mg/kg/day[8] and net magnesium retention in human milk–fed premature infants meets intrauterine estimates. Hypomagnesemia may be present in infants of diabetic mothers and is frequently transient. Hypomagnesemia may reduce parathyroid hormone secretion and responsiveness. Thus, magnesium correction may be required to achieve normocalcemia.

Trace Elements

Premature infants are particularly susceptible to trace element deficiencies given that most accrual occurs during the last third of

pregnancy. The literature on trace mineral requirements for premature infants is sparse, as are studies to support evidence-based guidelines for provision of these nutrients.[15]

Zinc

Several factors affect the zinc needs of the enterally fed premature infant. Fetal accretion of zinc is approximately 0.85 mg/kg/day. Growth is a major determinant of zinc needs. An enteral zinc intake of 2 to 3 mg/kg/day is recommended for preterm infants (inclusive of zinc supplied in human milk fortifiers).[16] A recent RCT found that higher enteral doses of zinc may reduce mortality and other neonatal morbidities.[17]

The major excretory route of zinc is via the gastrointestinal (GI) tract. Infants with large GI fluid losses may become zinc deficient, and patients with short bowel syndrome may require zinc intake of 400 to 800 µg/kg/day. The classic signs of zinc deficiency include an erythematous dermatitis over mucous membranes, facial areas, and the extremities. Symptomatic zinc deficiency presents with susceptibility to infection, impaired wound healing, and failure to thrive.

Plasma zinc values lower than 50 µg/dL are highly suggestive of deficiency, but this is not a reliable biomarker for marginal deficiency, and levels may be falsely elevated in times of bone remodeling. Zinc levels should be measured in the context of large stool or ostomy losses. A very low activity of serum alkaline phosphatase, a zinc-dependent enzyme, is also suggestive of deficiency. Reports of symptomatic zinc deficiency in unsupplemented human milk–fed preterm infants serve as a reminder of the decline in milk zinc concentration as lactation advances.

Copper

No universally accepted methods exist to assess copper status clinically. Balance study data provide only an estimate of copper retention at one point in time. Premature infants receiving pooled pasteurized human milk (copper intake of approximately 85 µg/kg/day) are in negative copper balance for 30 days postnatally and never meet the intrauterine accretion rate. Copper concentration is high in early breast milk and decreases throughout lactation. Human milk fortifier supplies additional copper. Symptoms of copper deficiency include osteopenia, neutropenia, and hypochromic anemia. Copper retention is negatively correlated to zinc intake and postnatal age.[8] Copper deficiency may also present as metabolic bone disease with osteoporosis, metaphyseal changes, and physeal disruption. Because copper is excreted in bile, cases of severe cholestasis warrant limitation of copper intakes.

Selenium

Selenium is an essential trace element and is actively transported from the mother to the fetus and may have an important role in oxidative damage from organic hydroperoxides. Low maternal selenium status in early gestation may increase the risk of preterm premature rupture of membranes.[18] In randomized controlled trials, the incidence of premature rupture of membranes was lower in a group of pregnant women who received selenium supplementation.[19] Further research is needed to clarify the role of selenium in adverse pregnancy outcomes as most study designs are limited in the ability to determine causal relationships. A Cochrane review of three eligible trials (two of which were done in geographic regions with low population selenium levels) reported an association between selenium supplementation to premature infants and a reduced risk of sepsis.[20]

Iron

The iron needs of the premature infant are determined by birth weight, initial hemoglobin concentration, rate of growth, and magnitude of iron loss and/or volume of transfused blood. Iron endowment may be diminished by growth restriction and placental insufficiency in preterm infants.[21] Optimization of umbilical cord clamping practices results in increased iron stores.[22,23] Postnatal iron metabolism occurs in three phases. In the first phase, there is decreased erythropoiesis. The hemoglobin concentration declines to a nadir, which is at approximately 2 to 3 months of postnatal age. This time represents a physiologic anemia of prematurity. In the second phase, the hemoglobin concentration rises as active red cell production is occurring. In this phase, iron is needed. The third phase is an exhaustion of iron stores, or late anemia of prematurity, observed if iron supplementation is inadequate.

The concentration of iron in human milk declines throughout lactation. Premature infants fed human milk are in negative iron balance, which, in the absence of transfusion, can be corrected with iron supplements which have been shown to decrease the prevalence of iron-deficiency anemia.[24] Iron absorption also appears to be facilitated by a modest degree of anemia. The most recent recommendation from the AAP Committee on Nutrition is to begin enteral iron supplementation of 2 mg/kg/day by 1 month of age in preterm infants fed human milk. Formula-fed preterm infants should receive iron-fortified formula from the onset of milk feeding. Term infants should receive 11 mg/day beginning at 6 months of age.[25]

Iron supplementation may be delayed in preterm and sick term infants, who have received multiple erythrocyte transfusions, as each milliliter of transfused blood delivers 1 mg of elemental iron, leading to risk of excessive iron stores.[12,26] Serum ferritin measurements may be a clinically useful means to gauge timing of enteral iron supplementation in infants who have received multiple transfusions.

Although preterm infants will require iron supplementation, the optimal timing of initiation of supplementation and the duration of use are less clear.[27] There is mixed evidence on the effects of iron supplementation and improved neurodevelopmental outcomes.[24] Dysregulation of the iron ion homeostasis pathway mediating oxidative damage may contribute to the mechanism for retinopathy of prematurity pathophysiology.[28] Latent iron deficiency is associated with abnormal auditory neural maturation in late preterm infants.[29] Early iron supplementation results in a higher nadir of hemoglobin and serum ferritin concentrations but may also result in iron overload.[30]

Sodium and Potassium

Preterm infants generally need more sodium per unit of body weight than is needed by term infants. Historical studies proposed that this increased sodium need was due to immature renal sodium conservation mechanisms, but more recent studies of very preterm infants have revealed defective aldosterone secretion with conserved renal aldosterone sensitivity.[31] Sodium wasting is inversely related to gestational age. A study comparing daily sodium intakes of 2.9 and 1.6 milliequivalent (mmol)/kg in

preterm infants suggested that the former intake provided more appropriate serum sodium concentrations. Hyponatremia also may occur in premature infants primarily fed human milk because the sodium content of preterm milk continues to decline throughout lactation.[32] This can be problematic in the use of donor milk, although studies have revealed that there is a significant increase in the sodium concentration of donor breast milk after 1 year postpartum.[33] Early sodium supplementation prevents hyponatremia in very preterm infants and may enhance weight gain.[34] The need for electrolyte supplementation, particularly potassium, may increase during or after diuretic use. Infants with short gut or ostomies may have increased sodium needs which can contribute to growth faltering. Urinary sodium measurements are a good correlate for growth and may help guide which infants would benefit from supplementation.[35]

Vitamins

The fat-soluble vitamins A, D, E, and K are stored in the body, and large doses may result in toxicity. Water-soluble vitamins—thiamine, riboflavin, niacin, vitamin B_6, folate, vitamin B_{12}, pantothenic acid, biotin, and vitamin C—are not stored in the body, and excess intakes are excreted in the urine or bile (vitamin B_{12}). The intake of water-soluble vitamins should therefore be monitored at frequent intervals to avoid deficiency states. Vitamin A and riboflavin concentrations decline in human milk under conditions of light exposure and after passage through feeding tubes. Because of exposure to air, ascorbic acid concentrations are lower in pooled human milk. Pasteurization of donor breast milk leads to reduction in vitamin D content.[36] Supplementary vitamins are provided in HMFs and in preterm formulas. A multivitamin supplement should be given to preterm infants once feedings change to unfortified human milk or standard formula. Infants with cholestasis and short bowel syndrome often have difficulty absorbing sufficient fat-soluble vitamins. These vitamin deficiencies can have long-lasting effects on retinal development and visual acuity, wound healing and skin integrity, bone density and fracture risk, and clotting abnormalities and neurodevelopmental outcomes.[37] Early assessment and supplementation of vitamins A, D, E, and K are especially vital for these patients.

Vitamin A

The reported vitamin A content of preterm breast milk is variable but is similar to that of term breast milk. The content is highest in colostrum (400 to 600 IU/dL) and in breast milk with a higher fat content and declines in mature lactation (60 to 200 IU/dL). The system of handling of breast milk, including bottle feeding, may decrease the concentration of retinol.[38]

The optimal dosing of vitamin A supplementation needs further study.[39] Meta-analyses reveal that vitamin A supplementation may result in a modest reduction in the risk of BPD or death, as low tissue vitamin A and retinol-binding protein levels are associated with decreased clearance of lung secretions and decreased ability to repair lung tissue.[40] However, these studies are confounded by the use of dexamethasone in preterm infants, which results in a transient rise, then fall, in serum retinol and retinol-binding protein levels. Furthermore, there is evidence of a higher risk of sepsis with vitamin A administration.[41,42] There is emerging evidence that retinoic acid, a vitamin A metabolite, may have a role in prevention of retinopathy of prematurity.[41,43]

Vitamin D

Conflicting guidelines are proposed by different professional organizations regarding the optimal dosing of vitamin D. The AAP Committee on Nutrition issued guidelines establishing the amount of recommended vitamin D as 400 IU/day for infants. The recommendation applies to infants receiving human milk and those who are consuming less than 1 quart of infant formula per day and is based in part on the risk of rickets in exclusively breastfed infants who do not receive supplementation with 400 IU of vitamin D per day. This level of supplementation is sufficient to meet a target plasma 25-hydroxyvitamin D concentration of 50 mmol/L in most infants.[10,44] However, the Endocrine Society recommends that infants may require up to 1000 IU/day to meet a target plasma 25-hydroxyvitamin D concentration of 75 mmol/L for nonskeletal health benefits.[45]

Antiepileptic drugs such as phenytoin and phenobarbital may affect vitamin metabolism. Ethnicity has a role in serum 25-hydroxyvitamin D levels, with Hispanic infants having a lower umbilical cord blood level.[46] Preterm infants are at higher risk of being born with lower 25-hydroxyvitamin D umbilical cord serum levels.[47] Certain vitamin D receptor polymorphisms are associated with increased frequency of BPD.[48] Lower maternal and neonatal serum 25-hydroxyvitamin D levels are associated with BPD in preterm infants.[49,50] Recent studies have revealed that vitamin D supplementation in black preterm infants is associated with more recurrent wheezing.[51]

Vitamin E

A single enteral dose of vitamin E supplementation raises the serum levels of α-tocopherol, the biologically active form, in preterm infants.[52] Colostrum has high concentrations of α-tocopherol in both term and preterm milk.[53] The system of handling breast milk, including bottle feeding, may decrease the concentration of α-tocopherol.[38] There is a high prevalence of vitamin E deficiency in VLBW infants until term-corrected gestational age.[54] Vitamin E supplementation reduces the risk of retinopathy of prematurity and intracranial hemorrhage and increases the risk of sepsis in VLBW infants.[52,55] Long-term (>6 months) α-tocopherol supplementation in extremely low birth weight (ELBW) infants may increase the performance intelligence quotient.[56]

Vitamin K

In the United States, 0.5 to 1.0 mg phytonadione (vitamin K) is routinely administered at birth by intramuscular injection to prevent hemorrhagic disease of the newborn. There are oral dosing regimens reported in the literature, but there is a lack of evidence to support routine alternative use.[57] Genetic polymorphisms in the vitamin K–dependent coagulation system may cause some preterm infants to be at higher risk of developing intraventricular hemorrhage.[58] Proteins induced by vitamin K absence are the most sensitive indicators of vitamin K status, but prothrombin time and coagulation studies are commonly used.

Options for Enteral Nutrition

When clinicians are considering enteral feeding in neonates, there are several basic choices that they must make. First and foremost is the choice of base diet for the infant, with three

options commonly used: maternal milk, donor human milk, or preterm formula. Once this decision has been made, clinicians must decide (1) when to initiate enteral feeding, (2) concomitant medical conditions that may affect feeding, (3) how to advance the feeding volumes, and (4) how to feed the infant, for example, by mouth, by gravity bolus via a nasogastric (NO)/orogastric (OG) tube, or by timed or continuous infusion via NG/OG tube. Because of the specific nutritional needs of preterm infants (primarily the requirement for higher protein and mineral intake than that provided by human milk alone) and critically ill late-preterm/term infants (increased caloric and protein requirements), there is an additional decision to be made, and that is determining when human milk fortification will be initiated, what to fortify the milk with, and how to manage ongoing milk fortification.

Human Milk

Exclusive breastfeeding is recommended for all infants through 6 months of age. Continued breastfeeding for 12 months or beyond is advocated by the World Health Organization (WHO) and the AAP. Not all those who give birth and lactate are female or identify themselves as female. It is therefore appropriate to ask parents what pronouns they prefer when addressing issues surrounding lactation and breastfeeding. Some suggested terms include "lactating person/parent," "mother's own milk," "parent's milk," and "father's milk."[59] The term "mother's own milk" in this chapter refers to any parent's milk belonging to that infant.

Benefits of Human Milk

Human milk (HM) is considered the ideal source of nutrition for all infants.[60] HM feeding has been associated with a greatly reduced incidence of gastroenteritis, otitis media, respiratory illnesses,[61] and allergic and autoimmune disease,[62] and is recommended as the exclusive diet for infants less than 6 months of age.[60] In premature infants, a HM diet has been associated with a decreased incidence of late-onset sepsis, increased intestinal motility and gastric emptying, improved feeding tolerance, and general antiinflammatory effects.[63,64] Most notably, breast milk has been associated with a 6- to 10-fold decrease in the risk of developing NEC than those fed formula).[65–67] Human milk diets have also been associated with a reduction in bronchopulmonary dysplasia with proportionate decrease based on percentage of MBM.[68] Furthermore, HM diets are associated with decreased time to full enteral feeds, decreased hospital length of stay (LOS),[64,68–70] and decreased rates of rehospitalization in preterm infants.[71] These beneficial effects on time to full enteral feeds, LOS, and time on parenteral nutrition have also been shown in late preterm/term infants with surgical intestinal disorders.[72]

Neurodevelopmental Outcome Effects

Longer duration of breastfeeding and greater exclusivity of breastfeeding are associated with better receptive language at age 3 years and with higher verbal and nonverbal IQ (intelligence quotient) at age 7 years,[73] as well as enhanced white matter development in exclusively breastfed infants.[74] In ELBW preterm infants, maternal milk was associated with higher motor, cognitive, and behavioral scores on BSD-II at 18-month and 30-month neurodevelopmental follow-up. This was a dose-dependent response with an estimated increase of 0.5 in IQ for every 10 mL/kg increase in breast milk in the diet.[71] Similarly, there was a dose-dependent increase in hippocampal and gray matter volume, as well as overall intracranial volume for each day VLBW infants were fed greater than 50% mother's own milk (MOM).[75]

Human Milk Nutrient Content

Protein

Human milk is comprised of approximately 70% whey proteins and 30% casein compared to bovine milk, which is predominantly casein with less than 20% whey. The percentage of whey:casein in human milk varies by the stage of lactation, and wanes to 50:50 late in lactation.[76] The inverted ratio of whey:casein in human milk, as compared to bovine milk, lends to a very different amino acid profile. Glutamine is the most abundant free amino acid in human milk and has several key functions, including providing ketoglutaric acid for the Krebs cycle, a key energy source for intestinal epithelial cells and perhaps providing a substrate for neurotransmitters.[77] Glutamine levels rise over 20-fold from colostrum to mature milk. Whey proteins in HM include α-lactalbumin, β-lactoglobulin, serum albumin, immunoglobulins, lactoferrin and peptide hormones such as growth hormone and insulin-like growth factors, epidermal growth factor, b-cellulin, TGF-α and platelet-derived growth factor. Other proteins include lysozyme, casein, lipase and amylase, bifidus factor, folate-binding protein, α_1-antitrypsin, antichymotrypsin, and haptocorrin.[78] The most abundant protein in HM is α-lactalbumin, which functions both as a nutritional protein source for the infant as well as an essential component for lactose synthesis in the mammary gland itself. Several proteins, such as lactoferrin, lysozyme, and immunoglobulins, play a role in innate host defense and are particularly resistant to acid hydrolysis in the GI tract.

Protein content of preterm human milk is higher than that of term milk (2.2 g/dL vs. 1.2 g/dL) and both show a significant decrease in the first month and continued decline over the course of lactation, both leveling out around 0.9 to 1.0 g/dL by 3 months of lactation (Table 59.2).[79]

Colostrum

The protein content of colostrum is very high due in part to the passage of larger bioactive proteins and trophic factors, such as IgA and growth factors, through the mammary epithelium than found in mature milk. Colostrum is also high in cellular content, human milk oligosaccharides (HMOs), lactobacillus, and antioxidant compounds, all of which provide a trophic environment for the newly colonizing neonatal intestine. In the animal model, colostrum and colostrum protein concentrate have been shown to stimulate mucosal growth and increased tight junctions in the epithelium.

Carbohydrate

The two main sources of carbohydrates in human milk are lactose and HMOs. Lactose is a disaccharide comprised of galactose and glucose monosaccharides produced in the mammary gland by the enzyme system lactose synthase, a complex of galactosyltransferase and α-lactalbumin. The transcription of α-lactalbumin, which is essential to human milk synthesis, is regulated by the hormone prolactin, and is only active in the mammary gland during pregnancy and lactation. Unlike protein and fat, lactose content is not influenced by maternal diet, nor does it vary or decline during lactation, and is similar between preterm and term human milk.[80]

TABLE 59.2 Composition of Preterm and Term Human Milk

	Energy (kcal/dL)	Protein (g/dL)	Fat (g/dL)	Lactose (kcal/dL)	Oligosaccharides (g/dL)
Preterm					
Week 1	60 (45–75)	2.2 (0.3–4.1)	2.6) (0.5–4.7)	5.7 (3.9–7.5)	2.1 (1.3–2.9)
Week 2	71 (49–94)	1.5 (0.8–2.3)	3.5 (1.2–5.7)	5.7 (4.1–7.3)	2.1 (1.1–3.1)
Weeks 3–4	77 (61–92)	1.4 (0.6–2.2)	3.5 (1.6–5.5)	6.0 (5–7)	1.7 (1.1–2.3)
Weeks 10–12	66 (39–94)	1.0 (0.6–1.4)	3.7 (0.8–6.5)	6.8 (6.2–7.2)	NA
Term					
Week 1	60 (44–77)	1.8 (0.4–3.2)	2.2 (0.7–3.7)	5.8 (4.2–7.4)	1.9 (1.1–2.7)
Week 2	67 (47–86)	1.3 (0.8–1.8)	3.0 (1.2–4.8)	6.2 (5–7.3)	1.9 (1.1–2.7)
Weeks 3–4	66 (48–85)	1.2 (0.8–1.6)	3.3 (1.6–5.1)	6.7 (5.3–8.1)	1.6 (1–2.2)
Weeks 10–12	68 (50–86)	0.9 (0.6–1.2)	3.4 (1.6–5.2)	6.7 (5.3–8.1)	NA

Values are given as the mean ± 2 standard deviations.
NA, Not available.
Modified from Gidrewicz DA, Fenton TR. A systematic review and meta-analysis of the nutrient content of preterm and term breast milk. *BMC Pediatr*. 2014;14:216.

Human Milk Oligosaccharides

HMOs are complex sugar molecules found in human milk which are unique to each mother. HMOs are comprised of five monosaccharide building blocks: galactose (Gal), glucose (Glc), *N*-acetylglucosamine (GlcNAc), fucose (Fuc), and the sialic acid (Sia) derivative *N*-acetylneuraminic acid (Neu5Ac). All HMOs consist of a lactose backbone (Galb1–4Glc) at the reducing end, which can then be elongated/branched by the addition of a variety of disaccharides. These elongated/branched chains can then be fucosylated or sialylated. HMOs are often classified by the presence or absence of Neu5Ac, which results in either a sialylated (acidic) or nonsialylated (neutral) HMO, both of which can be fucosylated.[81] The presence of fucosylated HMOs is genetically determined by the mother's secretor (expression of Se gene) and Lewis blood group status. One particular HMO, disialyllacto-N-tetraose (DSLNT), seems to confer particular protection against NEC. Although the exact mechanism of this protection remains to be elucidated, it suggests a very structure-specific and potentially host receptor-mediated effect.[81] HMOs in bovine milk are not structurally similar to those found in human milk; hence, formula is not a source of HMOs for the infant.

Colostrum HMO content is higher than in mature milk and can reach up to 20 to 25 g/L. As the milk matures, this concentration declines to 5 to 20 g/L, which still exceeds the total milk protein concentration. HMOs are generally resistant to the stomach's acidic environment and degradation from pancreatic enzymes and arrive at the colon intact.[82]

Roles of Human Milk Oligosaccharides

Prebiotics: Promote the growth of certain but not all *Bifidobacterium*, such as *B. infantis*, which may keep potentially harmful bacteria in check as they compete for limited nutrient supply.[83]

Antiadhesive antimicrobials: HMOs resemble intestinal cell-surface glycan molecules and act as decoy receptors to prevent viral, bacterial, and protozoan pathogen binding.

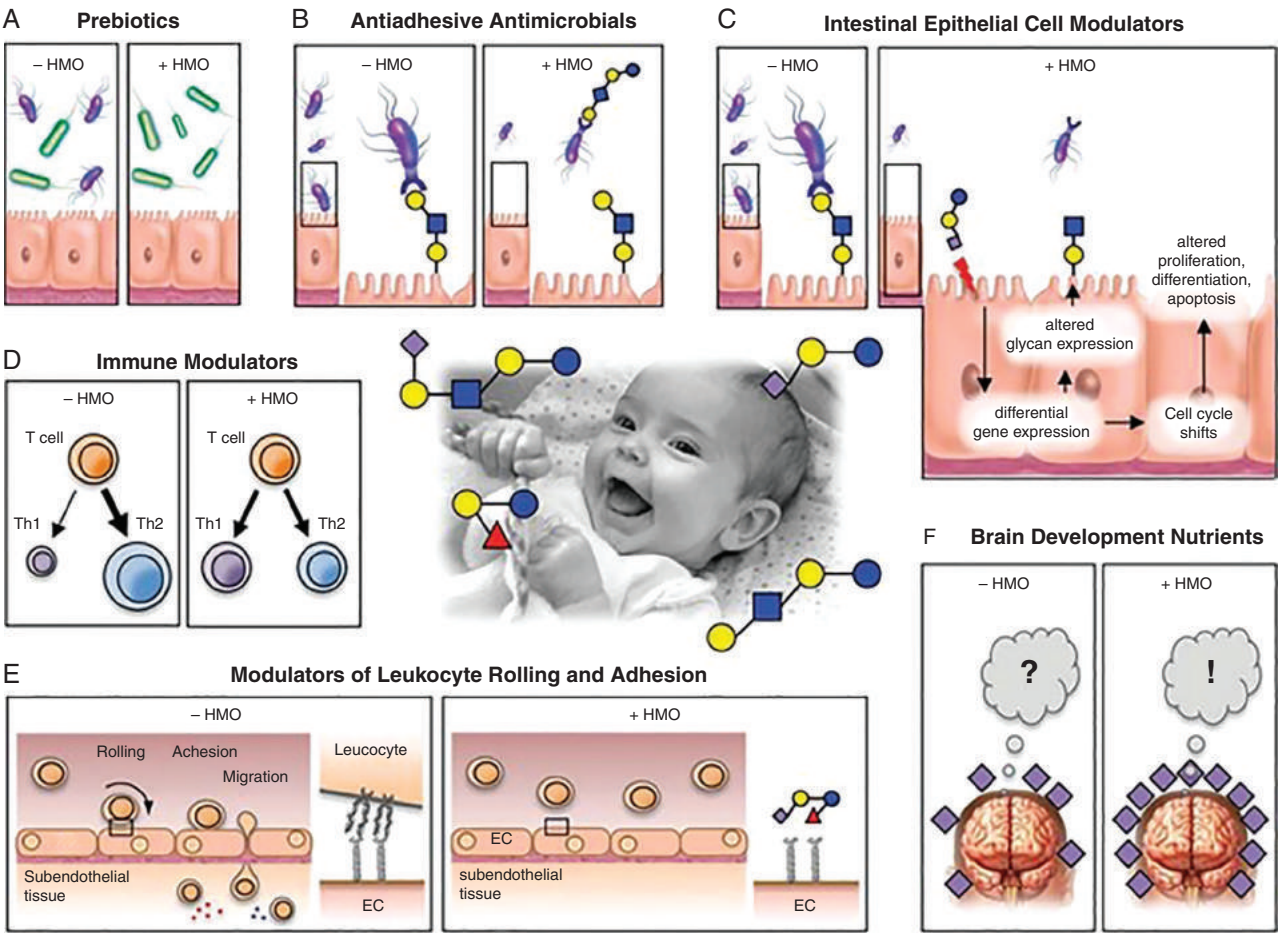
Modulators of intestinal epithelial cell responses: HMOs may also directly modulate host intestinal epithelial cell responses by altering expression of sialylated cell surface glycans which many pathogenic bacteria such as *Escherichia coli* use to adhere to the host's intestinal epithelial cells.

Immune modulators: In addition to local effects of HMOs on mucosa-associated lymphoid tissue, HMOs may also act to modulate the systemic immune response as approximately 1% of HMOs are absorbed into the systemic circulation. Here they have been postulated to influence lymphocyte maturation and enhance the shift towards a more balanced Th1/Th2 cytokine response and decrease production of IL-4 which may contribute toward food allergy prevention (Fig. 59.1).[82]

Fat

Fat provides 50% of the energy in human milk. The lipid system in human milk is structured in a way that facilitates fat digestion and absorption. In human milk, fat exists as organized fat globules containing an outer protein coat and an inner lipid core. The type of fatty acids (high palmitic 16:0, oleic 18:1, linoleic 18:2ω-6, and linoleic 18:3ω-3), their distribution on the triglyceride molecule (16:0 at the 2-position of the molecule), and the presence of bile salt–stimulated lipase are important components of the lipid system in human milk. Fat content of preterm milk is higher than that of term milk in the first 2 weeks (2.2 to 3.5 g/dL in preterm milk vs. 1.8 to 3.0 g/dL in term milk) (see Table 59.2).[9] Fat content of human milk differs among women, changes during the day, rises slightly during lactation, and increases dramatically within a single milk expression. The variability in total fat content is unrelated to maternal dietary fat intake. Because it is not homogenized, the fat separates out of human milk on standing. The separated fat may adhere to collection containers, feeding tubes, and syringes and thus may not be delivered to the infant, compromising energy intake.

The variability in the fat content of human milk may be used to advantage in the premature infant. Most milk transfer during



• **Fig. 59.1** Postulated Human Milk Oligosaccharides *(HMO)* Effects. HMOs may benefit the breast-fed infant in multiple different ways. (A) HMOs are prebiotics that serve as metabolic substrates for beneficial bacteria *(green)* and provide them with a growth advantage over potential pathogens *(purple)*. (B) HMOs are antiadhesive antimicrobials that serve as soluble glycan receptor decoys and prevent pathogen attachment. (C) HMOs directly affect intestinal epithelial cells and modulate their gene expression, which leads to changes in cell surface glycans and other cell responses. (D) HMOs modulate lymphocyte cytokine production, potentially leading to a more balanced Th1/Th2 response. (E) HMOs reduce selectin-mediated cell–cell interactions in the immune system and decrease leukocyte rolling on activated endothelial cells, potentially leading to reduced mucosal leukocyte infiltration and activation. (F) HMOs provide sialic acid as potentially essential nutrients for brain development and cognition. (Bode L. Human milk oligosaccharides: every baby needs a sugar mama. Glycobiology. 2012;22[9]:1147–1162.)

a feeding occurs in 10 to 15 minutes, but continued milk expression yields a milk with a progressively higher fat content. Thus, the "hindmilk" has a higher fat content than the earlier "foremilk." The fat content of hindmilk may be 1.5- to 3-fold greater than that of foremilk. The use of hindmilk in selected cases may provide the infant with additional energy. Hindmilk and foremilk contain similar concentrations of nitrogen, calcium, phosphorus, sodium, and potassium. Copper and zinc concentrations decline by approximately 5% from foremilk to hindmilk.

The differences between foremilk and hindmilk should also be considered in terms of the distribution of calories. Fat and protein account for 42% and 12%, respectively, of the calories in foremilk and 55% and 9% of the calories in hindmilk. The long-term feeding of hindmilk thus could have a negative effect on protein status. A greater proportion of protein calories (10% to 12%) is recommended for premature infants.

Essential Fatty Acids

The essential fatty acids, linoleic and linolenic acids, are present in ample quantities in human milk and commercial formula. Without an adequate intake of these fatty acids, essential fatty acid deficiency (thrombocytopenia, dermatitis, increased infections, and delayed growth) can develop in as little as 1 week. Only 0.5 g/kg/day of essential fatty acids (~4% of total energy intake) will prevent the deficiency. α-Linolenic acid is an important precursor for synthesis of both eicosapentaenoic acid and docosahexaenoic acid (DHA). The very long chain polyunsaturated fatty acids arachidonic acid (AA) (20:4ω-6) and DHA (22:6ω-3) are found in human milk but not bovine milk and are components of phospholipids found in brain, retina, and red blood cell membranes. AA and DHA functionally have been associated with body growth, vision, and cognition. In addition, the fatty acids are integral parts of prostaglandin metabolism. When their diet was supplemented

with polyunsaturated fatty acids, formula-fed premature infants had red blood cell concentrations of DHA paralleling those of similar infants fed human milk. Follow-up studies of such supplemented infants suggest improvements in visual acuity compared with infants that received no supplementation but of similar magnitude to that in infants fed human milk.[84] Improvement in cognitive measures during the first year of life has also been shown. Both AA and DHA are now added to premature formula. The recommended intakes for DHA and AA are 11 to 27 mg/100 kcal and 16 to 39 mg/100 kcal, respectively.[85–87]

Carnitine

Carnitine is synthesized from lysine and methionine and serves as an important effector of fatty acid oxidation in the mitochondria. The provision of carnitine in the diet results in improved fatty acid oxidation. Human milk contains abundant carnitine, and all infant formulas are supplemented with carnitine.

Human Milk Enzymes

Human milk contains enzymes that aid the infant in nutrient digestion. α-Amylase, the enzyme responsible for most of polysaccharide digestion, is not fully developed at birth, even in term infants, who have only 0.2% to 0.5% of adult activity. Mammary amylase is active at the pH of both the stomach and the duodenum and can aid in the digestion of glucose polymers and starches. Although human milk does not contain substrate for α-amylase, this enzyme may aid in digestion of feedings, including infant formula or HMFs that contain complex carbohydrates. Lipases (similar to pancreatic lipase) are present in human milk and aid in digestion of triglycerides such that a significant fraction are broken down into free fatty acids and glycerol before digestion in the small intestine. Bile salt–stimulated lipase, a lipase present in human milk, is highly active because of its wide substrate specificity: it hydrolyzes monoacylglycerols, diacylglycerols, and triacylglycerols, as well as cholesterol esters. This enzyme is also stable in the duodenum and resistant to the low pH of the stomach.[76]

Vitamins and Minerals

Some vitamins and minerals such as thiamine, riboflavin, vitamin B_6, vitamin B_{12}, choline, vitamin A, vitamin C, vitamin D, selenium, zinc, and iodine appear to be rapidly secreted into milk. Maternal dietary intake and states of depletion can substantially affect concentrations of these components in the breast milk. Maternal intake, however, has little effect on concentrations of calcium, magnesium, and iron secreted into breast milk.[80] The vitamin and mineral content of preterm MOM multicomponent human milk fortifiers are shown in Table 59.3.

Preterm Milk

Recent studies of preterm milk analysis show a similar decline in protein content of mother's own milk from ~1.6 to 2.2 g/dL on the first day after delivery to 1.2 to 1.6 g/dL by day 28. This decline was more pronounced in white mothers compared to black mothers.[88] Of note, maternal factors including parity, mode of delivery, prepregnancy body mass index (BMI), previous breastfeeding status, and maternal diet, as well as neonatal factors such as umbilical artery Doppler flows, neonatal AGA or SGA status, gestation, and weight at birth appear to have no impact on the macro- and micro-nutrient content of the breastmilk.[79] Micronutrients such as vitamin D, zinc, calcium, and phosphorus also decline over the first month post-partum and highlight the need for multi-nutrient fortification for preterm infants. Sodium content was significantly lower in milk of mothers of infants born less than 28 weeks' gestation compared to those born greater than 28 weeks' gestation, which can be particularly problematic as the sodium losses for those infants in urine and stool are greater and this can contribute to growth failure.[88]

Special Issues/Contraindications to Mother's Own Milk

Contraindications to breastfeeding and the use of mother's own milk vary throughout the world depending on the risks/benefits to the infant to not breastfeed/receive mother's own milk. In the United States, the following sets of guidelines are generally endorsed (Table 59.4).

Breastfeeding and Substances of Abuse

In addition to the direct risks of contamination of breastmilk by alcohol or drugs, substance use disorders often expose the infant to associated behaviors or conditions that place them independently at higher risk. Although substance use crosses all socioeconomic boundaries, low socioeconomic status, low levels of education, poor prenatal care, food insecurity, and poor nutrition also play a role. Polysubstance abuse is common (drugs, alcohol, tobacco), and adulterants to the drugs, infectious diseases, and mental illness add to the burden of risk to the breastfeeding infant. Despite these multifaceted risks, the proven benefits of human milk and breastfeeding must be carefully considered and weighed.[89] Studies evaluating the outcomes of these risks/exposures are inherently flawed as the infant has already likely been exposed to these circumstances in utero.

Cocaine and phencyclidine hydrochloride (PCP) have both been detected in high concentrations in human milk and have been reported to cause infant intoxication.[90] Other than the drugs discussed below, there is little to no data on other drugs of abuse, as ethical considerations preclude controlled studies.

Opioids

Short courses of most low-dose prescription opioids can be safely used for episodic pain by a breastfeeding mother. Codeine, however, should be used with caution as CYP2D6 ultra-rapid metabolizers may experience high morphine (metabolite) blood levels, potentially placing the infant at increased risk. Information is lacking on the safety of breastfeeding with the use of moderate to high doses of opioids for longer periods of time, nor is there data available for transitioning mothers from short-acting opioids to opioid maintenance therapy while breastfeeding.

Methadone

As the concentration of methadone excreted in human milk is low, women on stable methadone maintenance regimens should be encouraged to breastfeed regardless of their methadone replacement dose. Despite this low excretion rate, provision of breastmilk and breastfeeding have been shown to reduce the severity and duration of neonatal opioid withdrawal syndrome (NOWS)

TABLE 59.3 Comparison of Nutrient Content of Preterm Mother's Own Milk + Multicomponent Fortifiers

Per 100 kcal	Similac HMF Hydrolyzed Protein[a]	Enfamil HMF High Protein[b]	Enfamil HMF Standard Protein[c]	Prolact +4 H²MF[d]
Protein (g)	3.58	4	3.4	3
Fat (g)	4.98	6	6	5.7
Carbohydrate (g)	10.4	7.9	8.7	9.2
Vitamin A (IU)	1238	1240	1240	93.2
Vitamin D (IU)	149	200	200	10
Vitamin E (IU)	5.3	6.2	6.2	0.5
Vitamin K (μg)	10.3	7.9	7.9	0.2
Thiamin (vitamin B_1) (μg)	224	200	200	10.1
Riboflavin (vitamin B_2) (μg)	362	300	300	30.8
Vitamin B_6 (μg)	226	150	150	6.1
Vitamin B_{12} (μg)	0.6	0.68	0.68	0
Niacin (μg)	4279	4000	4000	223.4
Folic acid (μg)	32.9	35	35	6.1
Pantothenic acid (μg)	1489	1190	1190	264.7
Biotin (μg)	24.8	4.1	4.1	0.5
Vitamin C (ascorbic acid) (mg)	43.7	20	20	4.2
Sodium (mg)	47	57	57	70.8
Potassium (mg)	148	98	98	108.3
Chloride (mg)	113	88	88	83.5
Calcium (mg)	152	145	145	139.4
Phosphorus (mg)	85	80	80	78.5
Magnesium (mg)	12.1	5.3	5.3	10.3
Iron (mg)	0.59	1.9	1.9	0.1
Zinc (mg)	1.66	1.37	1.37	1.1
Copper (μg)	131	101	101	112.4
Manganese (μg)	9.9	10.7	10.7	112.4
Osmolality (mOsm)	450	350	330	360

[a]http://abbottnutrition.com/brands/products/similac-human-milk-fortifier-hydrolyzed-protein-concentrated-liquid.
[b]https://www.hcp.meadjohnson.com/s/product/a4R4J000000PpQRUA0/enfamil-liquid-human-milk-fortifier-high-protein.
[c]https://www.hcp.meadjohnson.com/s/product/a4R4J000000PpQmUAK/enfamil-liquid-human-milk-fortifier-standard-protein.
[d]http://www.prolacta.com/Data/Sites/14/media/PDF/mkt-180-prolact-hmf-nutrition-labels.pdf.
HMF, Human milk fortifier; *IU*, international unit.

treatment.[91] Similar results and recommendations apply for buprenorphine treatment for maternal opioid use disorder.

Marijuana

Marijuana is a particularly difficult substance to establish breastfeeding policy for given the differences in legality across state lines, although it currently remains illegal at the federal level. It is also difficult to assess the risk/benefit balance across levels of use from occasional use to heavy use. Δ9-Tetrahydrocannabinol (THC), the psychoactive component found in marijuana, is concentrated up to eight times that found in maternal serum.[92] Once ingested or inhaled, it is rapidly distributed to fat tissues such as adipose and brain, where it may be stored for weeks to months. Because of this long half-life, metabolites may be found in neonatal urine and feces for several weeks, making it extremely difficult to differentiate the occasional versus chronic user, although number of daily uses and time to last use correlate with levels of Δ9-THC in the milk.[93] Also concerning is the increase in potency of marijuana from approximately 3% in the 1980s to 12% in 2012. These

TABLE 59.4 Contraindications to Breastfeeding and the Use of Human Milk

Contraindications to Breastfeeding *and* Use of Expressed Breast Milk

Infant is diagnosed with classic galactosemia.

Mother is infected with the human immunodeficiency virus.[a]

Mother is using an illicit street drug, such as PCP (phencyclidine) or cocaine. *(Narcotic-dependent mothers who are enrolled in a supervised methadone program and have a negative screening for human immunodeficiency virus (HIV) infection and other illicit drugs should be encouraged to breastfeed.)*

Mother has suspected or confirmed Ebola virus disease.

Temporary Restrictions on Breastfeeding *and* Use of Expressed Breast Milk

Mother is infected with untreated brucellosis.

Mother is taking certain medications.[b]

Mother is undergoing diagnostic imaging or treatment with radiopharmaceuticals.

Mother has an active herpes simplex virus (HSV) infection. *(Can breastfeed directly from the unaffected breast if lesions on the affected breast are covered completely to avoid transmission.)*

Temporary Restrictions on Breastfeeding, but *May* Use Expressed Breast Milk

Mother has active, untreated tuberculosis. (*The mother may resume breastfeeding once she has been treated appropriately for 2 weeks and is documented to be no longer contagious.*)

Mother has active varicella (chicken pox) infection that developed within 5 days prior to delivery to 2 days following delivery.

[a]See section on HIV/undetectable viral load for additional considerations.
[b]For the most up-to-date information available on medications and lactation, refer to LactMed.
https://www.cdc.gov/breastfeeding/breastfeeding-special-circumstances/contraindications-to-breastfeeding.html.

issues complicate long-term neurodevelopmental studies. In utero exposure during periods of brain development can have profound effects on brain maturation, leading to long-lasting changes in cognitive function and behavior. Given the long-term neurobehavioral concerns, a mother wishing to breastfeed should be counseled to eliminate or reduce their use/exposure to marijuana.

Alcohol

Alcohol use during pregnancy has been well-documented to be associated with fetal alcohol syndrome, birth defects, preterm birth, spontaneous abortions, and immune dysregulation. Despite the fact that many women reduce or completely eliminate alcohol use during pregnancy, more than half of women in the United States return to consuming alcohol at least occasionally while breastfeeding.[94] Levels of alcohol in human milk parallel those found in maternal serum and effects on the neonate range from somnolence to poor feeding to concern for effects on psychomotor development. Typical recommendations for consumption of alcohol and breastfeeding involve the 2/2/2 rule: no more than 2 (4 oz) glasses of wine or 2 beers, followed by at least a 2-hour waiting period before resuming breastfeeding. A more detailed nomogram based on maternal weight and amount of alcohol consumed has been published and is available online at the Canadian Motherisk program.[95,96]

Human Immunodeficiency Virus/Undetectable Viral Load in Human Immunodeficiency Virus

Breastfeeding in high-income countries (HICs) by women living with human immunodeficiency virus (HIV) remains a contentious issue. There is a dichotomy of advice regarding infant feeding and HIV in the US. The WHO advocates that all new mothers should breastfeed regardless of their status, while the AAP, American College of Obstetricians and Gynecologists, and Centers for Disease Control and Prevention (CDC) continue to recommend formula feeding by mothers living with HIV to eliminate the risk of postnatal transmission.

Approximately 8700 HIV-infected women give birth in the United States in 2006. With current interventions, mother-to-child HIV transmission during pregnancy and labor is very low: under 1%. In the absence of antiretroviral prophylaxis, postnatal infection risk appears to be highest in the first 4 to 6 weeks of life, ranging from 0.7% to 1% per week. The risk continues for the duration of breastfeeding. Two large studies showed that late postnatal transmission risk, after 4 to 6 weeks of age, was 8.9 infections per 100 child-years of breastfeeding (approximately 0.17%/week) and was constant throughout this period. Breastfeeding transmission rates with antiretroviral prophylaxis administered to either the infant or the mother, although low, are still 1% to 5%, and transmission can occur despite undetectable maternal plasma RNA concentrations.

Factors associated with increased risk of HIV transmission via human milk include high maternal plasma and human milk viral load, low maternal CD4+ cell count, longer breastfeeding duration, breast abnormalities (e.g., mastitis, nipple abnormalities), oral lesions in the infant, mixed breastfeeding and formula feeding in the first few months of life (compared with exclusive breastfeeding), and abrupt weaning. Antiretroviral drugs taken by the mother have differential penetration into human milk, with some drugs achieving concentrations much higher or lower than maternal plasma concentrations. The decision to breastfeed with an undetectable HIV viral load is a multifaceted one and requires a thoughtful discussion between the clinician and parent on medication compliance, duration of zero viral load, commitment to

exclusive breastfeeding, and the overall risks/benefits to the infant and mother.

COVID-19

COVID-19 was declared a public health emergency of international concern by the WHO in early 2020 and spread into a worldwide pandemic soon thereafter. Concern for possible transmission of the virus via breast milk led to initial restrictions on breastfeeding and use of MOM.

To date, live, replicatable virus has not been isolated from colostrum or breast milk of mothers positive for SARS-CoV-2 and there has been no convincing evidence for infant infection from breastmilk.[97] The risk of SARS-CoV-2 transmission to the neonate is primarily via contact with infectious respiratory secretions from the mother, caregiver, or other person with SARS-CoV-2 infection. Breastfeeding and provision of breastmilk should continue to be encouraged as the benefits to both the infant and mother outweigh the risks. The CDC has published guidance on safe breastfeeding and handling of breastmilk with COVID-19. Donor milk banks perform extensive screening of their donor mothers for travel and illnesses, including viruses such as COVID-19. In addition, the milk is then pasteurized under conditions which have been shown to kill other viruses such as influenza and SARS-CoV, as cold storage alone is insufficient to kill these viruses.[98,99]

COVID Vaccination

Women receiving SARS-CoV-2 mRNA vaccines have shown robust secretion of IgA, IgM, and IgG antibodies against the virus in their breastmilk for 6 weeks after vaccination.[100,101] The second dose of vaccine further increased levels of IgG in the breastmilk, while IgA remained constant. These antibodies showed neutralizing effects against SARS-CoV-2, providing passive immune transfer to neonates through breastmilk, which may indicate a potential protective effect against infection in the infant. This suggests a critical role for breastmilk IgG in neonatal immunity against SARS-CoV-2 which is similar to the mechanism of protection from several other viral pathogens such as HIV, respiratory syncytial virus, and influenza. The difference in antibody isotype transfer in breastmilk (IgG in vaccine, IgA in natural infection) likely reflects differences in antibody profile programming between naturally acquired SARS-CoV-2 infection (mucosal) versus vaccination (intramuscular).[100] Whether breastmilk IgG or IgA will provide greater neonatal protection remains unclear.

Donor Human Milk

Although MOM is the ideal source of nutrition for at-risk infants, especially the VLBW infant, access to sufficient MOM is often problematic. Admission to an intensive care unit has been shown to impact initiation of pumping, volume of milk pumped per day, and rates of breastfeeding at discharge. A recent study from Children's Hospital of Philadelphia examined these issues in their cardiac ICU. Rates of initiation of pumping were higher among mothers whose babies were inborn (96%) versus mothers who were separated from their infant after birth because of transport to a tertiary care center (67%).[102] Factors that affect provision of maternal milk include separation of mother and infant after delivery, stress of having a critically ill infant, lack of lactation support, and clinician opinion. There is now general consensus from multiple expert panels that pasteurized donor human milk should be provided to VLBW infants as a supplement or alternative to MOM when maternal milk is insufficient in supply.[103,104] Newer data on the advantages of donor milk as a supplement/alternative for MOM in late preterm and term infants with high level of disease severity (CHD, CDH, surgical intestinal disorders) is also evolving.

The majority of donor human milk currently used in NICUs in North America is processed and dispensed from the 31 member banks of the nonprofit Human Milk Banking Association of North America (HMBANA). With expanded criteria for donor milk usage, and the availability for families to buy milk directly from these milk banks for home use, there has been an exponential increase in the amount of milk processed over the past 20 years, from less than 500,000 oz in 2000 to over 7.4 million ounces of milk dispensed in 2019. Human milk processed and dispensed by HMBANA is obtained from healthy donors, most of whom delivered term infants and who undergo extensive screening by HMBANA milk banks, both verbally and in written questionnaires. Donor screenings include detailed inquiries regarding international travel as well as recent illness history including family members in the home. They also require a medical release form to be completed by each donor's licensed healthcare provider. Serologic testing of donors includes human immunodeficiency virus, human T-lymphotropic virus 1 and 2, hepatitis B, hepatitis C, and syphilis. Pooled milk is then processed by a Holder pasteurization, where the milk is heated to 62°C for 30 minutes, allowed to cool, then aliquoted and frozen for shipping. Samples of each batch of pasteurized milk undergo bacteriologic screening. Holder pasteurization is not only highly effective in eliminating all bacterial contamination but eliminates all viruses as well, including members of the SARS (severe acute respiratory syndrome) and MERS (Middle East respiratory syndrome) families. Since the inception of these screening practices in 1985, there has never been an incident of disease transmission or a negative outcome in an infant due to the processing or distribution of pasteurized donor human milk by an HMBANA member bank.

There are several for-profit companies that also supply donor human milk to NICUs and families. Prolacta Bioscience (City of Industry, CA) uses a process similar to Holder pasteurization to process the donated milk. Their screening process is more extensive and, in addition to serologic testing of the mother, the milk is DNA fingerprinted against the mother and tested for drugs of abuse and adulteration. Prolacta is currently the only source of human milk-based fortifiers for both preterm and term infants.

Medolac Laboratories (Lake Oswego, OR) and Ni-Q (Wilsonville, OR) both use a proprietary version of retort processing which exposes the milk to sterilization by heating to 121°C for 5 min, with added pressure of 15 pounds per square inch above atmospheric pressure. This shelf-stable milk does not require refrigeration until after opening and has a shelf life or 1 to 2 years. This milk is an alternative for NICUs that may not have the storage space or volume of usage for a dedicated -80°C freezer and is an option for families who wish to supplement their own milk supply without the use of informal milk sharing.

Differences Between Maternal and Donor Human Milk

Donor human milk has several major differences when compared to mother's own milk. Most milk is donated by mothers of term infants and generally obtained later in the course of lactation. Consequently, donor milk is less calorically dense and contains less protein than mother's own milk from term and preterm infants. Analysis of 415 sequential milk samples from 273 donors showed marked reduction in both energy content and protein. Fat content

was the most variable, leading to a mean energy content of 19 kcal/oz, while 25% of samples were less than 17 kcal/oz and 65% were less than 20 kcal/oz. Processing, container changes, and tube feeding also lead to further decreases in fat content, as human milk fats adhere to the plastics typically used to manufacture these products.[105] Protein content was decreased from estimates of ~1.4 g/dL in mother's own milk to 0.9 to 1.1 g/dL, with over a third of the samples having a protein content of less than 1 g/dL.[106]

A recent metaanalysis by Perrin et al. showed substantial differences between the AAP and American Dietetic and Nutrition published nutritional content values for DHM (donor human milk) and published results from 14 studies. Protein and fat content, as well as total energy, were the most variable between samples, and lower than published norms, and may reflect donor pools and methodological differences in measurements.[107]

Micronutrients such as vitamin D, zinc, calcium, and phosphorus also decline over the first month postpartum and highlight the need for multinutrient fortification for preterm infants. Sodium content was significantly lower in milk of mothers of infants born less than 28 weeks' gestation compared to those born greater than 28 weeks' gestation, which can be particularly problematic as the sodium losses for those infants in urine and stool are greater, and this can contribute to growth failure.[88]

Commercial suppliers of DHM have proprietary processes for balancing macro- and micro-nutrient content between batches and label their products with nutritional content information. Some HMBANA milk banks have pools specifically from mothers who deliver preterm to deliver higher protein content to the smallest infants. Many milk banks also label their pasteurized DHM (PDHM) with total energy and/or nutritional content information.

There are also substantial effects of the pasteurization and sterilization methods on the growth factors and immune components of donor human milk. In both methods, *Lactobacillus* and lymphocytes including B and T cells are destroyed. There is also marked reduction in lactoferrin, erythropoietin, IL-10, IL-1β and IFN-γ. Notably, there is little to no change in electrolytes, vitamins, and iron, as well as lysozyme and HMO integrity, although the composition will be very different to each baby than their MOM. The impact of these differences is highlighted in a recent study by de Halleux et al., in which babies were fed diets of raw MOM, pasteurized MOM (P-MOM), and PDHM and individually fortified, giving equal caloric, protein, and fat content among the groups. The groups fed MOM and P-MOM had substantial increases in weight gain and length and most of that increase was attributable to the raw MOM.[108] These findings raise interesting questions about the effects of noncaloric/macronutrient components of human milk on growth and development.

Donor Milk as a Bridge to Breastfeeding for Term and Late-Preterm Infants

Late preterm infants (LPIs), born between 34 and 36 6/7 weeks. gestational age, are at increased risk of morbidity and mortality, much of which is related to feeding difficulties. They have immature sleep-wake cycles that interfere with their feeding cues, weaker sucks, and early fatigue which lead to poor milk transfer and poor thermoregulation, all of which contribute to hypoglycemia, hyperbilirubinemia, and excessive weight loss that prompts readmission to the hospital and breastfeeding failure. Hospital policies often delineate formula as the only option to supplement breastfeeding and exclude LPIs from receipt of PDHM.[109] A recent study from Mannel et al.[110] examined the type of milk supplementation with LOS and breastfeeding status at discharge in LPIs supplemented with PDHM versus formula. Breastfed infants supplemented with expressed human milk and/or PDHM had a similar LOS to exclusively breastfed infants who required no supplementation. Exclusively formula-fed infants had significant longer LOS. In addition, formula supplementation of breastfed infants led to a 16% decrease in likelihood of breastfeeding at discharge compared to those that received PDHM supplementation. Supplementation with PDHM must be accompanied by robust lactation support in order to produce the desired effect of exclusive breastfeeding success, and policies must evolve to address both of these issues.[111] Meeting the increased demand for PDHM to include this growing population of infants may stress an already limited resource and will need consideration.

Informal Milk Sharing

Wet nursing and cross nursing have existed for thousands of years, being referenced in the Babylonian Code of Hammurabi and ancient Greco-Roman texts. Between the 11th and 18th centuries, the majority of aristocratic infants were fed by wet nurses, as breastfeeding was deemed "indecent." The use of wet nurses declined in the 19th to 20th centuries, as did breastfeeding overall, with the advent of alternate milk sources and formula. With the renewed emphasis on the benefits of breastfeeding for the infant and the mother's health, many families are again exploring the issue of milk sharing through direct wet nursing or cross nursing or attaining donor milk through informal sources such as the Internet or community-based milk sharing groups. Several studies have documented that milk sold for profit on Internet-based sites can pose greater risk than other milk sharing sites. Issues such as milk adulteration (mixing with other substances such as cow's milk to extend the volume), improper storage/freezing methods, bacterial contamination, and lack of transparency of the donor's health, medication, and social histories can greatly increase the risk to the infant.[112,113]

The 2017 Academy of Breastfeeding Medicine Position Statement addresses these concerns and offers recommendations for healthcare providers and families on the strategies to reduce the risk in obtaining milk from informal sources, as well as instructions for home pasteurization. Some HMBANA milk banks and for-profit companies now offer families the opportunity to purchase pasteurized/sterilized donor milk for home use.

Human Milk Fortification

For the term infant, mother's own milk will likely provide adequate protein and energy intake as long as feeding volumes are not restricted and the infant can consume approximately 180 to 200 mL/kg/day. Preterm infants, especially the very low birthweight (VLBW, <1500 g), have several key disadvantages compared to their term counterparts. They are born during what would have been a period of rapid fetal growth in utero and have greatly increased caloric, macro- and micro-nutrient needs to meet this growth potential, as well as contend with severity of illness, after birth. Often respiratory issues and concern for risk of NEC makes larger total daily feeding volumes untenable. Postnatal growth failure is estimated to occur in up to 50% of preterm infants and has been associated with adverse effects on neurodevelopment, renal function, retinopathy of prematurity, BPD, and long-term metabolic function.[113–115] In addition, they have missed much of the

active transport phase of calcium and phosphorus that occurs in the third trimester, placing them at increased risk for metabolic bone disease and fractures.

To meet the nutritional needs of VLBWs, it is standard practice to add a multicomponent fortifier (MCF) to mother's own milk and/or donor milk to provide adequate calories, protein, and minerals. A recent Cochrane review of 18 trials comparing growth in preterm infants fed fortified versus unfortified human milk showed increased gains in head circumference, body length, and weight in those infants receiving fortified feeds while hospitalized. Long-term effects of these fortified feedings on later growth and neurodevelopmental outcome are limited.

MCFs can be divided into two groups: bovine-based (BOV) or human milk-based fortifiers (HMBF). BOV-MCFs provide a hydrolyzed or intact source of bovine protein, as well as added fats and carbohydrate. The BOV-MCFs have evolved from bovine-based powdered packet doses to the newer liquid versions which are designed to provide an increased amount of total protein (1.0 to 1.8 g/100 mL vs. 0.8 to 1.6 g/100 mL) to better approximate the in utero protein accretion and improve linear growth,[116] and are currently the most commonly used in the United States. These liquid MCFs also address the concerns for potential contamination of powdered preparations with *Cronobacter sakazakii*.[117] HMB-MCFs, available for both preterm and at-risk term infants, are produced from PDHM and contain only human milk proteins, fats, and carbohydrates. Both of these fortifier types are enriched with electrolytes, minerals, and vitamins to better meet the needs of the preterm or at-risk infant. The macro- and micro-nutrient content of preterm milk fortified with the most widely used fortifiers are provided in Table 59.3.

Initiation of fortification varies by institution and product used, and range from 20 to 120 mL/kg/day, with little evidence to support differences in practice which are often driven by a balance of concern for NEC and growth restriction at birth. Fortification strategies are typically stratified into standard, adjustable, and targeted, and each is described below.

Standard Fortification

Standard fortification of human milk refers to the addition of fixed amounts of MCFs to HM with the goal of attaining desired caloric and/or protein intakes. This typically results in milk that delivers an estimated 24 kcal/oz, although the total protein intake can range from 3.5 to 5.4 g/kg/day and the micronutrient content vary by product used. The advantages of standard fortification, using either BOV or DMB MCFs, are that it has been shown to be safe and effective and is easy to implement on a large scale.[118,119] A major shortcoming of standard fortification is that it does not account for the differences in protein content and caloric density of the base human milk, especially in the case of DM or as lactation progresses. Preterm infants with in utero growth restriction, increased levels of illness such as BPD, or surgical conditions may require more than the 120 to 130 kcal/kg/day and 3.5 to 4.5 g/kg/day of protein standard fortification provides, so close monitoring is essential.

Adjustable Fortification

Adjustable fortification attempts to achieve optimal protein intake by customizing fortification using blood urea nitrogen (BUN) as a marker of each infant's metabolic response. Although BUN can be affected by hydration status, renal function, sepsis, metabolic, and cardiac disease, it can be a useful marker in the clinically stable, enterally fed infant. BUN is typically targeted to 9 to 16 mg/dL and protein intake is adjusted to attain that target level.[120,121] Modifications of this approach also include targeting both BUN and weight gain with the addition of protein or energy depending on the BUN and growth velocity.[122] When compared with the use of standard fortification, this approach has shown increased short-term growth, as well as higher Bayley Scales of Infant and Toddler Development scores at 18 months.[119,122]

Targeted (or Individualized) Fortification

A major drawback in both standard and adjustable fortification is that the nutrient content of the base HM (MOM or PDHM) is estimated using standard assumptions, which are imprecise at best. Targeted fortification utilizes bedside HM analyzers to more accurately assess the nutrient content of the base HM to which specific amounts of MCF and/or single component nutrients are added to achieve the desired targets for protein, fat, carbohydrates, and overall energy. This method has been shown to produce greater increases in both weight and length, as compared to standard fortification,[123,124] but is more labor and staff intensive, as it requires measurements several times a week for optimal effectiveness.[123] A recent double-blind controlled trial of infants fewer than 30 weeks' gestation, randomized to either standard fortification or targeted fortification using modular protein, carbohydrate, and fat components, showed higher macronutrient intakes, weight gain (~2 g/kg/day), and overall body weight in the intervention group. The impact was most apparent at 36 weeks in the group whose MOM had below-average protein content with significant increases in weight, length, head circumference, fat, and fat-free mass compared to the controls. There were also improved biochemical markers of higher BUN and lower triglycerides and positive effects on feeding tolerance.[124]

Initiation, Mode, and Advancement of Enteral Feedings

The initiation, mode, and rate of advancement of enteral feeding all remain a topic of controversy in neonatology. Substantial variation in the practice of enteral feeding exists among NICUs and, in the absence of standardized feeding protocols, can often vary between clinicians in the same unit. While the fear of necrotizing enterocolitis (NEC) is often cited as the reason for a conservative approach to enteral feeding, standardized feeding protocols have been shown to significantly reduce the incidence of NEC in preterm infants.[125] What is interesting is that, irrespective of the individual components of the feeding protocol, implementation of standardized feeding guidelines not only reduces NEC, but also improves important outcomes such as growth, time to reach full enteral feedings, and duration of parenteral nutrition.

Current evidence supports early initiation of low volume enteral nutrition within the first 48 hours of birth for preterm infants.[126] Low-volume enteral feedings are also referred to as trophic feeding or GI priming. Withholding enteral feeding has been shown to delay gut maturation in preterm infants and results in negative effects. Fasting results in intestinal atrophy, diminished intestinal weight and size (in animal models), delayed maturation of intestinal enzyme function, increase in gut permeability and bacterial translocation, and delay in maturation of the intestinal motor function. Additionally, delay in enteral feeding can extend the duration of parenteral nutrition, with its well-described risks, including metabolic disturbance, direct hyperbilirubinemia and cholestasis, and late-onset sepsis.

Several Cochrane reviews have investigated various aspects of feeding initiation and advancement in preterm infants, with underwhelming results. These issues have been difficult to study in a rigorous manner but have been addressed in a series of Cochrane systematic reviews.[127,128] The primary goal is to determine the optimal feeding regimen that does not increase the incidence of NEC. Comparisons of (1) early (<4 days of age) trophic versus delayed initiation of feeding, (2) early (<4 days) versus delayed progressive feeding advancement, and (3) slow (<24 mL/kg/day) versus faster feeding volume advancement yielded no association between any of these interventions and the incidence of NEC. Early progressive feeding was associated with a 2-day shorter hospital stay, and slow feeding volume advancement was associated with increased risk of invasive infection, not including NEC. They urge caution in interpretation of studies and recommend further randomized trials.[129–131] A recent single center study found that a shorter duration of trophic feeding (less than 3 days) reduced the time to reach full enteral nutrition without an increase in NEC.[132] In addition, the Speed of Increasing Milk Feeds (SIFT) trial conducted in the United Kingdom found that a faster (30 mL/kg) increase in enteral feeding volume (compared to a more standard increment of 18 mL/kg) did not adversely impact the risk of NEC or mortality.[133]

Tube Feeding

Preterm infants are often unable to feed by mouth at the breast or via a bottle at birth because of developmental immaturity. A meta-analysis of small studies suggested that infants with postmenstrual age as low as 28 weeks could safely be exposed to breastfeeding[134] and that introduction of oral feeding can be accomplished at 30 to 31 weeks' postmenstrual age.[135] However, extremely preterm infants may be unable to even begin oral feeding attempts for many weeks after birth and thus are dependent on NG or OG tube feeding. NG/OG feedings may be administered via a timed infusion, with use of an electronic pump, or via gravity bolus. Timed infusions can be periodic, occurring at a specified interval and lasting a specific duration, with a period of rest between feedings, or continuous, in which milk is infused slowly 24 hours a day with no rest period. There are theoretical benefits and risks from both intermittent/bolus and continuous feeding methods, although research is sparse.

Bolus feeding promotes the normal physiologic cyclical surges of GI hormones, which may not only enhance maturation of the preterm GI tract but also increase protein synthesis rates. Immature GI function, including delayed gastric emptying and abnormal intestinal motility, seen not only in preterm infants but also those with GI anomalies and short bowel syndrome, may result in feeding intolerance. With both tube feeding methods, nutrient delivery can be impaired by losses of nutrients that remain in feeding tubes and infusion systems rather than reaching the infant. Fat, particularly, is known to adhere to feeding equipment, decreasing energy delivery, which is worsened with continuous versus intermittent feeding methods.[136]

Infant Nutrition and Growth

A growing body of literature suggests that early nutrition has long-term implications for health and development of all infants and particularly preterm infants. In most scenarios the primary goal of postnatal nutrition for the preterm infant is to, as closely as possible, match expected in utero growth and development. Ideally, this would also optimize long-term developmental outcomes. However, feeding the preterm infant is associated with several challenges. Physiologic weight loss in the first few days after birth sets preterm infants up for "catch-up growth," and it is extremely difficult to provide nutrition (and minerals, in particular) at a rate that matches in utero accretion rates. On the other hand, potential risks of overnutrition may include short-term risk such as increased rates of NEC and longer-term risks such as metabolic syndrome. Most data supporting current nutritional practice in this population are derived from observational studies. The inherent limitations of this type of study design influence the conclusions that can be drawn about the links between nutrition, morbidities, growth, and outcomes.

During the first few weeks after birth, preterm infants may experience weight loss that corresponds to a drop of 0.8 Z score.[137] After this physiologic weight loss and regain of birth weight, it is important to closely monitor growth trajectories, including weight, length, and head circumference. Ideally, preterm infants should track along the percentile they are at after the physiologic contraction of extracellular fluid.

Although postnatal growth failure has historically been defined as body weight or length below the 10th percentile of expected intrauterine growth at the time of hospital discharge, this definition does not adequately account for the growth trajectory during the preterm infant's time in the NICU. The rates of reported growth failure depend on the population studied, and generally increase with decreasing gestational age and birth weight and increase with the severity of illness. Significant variation between hospitals in the degree of postnatal growth failure suggests that this outcome is modifiable and may at least in part reflect differences in nutritional status. Extremely preterm infants with a lower growth trajectory while in the hospital are more likely to have weight, length, and head circumference below the 10th percentile at 18 months' corrected age.[115]

Longitudinal growth is slower in infants with morbidities of prematurity. On the other hand, infants with morbidities such as lung disease or NEC may be fed less aggressively because of their underlying illness. Infants with morbidities of prematurity continue to experience poor growth through at least 2 years, which is only in part explained by the lower growth velocity during the first several months of life.

Extrauterine growth failure among preterm infants has been a target of multiple randomized trials and nutritional interventions in the past several decades. These have led to the current approach to nutrition for very preterm infants, including early intravenous amino acids, trophic enteral feeding—ideally with expressed maternal milk—as soon as possible, and early fortification of human milk feedings. This approach improves energy and nitrogen balance, promotes earlier regain of birth weight, and does not increase the risk of acidosis, NEC, sepsis, or other adverse clinical outcomes. Several studies suggest that lean growth and early catch-up are optimized with a high-protein regimen. In ELBW infants, early enteral provision of protein significantly increases weight, length, and head circumference at least until 36 weeks' postmenstrual age.[138] Shorter duration of dependence on parenteral nutrition is associated with improved longitudinal growth. Nevertheless, "normal" immediate postnatal weight loss is augmented by a suboptimal nutritional state during the first several weeks of life, and growth failure remains common. An observational study demonstrated that postnatal weight loss can be limited to the first few days (and limited to ~8% of birth weight) with optimized early parenteral and enteral nutrition, including a goal of achieving 120 kcal/kg/day and 3.8 g/kg/day of protein by the end of the first week.[139]

Infants with intrauterine growth restriction (IUGR) likely have different metabolic programming and therefore different

nutritional requirements from appropriately grown infants. Term infants born with IUGR have higher rates of adult-onset disease, including hypertension, heart disease, diabetes, and early death.[140,141] Unfortunately, similar data on IUGR preterm infants are not available. Little is known about how to identify or select these infants, and no studies have specifically targeted the nutritional needs of this population. Therefore, the nutrition and growth targets of the IUGR preterm infants are currently similar to those of the appropriate for gestational age preterm infant. The risks of slow growth and persistent small size for age, which are common in the IUGR population, likely outweigh the risks associated with rapid, early catch-up growth. Ultimately, adult weight and risk of long-term metabolic consequences are more likely to be related to parental weight, adult weight, and lifestyle choices than to aggressive early nutrition in the IUGR infant.

While growth failure in the preterm or IUGR infant remains a significant concern, growth that is too rapid in infancy is also associated with adult-onset diseases, including obesity. This phenomenon, in which early "programming" leads to future adverse effects, is commonly termed the Barker hypothesis. In a multicenter study of more than 19,000 term-born American children, growth in the first 4 months was associated with risk of overweight at 7 years.[142] However, it is unlikely these studies of term infants apply to preterm infants, in whom the benefits of brisk early growth may outweigh any potential risks. There is little evidence that current attempts to maximize growth in the NICU "programs" very preterm or critically ill infants for later adverse metabolic consequences. In fact, accelerated or catch-up growth in preterm infants may be associated with improved outcomes.

Assessing Growth and Body Composition in Infants

Infant growth is assessed by anthropometry, in which body weight, length, and head circumference are measured. The use of a length board to accurately measure linear growth is critical. These measurements are compared with established reference data or plotted on a set of growth curves. In general, growth curves for preterm infants are based on expected intrauterine growth. Others are based on postnatal growth data. Several sets of curves have been established for preterm infants; differences are related to population characteristics and sample size, as well as neonatal nutrition practices. In general, curves for both male and female infants allow the clinician to assess growth parameters relative to gestational age and sex-matched norms. Importantly, preterm infants do not always have proportional growth—or proportional growth failure—in the NICU. Linear growth often falters more than weight and may take longer to recover[143] published BMI curves for preterm infants. BMI curves allow the clinician to assess the proportion of weight to length and to distinguish proportionate from disproportionate growth, most commonly when weight increases faster than length. Similar to other growth and body composition parameters, BMI varies with sex and gestational age.

While a preterm infant is hospitalized, growth should be monitored serially and plotted on a set of standardized growth curves. These curves should include weight, length, head circumference, and possibly BMI. Nutrition should be adjusted to target expected intrauterine growth, which is about 15 to 20 g/kg/day for preterm infants with birth weight less than 1500 g.[137]

Changes in body composition over the first months and years of life are associated with nutritional programming of adult morbidities. Assessment of anthropomorphic measures, including BMI, does not inform the clinician about the relative contributions of bone mass, fat mass, and lean mass to body size. Data on the contributions of bone, fat, and lean mass may better reflect the nutritional state of an infant and could potentially inform decisions about appropriate provision of nutrients and goals for growth.

Many preterm infants have poor growth into adolescence, with lower BMI and lower body weight. They have equal fat mass and waist circumference, with an increase in relative abdominal adiposity compared with term-born children. Adiposity may also differ in different groups of preterm infants. For instance, small for gestational age preterm infants have lower fat mass between term-corrected age and 3 months of life but catch up after that point to match appropriate for gestational age born preterm infants.[144] Critically ill preterm infants may have increased central or abdominal adiposity, which is a marker of insulin resistance, versus subcutaneous adiposity. This has unclear implications for short-term and long-term health. Increased abdominal adiposity, rather rapid increases in body weight, or the absolute body weight itself may be the factor that puts some preterm infants at increased risk of adult-onset diseases.[145,146]

Little else is known about the changes in body composition in preterm infants and how to distinguish "normal" from "abnormal." Yet, an understanding of body composition is essential for comparing quality versus quantity of weight gain. An appropriate technique for assessment of body composition must be standardized, valid, reliable, portable, inexpensive, and noninvasive. Ideally, such technology would be used serially to assess response to interventions. Several techniques for assessment of body composition, including dual-energy x-ray absorptiometry and magnetic resonance imaging, are available for research use but cannot practicably be applied in clinical settings, particularly in critically ill neonates. Until additional data on "normal" trajectories of body composition in preterm infants and appropriate technologies for assessment of body composition exist, body composition cannot be used to guide nutrition interventions in the NICU.

Growth and Developmental Outcomes in Preterm Infants

Optimal nutrition is not only essential to match appropriate growth trajectories and minimize risk for adult-onset diseases, early growth and nutrition impact developmental outcomes throughout childhood. The explanation for this long-term impact of infant nutrition is that the first year of life represents a "critical window" of brain growth. In a multicenter cohort study, Ehrenkranz et al. divided ELBW infants into quartiles of in-hospital growth velocity rates.[115] They identified correlations between the quartile of in-hospital growth velocity and the risk of cerebral palsy and developmental outcomes more than 2 standard deviations below the mean at 18 to 22 months' corrected age. These relationships persisted when they were adjusted for multiple potential confounders, such as center and severity of illness.

Postdischarge growth is likely at least as important for developmental outcomes as in-hospital growth. Preterm infants who experience catch-up growth by 8 months' corrected age have better developmental outcomes than those who do not catch up or fail to thrive by 8 months. By 8 to 9 years' corrected age, head growth less than 2 standard deviations below the mean for age at 8 months' corrected age is independently associated with lower intelligence quotient and lower reading, mathematics, spelling, and language scores. In a cohort of 62 VLBW infants followed up prospectively until 24 months' corrected age, poor linear growth

velocity in this period was associated with lower cognitive performance at 24 months.[143]

It remains unknown which specific nutritional interventions in hospital and after discharge would optimize both growth and developmental outcomes in the preterm infant. In two randomized trials of fortified versus term formula at discharge for preterm infants, infants fed fortified preterm formula had improved growth at 18 months.[147] However, there was no difference in developmental scores at 18 months in either trial. Continued research will be essential to establish which growth targets are associated with the best long-term developmental and health outcomes in preterm infants and which nutritional strategies best achieve these targets.

Post-Discharge Nutrition for the Premature Infant

When preterm infants are discharged from the NICU, they have typically accumulated energy, protein, and mineral deficits. In addition, they are often discharged before their original anticipated birth date. Together, these factors lead the preterm infant to have higher nutritional needs after discharge than healthy appropriate for gestational age term infants. Targeting of an appropriate trajectory is essential because postnatal growth through age 1 year in infants born before 32 weeks has a positive relationship with height, weight, and BMI until at least 19 years.[148]

At least one small, randomized trial[149] has demonstrated improved growth among preterm infants fed standard, rather than fortified, formula after discharge. On the other hand, several randomized trials have demonstrated improved growth and mineral accretion among both well former preterm infants and infants with BPD, when fed fortified formula feedings after discharge. However, infants who received the nutrient-enriched formula seemed to have improved proportional growth, because they weighed more at 6- and 12-months' corrected age, were longer at 6 months' corrected age, and had better head circumference growth at term and at 1, 2, 6, and 12 months' corrected age. The nutrient-enriched formula may have been particularly beneficial among infants with birth weight less than 1250 g.

Fewer data are available to support decisions about fortification of maternal milk after discharge of the preterm infant, particularly when families express a goal of nursing rather than bottle feeding their infants. Infants fed unfortified maternal milk have slower growth and lower bone mass than those fed formula after discharge. Fortification of maternal milk is most commonly accomplished by addition of a post-discharge formula to maternal milk to increase energy, protein, calcium, and phosphorus intake. Such fortification improves mineral accretion but may not impact growth in the long term. Nevertheless, on the basis of the existing literature, "fortified" or high-calorie/high-protein milk feeding has become the standard for the preterm infant at discharge, whether feeding is with human milk or formula. Fortification should be continued until at least 6 months' corrected age to optimize catch-up growth. After that time, overly aggressive nutrition may carry an increased risk of adult-onset disease.

Postdischarge formulas supply more energy (22 kcal/oz), protein (2.8 g/100 kcal), calcium, phosphorus, and zinc than term formulas. Compared with term formula, these formulas provide 49% more protein, 10% more calories, 48% more calcium, 62% more phosphorus, and 75% more zinc. Even at intake volumes of up to 200 mL/kg/day, neither maternal milk nor preterm formulas contain the recommended allowance of vitamin D. Thus, vitamin D should be provided as a supplement to all infants both during the hospitalization and after discharge. In infants younger than 1 year, 1 L of vitamin D–fortified infant formula or cow's milk per day will provide sufficient vitamin D to discontinue supplementation.

Post-discharge nutrition should be discussed during the discharge planning process with the families of premature infants. In addition, these plans should be discussed with the primary medical caregiver to ensure a smooth transition to the outpatient setting. Premature infants who are formula fed should be fed nutrient-enriched post-discharge formula for the first year of life. The duration of use will differ depending on the severity of postnatal growth failure, bone health, and proportional growth after NICU discharge. Likewise, growth of preterm infants discharged on human milk should be closely monitored to ensure optimal proportional growth.

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Parenteral Nutrition for the High-Risk Neonate

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KEY POINTS

- Early use of parenteral nutrition in the very low birth weight (VLBW) neonate minimizes nutrient store losses and improves growth outcomes.
- Initial support goals include glucose infusion of 4 to 8 mg/kg/min, amino acids at 2 to 3 g/kg/day, and lipids at 2 g/kg/day.
- Goal calorie intake for full parenteral nutrition for VLBW infants (1000 to 1500 g) is 90 to 100 kcal/kg/day (3 to 3.5 g/kg/day amino acids, glucose infusion rate 9 to 12 mg/kg/min, and lipids of 3 g/kg/day).
- Goal calorie intake for full parenteral nutrition in extremely low birth weight (ELBW) infants (<1000 g) is 100 to 115 kcal/kg/day (3.5 to 4 g/kg/day amino acids, glucose infusion rate of 9 to 12 mg/kg/min, and lipids of 3 to 4 g/kg/day).
- Complications of parenteral nutrition include parenteral nutrition-associated liver disease (PNALD). Intravenous (IV) lipid emulsions contribute to PNALD development. Fish oil-based lipid monotherapy is now approved for the treatment of PNALD in pediatric patients.

Early, effective nutritional support for the premature and critically ill neonate is largely dependent on parenteral nutrition. In practice, the supply of nutrients to preterm neonates—especially very low birth weight (VLBW) neonates—is often inadequate, and these neonates accumulate major deficits in early postnatal life.[1–3] This inadequate nutritional intake is associated with an increased risk of morbidities in VLBW neonates.[4] In addition, a high proportion of VLBW neonates exhibit poor growth during their neonatal intensive care unit (NICU) stay, with those at the lowest birthweight at greatest risk.[5] However, early use of parenteral nutrition may minimize nutrient losses and improve growth outcomes.[6–9] For example, Genoni et al. found that a change in nutritional protocol to include early, aggressive parenteral and enteral nutrition decreased growth failure.[8] In addition to improved growth outcomes, studies have also noted an association between increased early nutrient intake and improved neurodevelopmental outcomes, including both improved Bayley scores and brain growth.[10,11]

Parenteral nutrition solutions, although still imperfect, have improved markedly from the early days of use, with complications being less common. At present, however, improved outcomes in preterm neonates continue to require a consistent effort at providing parenteral nutrition support, especially in early postnatal life. This means initiating parenteral nutrition as soon as possible after birth, continuing until at least 75% of the total protein and energy requirements are supplied by enteral nutrition, and restarting parenteral nutrition quickly if enteral feeding is suspended.

Components of Parenteral Nutrition

Protein

The initial goal of parenteral nutrition is to minimize losses and preserve existing body stores, particularly for protein. Protein losses are significant if no parenteral amino acids are supplied, with losses being the highest in the most premature neonates. For example, a 26-week gestational age infant loses 1.5 g/kg/day of body protein when provided glucose alone over the first 2 days of life. This is compared to a term infant who loses only 0.7 g/kg/day of body protein over the same time period.[12] Extremely preterm neonates given no amino acid supply lose 1.5% of their body protein per day compared to the fetal accretion rate of 2% per day. After only 3 days of no protein intake, an extremely preterm infant will accumulate a 10% protein deficit compared to the fetus over this same time period.

Fortunately, there is good evidence that early amino acid intake can improve nitrogen balance and help compensate for the high rate of protein loss.[13,14] In addition, high versus low amino acid dosing has been shown to influence the nitrogen balance and decrease the risk for hyperglycemia; the influence on other outcomes, such as growth and neurodevelopment, however, is less clear.[15–19] With regard to the side effects of various amino acid dosing strategies, some studies have found no correlation between blood urea nitrogen (BUN) and early as compared to late amino acid administration.[13,14] Other studies, however, have shown a correlation between increased amino acid dosing and BUN concentrations during the first week of life.[15–17] BUN levels, however, are not only influenced by protein intake but also fluid status and renal function.[20] The available data indicate that providing parenteral amino acids at a rate of 2 to 3 g/kg/day as soon as possible after birth can preserve body protein stores in sick, premature, and VLBW neonates, even when given at low caloric intakes.

It is important to note that even though parenteral amino acid administration is beneficial at low caloric intakes, increasing caloric intake is likely to improve protein accretion. For example, even with a small increase in total calories from 49 to 62 kcal/kg/day over a short period, Vlaardingerbroek et al. demonstrated improved nitrogen balance with lipid supplement

added to parenteral glucose and amino acids.[21] Based on current data, a minimum intake of 30 to 40 kcal per 1 g amino acids is recommended to optimize protein accretion.[22] However, additional energy beyond this amount is necessary to produce appropriate growth.

The ultimate goal of parenteral amino acid administration is to achieve a rate of protein accretion similar to that of the fetus. Based on a variety of studies measuring protein losses and balance, 3 to 4 g/kg/day of amino acids is a reasonable estimate of the parenteral protein requirements of VLBW neonates.[23] Birth weight correlates with parenteral protein needs, with those neonates born less than 1000 g having estimated protein requirements of 3.5 to 4 g/kg/day. Estimates for term neonates are 2.5 to 3 g/kg/day. Parenteral protein intake recommendations for premature neonates are shown in Table 60.1.

The composition of currently available amino acid solutions is shown in Table 60.2. These amino acid solutions were designed to mimic plasma amino acid concentrations in healthy 30-day-old breastfed infants (TrophAmine, B. Braun Medical Inc.) or fetal or neonatal cord blood amino acid concentrations (Primene, Baxter Corporation). No convincing data exists to support the superiority of one neonatal amino acid solution over another.

Although the current neonatal amino acid solutions represent a substantial advance over previous mixtures, these solutions do not contain all amino acids. Glutamine, an amino acid supplied abundantly in breast milk and conditionally essential in premature neonates, is not included in any amino acid solutions due to instability (see Table 60.2). Meta-analysis of multiple studies investigating parenteral glutamine supplementation in premature neonates found no effect on mortality, invasive infection, or necrotizing enterocolitis.[24] Tyrosine, a conditionally essential amino acid in neonates, has limited solubility, so little is included in current amino acid solutions. Some solutions, including TrophAmine and Premasol (Baxter Corporation) contain N-acetyltyrosine, a soluble tyrosine derivative with poor bioavailability. A variety of studies in premature neonates suggest the tyrosine supply may be suboptimal in current amino acid solutions.[25] However, it is also important to note excess tyrosine supplementation should be avoided as elevated levels have been associated with negative neurodevelopmental outcomes.[22] Cysteine, another conditionally essential amino acid in neonates, is not included in most amino acid solutions as it is not stable for long periods. However, a cysteine hydrochloride supplement that can be added to parenteral nutrition solutions prior to delivery is commercially available. Evidence suggests that cysteine hydrochloride supplementation of parenteral nutrition improves nitrogen retention in premature neonates.[22,26] Cysteine supplementation of parenteral nutrition also improves the solubility of calcium and phosphorus. For these reasons, the addition of cysteine hydrochloride (30 to 40 mg/g of amino acids, up to a maximum of 120 mg/kg) is recommended. Cysteine hydrochloride can result in metabolic acidosis, but this can be countered by using acetate in the parenteral nutrition solution.[26]

Energy

The initial goal of parenteral nutrition in early postnatal life is to provide sufficient energy intake to at least match energy expenditure to preserve body energy stores. Estimates of energy expenditure in premature infants over the first few weeks of life range from 30 to 75 kcal/kg/day.[27–29] An early intake of approximately 60 to 70 kcal/kg/day may be a reasonable clinical goal to achieve a neutral or slightly positive energy balance. However, because of glucose and lipid intolerance, this intake may not be achievable for a number of days after birth. Energy expenditure increases with advancing postnatal age, increased calorie intake, sepsis, and stimulant use; it can also be influenced by multiple environmental factors.[30–32] It is important to consider these increases in energy needs as lack of adequate compensation can further worsen energy deficits.

TABLE 60.1 Suggested Daily Parenteral Intake for Infants With Birth Weight Less Than 1000 g and 1000 to 1500 g

Component (units/kg/day unless noted)	<1000 g			1000 to 1500 g		
	Day 0*	Transition†	Growing	Day 0*	Transition†	Growing
Energy (kcal)	40–50	70–80	100–115	40–50	60–70	90–100
Protein (g)	2–3	3.5	3.5–4	2–3	3–3.5	3–3.5
Glucose (g)	6–9	9–15	13–17	7–12	9–15	13–17
Glucose infusion rate (mg/kg/min)	4–6	6–10	9–12	5–8	6–10	9–12
Fat (g)	2	2–3	3–4	2	2–3	3
Sodium (mEq)	0–1	2–5	3–7	0–1	2–5	3–5
Potassium (mEq)	0	0–2	2–3	0	0–2	2–3
Chloride (mEq)‡	0–1	2–5	3–7	0–1	2–5	3–5
Calcium (mg)	25–60	60–80	65–100	25–60	60–80	65–100
Phosphorus (mg)	18–30	45–60	50–80	18–30	45–60	50–80
Magnesium (mg)	0–3	3–7.2	3–7.2	0	3–7.2	3–7.2

*Recommended parenteral intakes on the first day of life.
†Period of transition to physiologic and metabolic stability. For most premature neonates, this occurs between 2 and 7 days.
‡Some consideration should be given to providing chloride at a slightly lower level than the sum of sodium and potassium to avoid iatrogenic metabolic acidosis.

TABLE 60.2 Composition of Commercial Parenteral Amino Acid Solutions for Infants

	CONCENTRATION (MG/DL)			
Amino Acid*	**Aminosyn-PF (Hospira/ICU Medical)**	**TrophAmine (B. Braun)**	**Primene (Baxter)†**	**Premasol (Baxter)†**
Essential Amino Acids				
Histidine	312	480	380	480
Isoleucine	760	820	670	820
Leucine	1200	1400	1000	1400
Lysine	677	820	1100	820
Methionine	180	340	240	340
Phenylalanine	427	480	420	480
Threonine	512	420	370	420
Tryptophan	180	200	200	200
Valine	673	780	760	780
Conditionally Essential Amino Acids in Neonate				
Arginine	1227	1200	840	1200
Cysteine	0	<16	189	<16
Glutamine	0	0	0	0
Glycine	385	360	400	360
Proline	812	680	300	680
Taurine	70	25	60	25
Tyrosine	44	240‡	45	240‡
Nonessential Amino Acids				
Alanine	698	540	800	540
Aspartic acid	527	320	600	320
Asparagine	0	0	0	0
Glutamic acid	820	500	1000	500
Serine	495	380	400	380

*All amino acid mixtures shown are 10% solutions.
†Primene available in Canada; Premasol available in the United States.
‡Mixture of L-tyrosine and *N*-acetyltyrosine.

To support normal rates of growth, a positive energy balance of 20 to 25 kcal/kg/day must be achieved.[12] This requires parenteral calorie provision of 90 to 100 kcal/kg/day in infants with a birth weight of 1000 to 1500 g and 100 to 115 kcal/kg/day for those infants with a birth weight less than 1000 g (see Table 60.1). For term infants, a parenteral intake of 80 to 90 kcal/kg/day is often sufficient. The majority of parenteral calories is best supplied by a balanced caloric intake of lipid and glucose. Of note, parenteral energy requirements are less than those of enteral requirements as no energy is lost in the stool.

Glucose

Glucose is typically the first parenteral nutrient provided to premature neonates via an IV dextrose solution. IV dextrose should be initiated minutes after birth to maintain normal blood glucose levels and preserve endogenous carbohydrate stores. Although the precise definitions of hypoglycemia and hyperglycemia remain a topic of debate, maintaining glucose concentrations above 40 mg/dL and below 150 to 200 mg/dL is a reasonable goal.[33–35] Hypoglycemia is easily avoided in preterm neonates by maintaining a constant IV glucose delivery. Hyperglycemia, however, is more often problematic, especially in VLBW neonates, with around 75% of these patients having glucose levels greater than 150 mg/dL and around 30% levels greater than 180 mg/dL.[36]

Glucose infusion rates of 4 to 8 mg/kg/min (70 to 120 mL/kg/day of 10% dextrose in water) are appropriate starting points for most neonates. These glucose infusion rates approximate or slightly exceed the rate of endogenous glucose release from the liver in premature and term neonates with the goal to preserve

endogenous stores. Premature infants have higher glucose utilization rates (approximately 6 to 8 mg/kg/min) than term infants, given their increased brain-to-body ratio. In extremely low birth weight infants (ELBW), however, glucose supplementation does not completely suppress endogenous glucose production despite elevated insulin and glucose levels, with glucose production continued at a rate of 2 to 3 mg/kg/min. Concurrent glucose supplementation without suppression of endogenous glucose production increases the risk for hyperglycemia in ELBW infants.[37] Because ELBW infants can have fluid requirements in excess of 100 mL/kg/day, beginning with 5% dextrose may be necessary to maintain appropriate glucose infusion rates and blood glucose levels.

A gradual increase in glucose intake over the first 2 to 7 days of life up to a glucose infusion rate of 7 to 10 mg/kg/min is usually tolerated when the glucose is combined with amino acid supplementation. A reasonable maximum glucose infusion rate of 12 mg/kg/min should be targeted as rates above this level exceed the glucose oxidation capacity.[34] Exceeding glucose oxidative capacity will cause lipid synthesis from glucose, an energy-expensive process. Recommendations for glucose supplementation during parenteral nutrition are provided in Table 60.1.

Some premature infants, especially ELBW infants, have difficulty tolerating glucose supplementation due to their continued glucose production leading to the development of hyperglycemia. This hyperglycemia can typically be treated with a reduction in the glucose infusion rate.[33–35] The use of insulin in this setting is controversial given the contradictory results from several studies. For example, with regard to mortality and insulin use, Zamir et al.[38] showed a decreased mortality rate at 28 and 70 days, while Beardsall et al.[39] noted early insulin therapy was associated with an increased risk for 28-day mortality. Some of this variation may be accounted for by the fact that hyperglycemia itself is associated with an increased risk of mortality.[40] One of the biggest morbidities associated with insulin use is the occurrence of hypoglycemia, highlighting the importance of close glucose monitoring in this setting. Based on current evidence, insulin therapy should be limited to the setting of continued hyperglycemia despite an adequate decrease in the glucose infusion rate.[34]

Meeting the goal of 9 to 12 mg/kg/min of IV glucose supplementation will result in a caloric intake of 45 to 60 kcal/kg/day, which is insufficient by itself to meet total energy needs. IV lipids are necessary to supply the rest of the needed nonprotein calories. A balanced glucose and lipid approach to supplying nonprotein calories has a number of advantages: it better approximates the carbohydrate-to-fat ratio in enteral feedings, it may improve overall protein accretion, and it minimizes energy expenditure.[41,42]

Lipids

IV lipids are composed of triglycerides, phospholipid from egg yolk to aid in emulsification, and glycerol added to achieve isotonicity. IV lipid solutions available in the United States are noted in Table 60.3. Within the last decade, newer generation lipid emulsions containing fish oil have become available, including Smoflipid (Fresenius Kabi) and Omegaven (Fresenius Kabi). With the increased availability of products in the United States, the choice of lipid emulsion for the treatment of the preterm infant has become less clear. A recent meta-analysis found no particular lipid emulsion, with or without fish oil, to be superior for prevention of parenteral nutrition associated liver disease (PNALD), growth, mortality, retinopathy of prematurity, or bronchopulmonary dysplasia (BPD).[43] In the setting of PNALD, this meta-analysis did note less cholestasis associated with fish oil lipid emulsion use as compared to soybean oil lipid emulsion. Despite the lack of convincing evidence regarding lipid superiority, a recent expert consensus noted that fish oil lipid emulsions have advantages over conventional lipid emulsions in infants, including reduced risk of cholestasis, reduced oxidative stress, provision of docosahexaenoic acid (DHA), anti-inflammatory effects, well-balanced ω-6 to ω-3 polyunsaturated fatty acids (PUFA) ratio, and provision of medium-chain fatty acids.[44] In the setting of PNALD, fish oil monotherapy has been shown to reverse cholestasis in pediatric patients in this setting, Omegaven has now been approved in the United States.[45,46]

Supplementation with these various IV lipid emulsions leads to different fatty acid profiles in the infant, with profiles reflecting the fatty acid profile of the individual product given. All available IV lipid products contain long-chain fatty acids, and the mixed emulsion Smoflipid also provides medium-chain fatty acids. The ratio of specific long-chain PUFAs does vary by product, with the fish oil-containing products (Smoflipid and Omegaven) having a decreased ω-6 to ω-3 PUFA ratio as compared to pure soybean oil-based emulsions. Fish oil lipid emulsions also have an increased amount of α-tocopherol (vitamin E) compared to soybean oil lipid emulsions (see Table 60.3). Of note, all the IV lipid products have a fatty acid profile different from that of human milk (Fig. 60.1).[47]

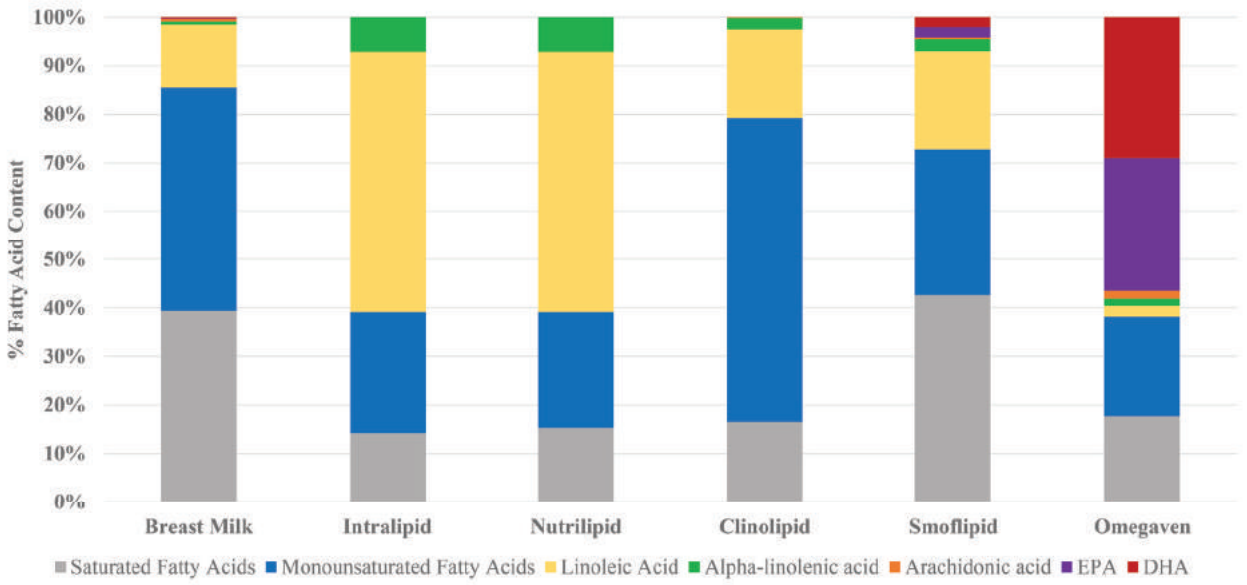
Considering the fatty acid profile of IV lipid products is important when considering dosing. Linoleic acid and α-linolenic acid cannot be synthesized endogenously and are considered essential fatty acids. With less ω-6 PUFA overall, the fish oil-containing lipid emulsions contain less linoleic acid (see Table 60.3). This raises concerns for an increased risk of essential fatty acid deficiency, which has been observed when the fish oil-containing lipid emulsions are given at a dose less than recommended.[48,49] To prevent essential fatty acid deficiency, it is recommended to give preterm infants a minimum dose of 0.25 g/kg/day of linoleic acid.[50] However, despite inadequate linoleic acid content, treatment with fish oil monotherapy at a minimum dose of 1 g/kg/day has been shown to prevent essential fatty acid deficiency.[51] This finding has led to the thought that perhaps the true "essential" fatty acids are the downstream products DHA and arachidonic acid (ARA).[52,53] Additionally, alterations in the DHA and ARA levels have been associated with such outcomes as BPD and late-onset sepsis.[54] Other studies comparing soybean lipid emulsion to the composite emulsion Smoflipid have shown no difference in neurodevelopmental outcomes.[55,56] More information is still needed to fully understand these alterations in neonate fatty acid profiles and outcomes before a clear recommendation can be made regarding lipid use.

After birth, preterm neonates can sustain about 72 hours with no lipid supplementation prior to developing biochemical evidence of essential fatty acid deficiency (triene to tetraene ratio >0.2).[57] There is evidence to show that early IV lipid administration improves growth in ELBW infants and improves nitrogen balance.[21,58] In general, early lipid administration is safe and well-tolerated, and consideration should be given to starting IV lipids at 2 g/kg/day on day 1. Intolerance to lipid infusion is related to the rate of lipid infusion, with better tolerance in preterm infants when lipids are given as a continuous infusion.[50] Elevated triglyceride levels are an indication of lipid intolerance, but given levels of 150 to 200 mg/dL have been noted in enterally fed infants, it is reasonable

TABLE 60.3 Composition of Parenteral Lipid Emulsions Available in the United States*

	Intralipid (Baxter)	Nutrilipid (B. Braun)	Clinolipid (Baxter)	Smoflipid (Fresenius Kabi)	Omegaven (Fresenius Kabi)
Oil (g/100 mL)					
Soybean	20	20	4	6	–
Medium chain triglyceride	–	–	–	6	–
Olive	–	–	16	5	
Fish	–	–	–	3	10
Fats (%)					
Linoleic	44–62	48–58	13.8–22	14–25	1.5
α-Linolenic	4–11	4–11	0.5–4.2	1.5–3.5	1.1
EPA	0	0	0	1–3.5	13–26
DHA	0	0	0	1–3.5	14–27
Arachidonic acid	0	0	0.125	0.25	0.2–2
Glycerol, g/100 mL	2.25	2.5	2.25	2.5	2.5
Egg phospholipid, g/100 mL	1.2	1.2	1.2	1.2	1.2
Phytosterols, mg/L	348	Not reported	327	48	0
α-tocopherol, mg/L	38	35	32	163–225	150–300

*All listed lipid emulsions are 20% solutions with the exception of Omegaven, which is only available as a 10% solution.
DHA, Docosahexaenoic acid; *EPA*, eicosapentaenoic acid.
Data from manufacturer information in addition to Lacaille F, Gupte G, Colomb V, et al. Intestinal failure-associated liver disease: a position paper of the ESPGHAN Working Group of Intestinal Failure and Intestinal Transplantation. *J Pediatr Gastroenterol Nutr*. 2015;60(2):272–283.



• **Fig. 60.1** Fatty Acid Content of Breast Milk and Intravenous Lipid Emulsions. All data presented as percent of total fatty acid content. *DHA*, Docosahexaenoic acid; *EPA*, eicosapentaenoic acid. (Data reformatted from manufacturer data for IV lipids and breast milk data from Koletzko B. Human milk lipids. *Ann Nutr Metab*. 2016;69:28–40.)

to tolerate levels of 250 mg/dL prior to decreasing IV lipid dose.[50] Intolerance to lipid infusion is also related to lipid content. In general, 20% lipid emulsions are better tolerated than 10% lipid emulsions given their lower phospholipid content, which can interfere with triglyceride clearance. For premature infants, 20% IV lipid emulsions are preferred. The clearance of lipids from the blood is dependent on the activity of lipoprotein lipase (LPL). While heparin has been noted to increase the activity of LPL, its routine supplementation in parenteral nutrition for this purpose is not recommended as it has not been shown to improve lipid utilization.

Carnitine aids in the oxidation of lipids by facilitating transport across the mitochondrial membrane. Premature infants receiving parenteral nutrition have low carnitine levels, but the clinical significance of this finding remains uncertain. Meta-analysis of studies evaluating carnitine supplementation in infant parenteral nutrition found no evidence of an effect on ketogenesis, lipid utilization, or weight gain.[59] Despite this lack of evidence, current expert recommendations include using 2 to 5 mg/kg/day of carnitine in parenteral nutrition of infants receiving no enteral source.[60]

IV lipid emulsions undergo peroxidation leading to the formation of free radicals, potentially leading to cellular injury. Light exposure—in particular, phototherapy—greatly increases the amount of lipid peroxidation. Photoprotection of lipid emulsions by shielding the bag and tubing from light can improve outcomes. A recent meta-analysis showed a 50% reduction in mortality for premature infants who received photoprotected lipids.[61] Given this finding, multiple European and American professional societies now recommend light exposure protection of lipid emulsion and parenteral nutrition given to preterm infants.[50,61a]

Electrolytes, Minerals, Trace Elements, and Vitamins

Sodium needs are low in the first few days of life because of expected extracellular fluid contraction and free water diuresis. For VLBW infants, the addition of sodium to the parenteral nutrition may not be necessary until day 3 of life. It is necessary during this time, however, to perform frequent monitoring of serum sodium concentration and an assessment of water balance. After the initial diuresis, 3 to 5 mEq/kg/day of sodium is usually sufficient to maintain serum levels within the normal range (see Table 60.1). However, in VLBW infants, higher levels of sodium supplementation may be needed to compensate for increased renal sodium losses. Potassium requirements are also low in the first few days of life, and supplementation should generally be omitted from parenteral nutrition until renal function in ELBW infants has been clearly established. Potassium intake of 2 to 3 mEq/kg/day is usually adequate to maintain normal serum levels (see Table 60.1).

Chloride requirements follow the same course as sodium, with usual requirements of 3 to 5 mEq/kg/day (see Table 60.1). Excessive chloride supplementation can contribute to the development of metabolic acidosis; however, attempts should be made not to eliminate all chloride supplementation when giving bicarbonate or acetate. The use of sodium acetate as compared to sodium chloride is associated with less metabolic acidosis in VLBW infants.[62]

Supplying adequate calcium and phosphorus in parenteral nutrition is a challenge because of limited solubility. The fetus has average calcium and phosphorus accretion rates of approximately 100 to 120 mg/kg/day and 50 to 65 mg/kg/day, respectively.[63] Presently, in the United States it is not possible to achieve these high rates of calcium and phosphorus supplementation with the solutions available. In other countries, organophosphate preparations are available (e.g., glycerophosphate), allowing for higher rates of supplementation. Precipitation of calcium and phosphorus remains an issue in the United States. However, the solubility is dependent on temperature, type and concentration of amino acids, glucose concentration, pH, type of calcium salt, sequence of addition of calcium and phosphorus to the solution, the calcium-to-phosphorus ratio, and the presence of lipid. As previously noted, adding cysteine to the parenteral nutrition solution lowers the pH and improves calcium and phosphorus solubility. In growing premature infants, intakes of 65 to 100 mg/kg/day (1.5 to 2.5 mmol/kg/day) of calcium and 50 to 80 mg/kg/day (1.5 to 2.5 mmol/kg/day) phosphorus have been recommended (see Table 60.1).[63] A parenteral calcium-to-phosphorus ratio of 1.7 to 1 by weight (1.3 to 1 by molar ratio) is likely optimal for bone mineralization in premature infants, although they seem to be able to tolerate a ratio as low as 0.8.[64,65] In general, calcium and phosphorus should be added to parenteral nutrition early to prevent abnormalities. Magnesium supplementation in parenteral nutrition is also necessary and should be supplied at a dose of 3 to 7.2 mg/kg/day. The timing of magnesium supplementation should depend on infant blood concentration, especially in those infants whose mother received magnesium prior to delivery.

Recommendations for trace element needs in term and preterm infants are derived from recent joint guidelines from multiple European nutritional societies[66] and international expert consensus.[67] It is important to note that selenium and zinc should be included early in parenteral nutrition solutions.[68] Other trace elements may not be needed until after the first 2 weeks of life. The recommended intakes of trace elements for preterm and term infants are shown in Table 60.4.

Multiple individual parenteral products exist to allow for the separate addition of trace elements to parenteral nutrition, including zinc, copper, and selenium. Several combination trace metal solutions are available that include zinc, copper, chromium, and

TABLE 60.4 Recommended Parenteral Intake of Trace Elements for Preterm and Term Infants

Trace Element	Preterm (μg/kg/day)	Term (μg/kg/day)
Chromium*,†	–	0.2
Copper‡	40	20
Iron§	200–250	50–100
Iodide¶	1–10	1
Manganese‡,§	1	1
Molybdenum§	1	0.25
Selenium*	7	2–3
Zinc**	400–500	250

*Renal dysfunction can cause toxicity.
†Due to chromium contamination in parenteral nutrition products, no additional chromium recommended in preterm infants.
‡Impaired biliary excretion can cause toxicity.
§Recommended in long-term (>4 weeks) parenteral nutrition.
¶Not currently supplied by any products in the United States; contamination data unclear.
**The only trace element recommended on day 1 of parenteral nutrition.
Data from Hardy G, Wong T, Morrissey H, et al. Parenteral provision of micronutrients to pediatric patients: an International Expert Consensus Paper. *JPEN J Parenter Enteral Nutr.* 2020;44(S2):S5–S23; Domellöf M, Szitanyi P, Simchowitz V, et al. ESPGHAN/ESPEN/ESPR/CSPEN guidelines on pediatric parenteral nutrition: Iron and trace minerals. *Clin Nutr.* 2018;37(6 Pt B):2354–2359.

manganese, and these solutions are typically given at a dose of 0.2 mL/kg/day. When these trace metal solutions are used, additional zinc is needed to provide the recommended intake for preterm infants, and excess manganese is given. Copper and manganese are excreted via bile and should be used with caution in the setting of cholestasis with levels monitored and supplement discontinued if elevated. In patients with cholestasis, it is now recommended to continue copper supplementation at 20 mcg/kg/day with levels monitored.[66,68] In the setting of renal failure, chromium and selenium levels should be monitored closely. Parenteral iron supplementation is not necessary for short-term parenteral nutrition in infants. For patients on long-term parenteral nutrition, iron status should be assessed using serum iron, transferrin, and ferritin levels prior to initiation of supplementation.

The recommended intakes of vitamins for preterm and term infants on parenteral nutrition are shown in Table 60.5. These recommendations are based on recent updated expert consensus guidelines, although there is still a paucity of data.[67,69] Currently, only two pediatric multivitamin preparations are available. These preparations are meant to approximate the recommended dose for term infants when 5 mL/day is given. However, for preterm infants these preparations provide higher amounts of most B vitamins relative to the recommendations.

TABLE 60.5 Recommended Parenteral Intake of Vitamins for Preterm and Term Infants

Vitamin	Preterm (dose/kg/day)	Term (dose/kg/day)*
Fat Soluble		
Vitamin A (IU)*	700–1500	500–1000 (or 2300 IU/day)
Vitamin D (IU)	80–400 (or 200–1000 IU/day)	40–150 (or 400 IU/day)
Vitamin E (IU)†	2.8–3.5	2.8–3.5
Vitamin K (μg)‡	10	10
Water Soluble		
Vitamin B_{12} (μg)	0.3	0.3
Vitamin C (mg)	15–25	15–25
Biotin (μg)	5–8	5–8
Folic acid (μg)	56	56
Niacin (mg)	4–6.8	4–6.8
Pantothenic acid (mg)	2.5	2.5
Pyridoxine (B_6) (μg)	150–200	150–200
Riboflavin (μg)	150–200	150–200
Thiamin (B_1) (μg)	350–500	350–500

*Maximum dose up to 2300 IU/day.
†Maximum dose up to 11 IU/day including all sources (i.e. lipid emulsions).
‡This does not include the 0.5 to 1 mg of vitamin K to be given at birth.
Data from Bronsky J, Campoy C, Braegger C. ESPGHAN/ESPEN/ESPR/CSPEN guidelines on pediatric parenteral nutrition: vitamins. *Clin Nutr*. 2018;37(6 Pt B):2366–2378; Hardy G, Wong T, Morrissey H, et al. Parenteral provision of micronutrients to pediatric patients: an International Expert Consensus Paper. *JPEN J Parenter Enteral Nutr*. 2020;44(S2):S5–S23.

Complications of Parenteral Nutrition

Although a wide variety of complications associated with parenteral nutrition were reported in the early days, most of these are now rare with current parenteral solutions. Many of the modern complications (e.g., electrolyte and glucose abnormalities) can be prevented or corrected by altering the contents of the prescribed solution. The primary complications of parenteral nutrition as currently used include cholestasis and those related to the infusion catheter.

PNALD is hepatic dysfunction with cholestatic jaundice in the setting of parenteral nutrition use with no other identifiable cause. The most common definition of PNALD is a direct or conjugated bilirubin greater than 2 mg/dL. Risk factors for developing PNALD in infants include prematurity, increased length of parenteral nutrition, lower birth weight, and diagnosis (in particular gastroschisis, jejunal atresia, and surgical necrotizing enterocolitis).[70] Historically, PNALD occurs in up to 85% of infants on parenteral nutrition greater than 100 days.[70] Approximately 15% of patients with PNALD progress to end-stage liver disease.[71] Mortality in patients with PNALD is associated with the degree of direct bilirubin elevation.[70,72]

The etiology of PNALD is unknown and may be multifactorial. Parenteral nutrition factors associated with the diagnosis of PNALD include carbohydrate excess, amino acid imbalances (deficiency in taurine or cysteine or excess methionine), manganese excess, and lipid emulsion components.[73] However, current evidence suggests that lipid emulsions play the largest role in the development of PNALD, probably due to their phytosterol content, high concentration of ω-6 PUFAs, and low concentration of antioxidants (i.e., α-tocopherol).[74] As was previously noted, because of these factors a recent expert opinion stated that fish oil-containing IV lipid emulsions have certain advantages over conventional, soybean-based lipid emulsions (see previous "Lipids" section). For treatment of PNALD, pure fish oil monotherapy (Omegaven) is now approved for use in pediatric patients in the United States. In addition to alternate lipid therapies, soybean lipid restriction has been investigated as a means to both prevent and treat PNALD. However, studies investigating this treatment strategy have shown mixed results.[75–77] Given the fundamental role of lipids in supporting caloric needs and brain development, lipid restriction should not be routinely used in ELBW or VLBW infants.

Central line-associated bloodstream infections (CLABSIs) are the most common serious complication associated with central venous catheter use in infants. The most common bacterial pathogen is coagulase-negative staphylococcus. Other organisms found in infants with CLABSIs include gram-negative bacilli and fungi, in particular *Candida*.[78] Risk factors associated with CLABIs include prematurity, duration of central venous access, and the duration of parenteral nutrition. In addition, recurrent episodes of CLABSIs and sepsis in general can contribute to and worsen PNALD. Such strategies as line insertion and maintenance bundles and dedicated line teams have shown success at decreasing the rate of CLABSIs.[79] Other strategies such as antibiotic-impregnated lines, antimicrobial line dressing use, antibiotics at catheter removal, antibiotic locks, and prophylactic antibiotic use have shown promise in some studies regarding a decrease in the rate of CLABSIs.[78,80–83] However, limited data and significant side effects have limited the recommendations for the use of these strategies in neonates at this time. At present, the best strategy to prevent CLABSIs in neonates includes sterile technique use for insertion

and catheter care, although the best agent to carry out this care remains unknown in neonates.[79]

Other catheter-related complications include line-associated thrombosis, line occlusion, and line dislodgement. Life-threatening complications can include extravasation of parenteral nutrition, including pleural effusion and pericardial effusion. In a single-center review of cases, pleural effusions were rare at 0.4 per 1000 line days. All cases, however, were related to migration and malposition of the central venous catheter.[84]

Use of Parenteral Nutrition in the Neonatal Intensive Care Unit: A Practical Approach

The previous portion of this chapter presented the scientific basis for recommendations regarding the provision of parenteral nutrition to neonates. The following section presents a practical approach to administering parenteral nutrition, with particular emphasis on ELBW infants.

Every clinician caring for ELBW infants must recognize the urgent need to start IV amino acids shortly after birth. As noted previously (see "Protein" section), ELBW infants lose 1.5% of total body protein each day amino acid supplementation is withheld. Consequently, the goal of early parenteral nutrition is to limit catabolism and preserve endogenous nutrient stores. Numerous studies have clearly demonstrated both the safety and efficacy of early amino acid supplementation, even at low caloric intakes.

We recommend starting 3 g/kg/day of amino acids on the first day of life. This can be accomplished simply by adding one of the crystalline amino acid solutions designed for use in neonates (Aminosyn-PF, Primene, Premasol, or TrophAmine) to glucose to use as initial maintenance fluid in ELBW infants. We recommend consideration for developing a neonatal amino acid stock solution made in advance by the pharmacy. Our solution contains amino acids in 7.5% dextrose, that when delivered at 60 mL/kg/day, provides 3 g/kg/day of amino acids. Additional fluids with or without electrolytes and varying concentrations of dextrose can also be given to allow for adjustments based on individual fluid, glucose, and electrolyte needs. It is important to note that this stock amino acid solution should not be increased beyond 60 mL/kg/day, and any needed fluid alteration should be via the ancillary fluids. This stock solution can be given via peripheral IV line, umbilical venous line, or another central line, such as a percutaneous central venous catheter. In our unit, strong consideration is given to percutaneous central venous catheter placement in ELBW infants early in their postnatal course.

To meet growth requirements, 3.5 to 4 g/kg/day of amino acids is required. Once administration of amino acids is initiated, intake can be advanced to meet requirements for growth over a short period. We typically advance to an amino acid dose of 3.5 g/kg/day on the second day of life. Given the available data, we also recommend adding cysteine to the amino acid solution (40 mg/g of amino acids, to a maximum of 120 mg/kg). However, we delay adding cysteine until other electrolytes are included in the parenteral nutrition solution in order to add acetate to buffer the acid load of cysteine supplementation.

Glucose supplementation should be supplied at a sufficient dose to maintain normal glucose concentrations. As noted previously, preterm infants have increased glucose utilization rates of 6 to 8 mg/kg/min as compared to term infants at approximately 3 to 4 mg/kg/min. Also previously mentioned, ELBW infants have continued glucose production despite adequate glucose supplementation. Our current practice includes starting dextrose 10% solution at 40 mL/kg/day to give a total glucose infusion rate of 6 mg/kg/min on the initial day of life when combined with the stock amino acid solution. Given their increased risk of excessive fluid losses and hyperglycemia, ELBW neonates may need 5% dextrose supplementation, especially when needing total fluids of more than 120 to 150 mL/kg/day.

Lipids should be started within the first 24 hours. We typically start lipids at a dose of 2 g/kg/day and advance by 0.5 to 1 g/kg/day to a usual maximum goal of 3 g/kg/day while monitoring and maintaining serum triglyceride levels less than 250 mg/dL. Given the previously mentioned advantages, we use 20% lipid emulsions as the first line over 10% solutions. For ELBW infants expected to need parenteral nutrition for a short period of time with normal direct bilirubin levels, we use soybean oil lipid emulsions as the first-line therapy. For those patients who develop persistent cholestasis concerning for PNALD with direct bilirubin greater than 2 mg/dL and have continued need for parenteral nutrition, we consider soybean oil lipid reduction versus the use of composite lipid-containing fish oil (Smoflipid). The initial therapy choice is dependent on caloric needs and the ability to tolerate enteral feeds. If PNALD continues to worsen despite changing to composite lipid-containing fish oil, we consider fish oil monotherapy.

Caloric requirements during parenteral nutrition are lower than with enteral feeds. For optimal protein retention, approximately 70 to 80 kcal/kg/day is a reasonable goal. However, to achieve optimal growth, a goal of 100 to 115 kcal/kg/day for ELBW infants is reasonable once on stable parenteral nutrition. This goal can be achieved using an amino acid intake of 3.5 g/kg/day, lipid intake of 3 g/kg/day, and maximum glucose infusion rate of 12, often with no more than 12.5% dextrose solution.

There is a paucity of data related to laboratory monitoring in the setting of parenteral nutrition and the ELBW neonate. Suggested monitoring is shown in Table 60.6. It is important to

TABLE 60.6 Suggested Monitoring During Parenteral Nutrition

Parameter*	Frequency
Anthropometric Measurements	
Weight	Daily
Length	Weekly
Head circumference	Weekly
Serum Laboratory Monitoring	
Glucose	1× per shift during week 1, then daily
Na, K, Cl, bicarbonate, BUN, Ca	Daily until stable, then 2× per week
Magnesium, phosphorus	2× per week
Triglycerides	2× per week
ALT, AST, alkaline phosphatase, GGT, fractionated bilirubin	Weekly if on parenteral nutrition >2 weeks

*Further laboratory testing would be recommended for patients on prolonged (>3 to 4 weeks) parenteral nutrition including micronutrient monitoring.

ALT, Aminotransferase; *AST*, aspartate aminotransferase; *BUN*, blood urea nitrogen; *Ca*, calcium; *Cl*, chloride; *GGT*, gamma-glutamyl transferase; *K*, potassium; *Na*, sodium.

consider the balance of close monitoring of parenteral nutrition supplementation with the volume of blood needed for this testing. This suggested testing is not comprehensive. More in-depth testing would be needed in patients on parenteral nutrition for greater than 3 to 4 weeks.

The use of parenteral nutrition in ELBW infants should be accompanied by the early initiation of enteral feeds (ideally on the first day). Parenteral nutrition should be continued until enteral feedings are well established. We continue parenteral nutrition until enteral intake is 120 mL/kg/day or approximately 96 kcal/kg/day. As enteral feeds are advanced, delivered parenteral nutrient and fluid supplementation should be weaned to maintain appropriate fluid and calorie intake needs. In addition, with periods of enteral feeding intolerance, prompt reinstitution of parenteral nutrition in the ELBW infant cannot be overlooked.

Suggested Readings

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Structural Anomalies of the Gastrointestinal Tract

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KEY POINTS

- Small inclusion cysts in the oral cavity are common in newborns and almost always resolve without treatment.
- Suspect the diagnosis of esophageal atresia when there is feeding difficulty with an inability to pass a tube from the nose (or mouth) into the stomach.
- In the most common type of esophageal atresia, there is a fistula from the trachea to the distal esophagus (tracheoesophageal fistula [TEF]) that accounts for the air in the gastrointestinal tract (GI) tract seen on radiographs. When no air is seen it suggests that there is only esophageal atresia and no TEF.
- The most important risk factors for mortality in esophageal atresia anomalies are associated prematurity and major congenital heart disease.
- Infants with pyloric stenosis present with nonbilious vomiting of feedings, intravascular volume depletion, and hypochloremic, hypokalemic metabolic alkalosis in the first few weeks of life.
- Bilious vomiting in newborns indicates mechanical obstruction of the GI tract and, specifically, intestinal malrotation with midgut volvulus until proven otherwise.
- Suspected midgut volvulus requires urgent surgical evaluation. It is best to make the diagnosis of volvulus when there are signs of intestinal obstruction and not to wait for signs of intestinal ischemia.
- If there is a concern of intestinal malrotation and midgut volvulus, then an urgent upper GI contrast study should be done to evaluate the position of the ligament of Treitz (the transition point between duodenum and jejunum).
- Failure to pass meconium in the first day of life should raise concerns about distal bowel obstruction.
- The diagnosis of Hirschsprung disease can typically be confirmed or ruled out by suction rectal biopsy.
- In cases of anorectal malformation, associated anomalies in the VACTERL spectrum (Vertebral, Anorectal, Cardiac, TracheoEsophageal, Renal, Limb) should be explored.

This chapter will provide an overview of structural anomalies of the gastrointestinal (GI) tract seen in newborns. Most of the conditions are congenital; however, some are acquired. Although there are an almost limitless number and variety of structural anomalies, we will concentrate on those that are relatively common and those that have important clinical neonatal presentations. The topics will be discussed in anatomic progression, starting from the mouth and ending at the anus. Several important conditions of the GI tract and abdomen such as biliary atresia, necrotizing enterocolitis (NEC), abdominal wall defects, and abdominal tumors are addressed in other chapters.

Disorders of the Oral Cavity

Mouth

Newborns commonly have transient inclusion cysts in the mouth that may be classified into three types: Epstein pearls, Bohn nodules, and dental lamina cysts.[1,2] Epstein pearls are small keratin-filled nodules found in the midline of the palate. They are thought to be epithelial inclusion cysts located at the developmental fusion line of the palate. Bohn nodules are firm white or gray nodules, usually found along the alveolar (dental) ridges and are remnants of developing salivary glands. Dental lamina cysts are small, raised, white papules in the midline of the palate or on the alveolar (dental) ridges. Although at times concerning in appearance, all three types of oral inclusion cysts are benign and usually asymptomatic. Their natural history is to spontaneously rupture and fuse with the oral epithelium within 5 months of birth, thus conservative management is appropriate and treatment is rarely necessary.[1] Oral inclusion cysts may sometimes be confused with an oral cavity tumor such as a small congenital epulis or with the eruption of a neonatal tooth.

Oral cavity tumors are rare in newborns. The two most common oral cavity tumors are congenital granular cell epulis (CGCE) and oropharyngeal teratoma. CGCE is a benign tumor that presents as a smooth, red or pink mass arising most often from the maxillary alveolar (dental) ridge.[3,4] CGCE enlarges prenatally but does not usually grow after birth, is not associated with any syndromes, and has an excellent prognosis.[3]

An oral cavity teratoma arises from the upper jaw or palate and is also known as an epignathus. Teratomas are tumors that arise from fetal germ cells during development and consist of cells and tissues derived from all three germ cell layers (ectoderm, mesoderm, and endoderm). In the newborn, they are commonly found in the sacrococcygeal region but may occur in the gonads or other midline, or near-midline, locations. Symptoms are typically caused by compression of normal structures, although malignant degeneration may occur.

Larger oral cavity teratomas may be seen on prenatal imaging, but granular cell tumors are typically smaller and discovered on physical examination after birth.[5] Granular cell tumors and oral

cavity teratomas vary in size and, when large, their mass effect may cause feeding difficulty and even upper airway obstruction. Most oral cavity tumors require surgical resection. The timing of surgery depends upon the severity or potential severity of the symptoms.[4,6]

Tongue

The tongue is critical for sucking and swallowing, so congenital disorders such as aglossia, ankyloglossia, and macroglossia are usually noted early in life. Aglossia, or congenital absence of part or all the tongue, is a very rare condition caused by failure of tongue embryogenesis in weeks 4 to 8 of gestation. It is usually associated with craniofacial and limb anomalies.[7]

Ankyloglossia is commonly known as "tongue-tie." Several classification schemes exist for ankyloglossia, including the Corylios classification which describes four subtypes based on the location of attachment.[8,9] By far the most common form of tongue-tie is ankyloglossia inferior, which is an abnormally short, thickened, and/or tight inferior frenulum.[10] Ankyloglossia can also be described as anterior or posterior. Anterior ankyloglossia refers to a lingual frenulum that extends to the tip of the tongue or near the tip of the tongue that restricts tongue mobility.[9]

There is significant debate about the degree to which ankyloglossia interferes with tongue movement, feeding, and speech with some classification systems incorporating function in addition to anatomy, that is, Hazelbaker Assessment Tool for Lingual Frenulum Function and the Bristol Tongue Assessment Tool.[8] Overall, most newborns with ankyloglossia have no difficulty breastfeeding; however, if they do have difficulties then lactation evaluation and counseling are the next steps.[9] If feeding difficulties persist, then frenotomy (the surgical division of the frenulum) may improve feeding. While ankyloglossia inferior may impact the articulation of speech, it does not usually delay overall speech and language development.[11] Ankyloglossia superior is a rare but more serious anomaly that refers to a fibrous or osseous connection between the tongue and the hard palate. Ankyloglossia superior is associated with other significant anomalies, such as cleft palate, microglossia, micrognathia, GI malformations, and deformed limbs.[12,13] Ankyloglossia superior can cause difficulty in feeding and even airway obstruction, making early recognition and treatment necessary.[12]

Macroglossia means enlargement of the tongue and is defined as tongue protrusion beyond the teeth or alveolar ridge during resting posture. Macroglossia is seen in 4.63/100,000 live births, with about half being isolated and half syndromic. Macroglossia is more common among female infants and African Americans.[14] Macroglossia may be classified as "true macroglossia" or "pseudomacroglossia."[15,16] True macroglossia can be due to muscle hypertrophy of the tongue, which is usually the result of a genetic abnormality, such as Beckwith–Weidemann syndrome.[15] Tissue infiltration from amyloidosis, infection, hemangiomas/lymphangiomas, or systemic diseases such as hypothyroidism and diabetes can also lead to macroglossia.[15,16] Pseudomacroglossia occurs when the tongue appears large because of a relatively small oral cavity such as in babies with mandibular hypoplasia (micrognathia) or when the tongue is displaced anteriorly.[16] Surgical treatment is needed if there is airway compromise, dysphagia, or dysarthria. Operations for macroglossia aim to reduce the size of the tongue and preserve its function.[15–17]

Salivary Glands

The salivary glands (the parotid, submandibular, and sublingual glands) may be the source of several newborn problems. A ranula is a mucus-filled cyst arising from the sublingual gland. In newborns, a ranula is caused by congenital obstruction of a sublingual gland duct.[18] Ranulas that are entirely intraoral are known as simple ranulas, while those that extend through the floor of the mouth beyond the mylohyoid muscle are known as "plunging" ranulas and present as a submandibular or neck mass.[19] A ranula may be suspected on physical examination, and diagnosis can be confirmed by ultrasound, computed tomography (CT), or magnetic resonance imaging (MRI). If physical examination and imaging are not definitive then aspiration of the cyst showing yellow fluid containing amylase and mucin confirms the diagnosis.[20] Ranulas may spontaneously resolve and thus may be observed for up to 6 months before excision or sclerotherapy.[20,21]

Neonatal suppurative sialadenitis and parotitis are rare infections of the salivary and parotid glands, respectively, but should be considered in any newborns presenting with unilateral inflammatory submandibular or parotid swelling.[22–24] Bacterial contamination of the glands is from the oral cavity via the draining ducts or from hematologic spread from transient bacteremia.[23,25,26] Risk factors for these infections include prematurity, prolonged nasogastric feeding, mechanical ventilation, dehydration, and maternal mastitis.[24] *Staphylococcus aureus* is the most common causative organism, though *Streptococcus* species, *Escherichia coli*, and *Haemophilus influenzae* have also been reported. Treatment with broad-spectrum intravenous antibiotics is recommended, including an anti-staphylococcal β-lactam and an aminoglycoside or third-generation cephalosporin.[22–24] Although rare, an abscess can form, causing airway compromise and warranting urgent surgical drainage.[27]

Parotid hemangiomas are the most common salivary gland tumors in newborns.[28] They present as a rapidly growing mass in the preauricular area.[29] They may be segmental, involving structures in the distribution of the V3 (mandibular) branch of the trigeminal nerve, including the parotid gland, the overlying skin, and the airway. Alternatively, they may be focal, located within and involving only the parotid gland.[29] Hemangiomas traditionally have a rapid growth phase in the first months of life followed by spontaneous involution over the next decade. Diagnosis is by physical examination and is confirmed by imaging with Doppler ultrasound and sometimes MRI.[30] Observation alone is appropriate for small, non-disfiguring lesions with no systemic manifestations such as congestive heart failure. Treatment options include intralesional corticosteroids, systemic nonselective β-blocker (i.e., propranolol), endovascular sclerotherapy, and surgical resection with intraoperative facial nerve mapping.[28,29]

Disorders of the Neck

Although most disorders of the neck are not structural anomalies of the GI tract, abnormalities of the developing oropharynx, including branchial cleft and thyroid anomalies, may present as neck masses. The branchial cleft anomalies usually present as lateral neck masses, and thyroid anomalies usually present as midline neck masses. We will consider these topics and other lateral and midline neonatal neck masses briefly before covering disorders of the esophagus in the next section.

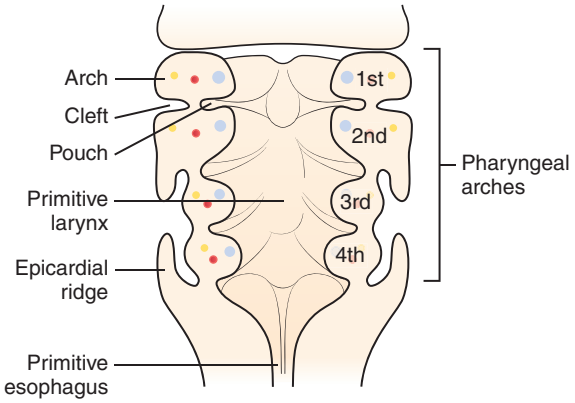
Branchial Anomalies

During early development, the flat, trilaminar embryonic disc forms into a cylindrical body, and in the head, neck, and upper chest this process involves the pharyngeal or branchial (Greek for *gills*) apparatus. (Branchial is the term most used in clinical medicine to describe these developmental structures, so we will use it here.) The branchial apparatus consists of a series of arches that fold and meet in what will be the ventral midline. The arches contain mesoderm and are separated by indentations that on the outer ectodermal surface are called clefts and on the inner endodermal surface are called pouches. The arches and separating clefts and pouches are numbered based on their cranial to caudal embryonic positioning (Fig. 61.1).[31]

Branchial cleft anomalies arise from incomplete obliteration of the embryonic clefts and pouches and result in an epithelial-lined cyst, sinus, or fistula. A cyst has no direct connection from its epithelial inner lining to the normal epithelium of the skin or pharynx. In contrast, a sinus is an abnormal epithelial extension from either the skin or pharynx, and a fistula is an abnormal epithelial connection between skin and pharynx. Most branchial cleft anomalies are cysts, and 95% of the cysts arise from the second branchial cleft. Although they are congenital anomalies present since birth, they often present in older children and adolescents or even adults. They usually present as neck masses on the anterior border of the sternocleidomastoid muscle.[31] These neck masses may be asymptomatic, or they may produce symptoms because of mass effect or infection. Diagnosis of the cyst is made by the characteristic position on physical examination and may be supported by ultrasound, CT, or MRI appearance. A true fistula may be demonstrated by contrast esophagram.[31,32] Treatment of branchial cleft cysts includes initial treatment of any complicating infection and then complete surgical excision when the infection has resolved. Surgical excision is well tolerated with minimal morbidity.[33]

Thyroid

The thyroid may be the source of a neck mass when remnants of its embryologic origin persist and enlarge, when the gland is in an abnormal location, or when it is enlarged in the normal location. During weeks 5 to 7 of gestation the rudimentary thyroid migrates from the foramen cecum (i.e., the base of the tongue) to its final position in the lower neck anterior to the trachea.[34] At the onset of normal migration, the endoderm of the primitive pharynx invaginates and forms the thyroglossal duct with the developing thyroid gland at its inferior aspect. The duct descends to the lower neck and is usually obliterated by week 10 of gestation. Incomplete obliteration of the duct may lead to a thyroglossal duct cyst that presents as a midline neck mass between the hyoid bone and the thyroid gland. Thyroglossal duct cysts have a propensity to become infected, and surgical excision is indicated.[35,36]



• **Fig. 61.1** Branchial Cleft Anomalies.

Simple excision of a thyroglossal duct cyst results in a high rate of recurrence, presumably because of small residual bits of thyroglossal duct tissue not in continuity with the main cyst. Therefore, the operation of choice is the Sistrunk procedure: an en bloc excision of the cyst and its tract, including the middle part of the hyoid bone. Previous infections increase the risk for cyst recurrence after surgery.[35] While rare, when identified later in life, thyroglossal duct cyst carcinoma is reported, with a mean age at diagnosis of 39.5 years.[37]

Disordered thyroid development may result in abnormally located or ectopic thyroid. In general, functional thyroid tissue not located anterior to the second, third, or fourth tracheal rings is considered to be ectopic. Ectopic thyroid tissue can be found anywhere between the foramen cecum and the mediastinum.[38] The most common location for ectopic thyroid is the tongue. Lingual thyroid results from the failure of normal migration of the thyroid during development and presents as a mass in the posterior midline of the tongue. Most patients with a lingual thyroid do not have additional thyroid tissue in the normal position. A large lingual thyroid may cause symptoms of airway obstruction.

A diffusely enlarged, nontender thyroid gland at birth is known as a congenital goiter. It may be associated with hyperthyroidism, hypothyroidism, or a euthyroid state. The diagnosis of congenital goiter can be confirmed with ultrasound. Thyroid function should be assessed to determine the need for thyroid hormone replacement.

Other Neck Masses

Neck masses are often distinguished by their location. As noted, the branchial anomalies usually present as lateral neck masses. The differential for lateral neck masses presenting in the first few weeks of life also includes cystic hygromas and those associated with torticollis.

The "sternocleidomastoid pseudotumor of infancy," or the neck mass associated with congenital muscular torticollis, is a firm, nontender thickening in the lower half of the sternocleidomastoid muscle. The mass is associated with tightness and shortening of the sternocleidomastoid muscle that leads to a characteristic positioning of the patient's head—the face turned away from the side of the lesion with the chin tilted up. The range of motion is reduced. The etiology is unknown, but it is associated with difficult deliveries. The condition is more common in males, more frequent on the right, and generally presents in the first few weeks of life.[39] Diagnosis is by characteristic examination findings. Treatment is directed at improving the range of motion and posture through physical therapy. Surgery is not usually required.

Cystic hygromas are congenital lymphatic malformations. Lymphatic malformations may be defined by the size of the cystic spaces (<2 cm is microcystic, >2 cm is macrocystic), and location is based on the de Serres staging system or the Mulliken/McGill system.[40] Cystic hygromas in the neck are classically macrocystic and located in the posterior triangle, more commonly on the left side. They are present at birth and typically grow with the child but may enlarge suddenly with internal hemorrhage or infection.

Treatment is by surgical excision or sclerotherapy. A systematic review in 2012 did not find conclusive evidence to support the superiority of one treatment modality over the other.[40]

In addition to thyroid anomalies, midline neck masses in newborns include dermoid cysts, teratomas, and, rarely, an undescended thymus. Dermoid cysts are subcutaneous cysts lined by keratinized, stratified, squamous epithelium with appendages such as hair follicles, sweat glands, and sebaceous glands.[41] They commonly occur on the face and scalp at lines of embryonic fusion and can also occur in the midline neck near the hyoid bone and be confused with thyroglossal duct cysts. Dermoid cysts are prone to infection and should be excised. Midline dermoid cysts or those over skull suture lines may connect to the dura, and imaging should be considered in these cases prior to excision. Imaging is not needed in most other cases. Teratomas in the neck are often found in the midline and are biologically the same tumor as oropharyngeal teratomas, described in the earlier section. They usually originate near the larynx and trachea and may cause airway obstruction. Almost all midline neck masses require surgical resection.

Disorders of the Esophagus

Newborns with disorders of the esophagus are challenging patients to manage, particularly if they have associated prematurity or congenital heart disease. This section will focus on esophageal atresia and its common variants and then briefly review related congenital conditions such as laryngotracheal cleft, esophageal stenosis, esophageal duplication cyst, and acquired esophageal perforation.

Esophageal Atresia

Esophageal atresia is the most common congenital anomaly of the esophagus and occurs when the esophagus is congenitally separated into segments that are not in continuity. Esophageal atresia is often associated with a tracheoesophageal fistula (TEF).

Epidemiology

The incidence of esophageal atresia is estimated at approximately 1 in 2500 to 1 in 4500 live births with some variation between regions of the world.[42–44] Esophageal atresia is usually a sporadic disorder, and the recurrence risk for a future sibling is less than 1%.[45]

Etiology and Associated Anomalies

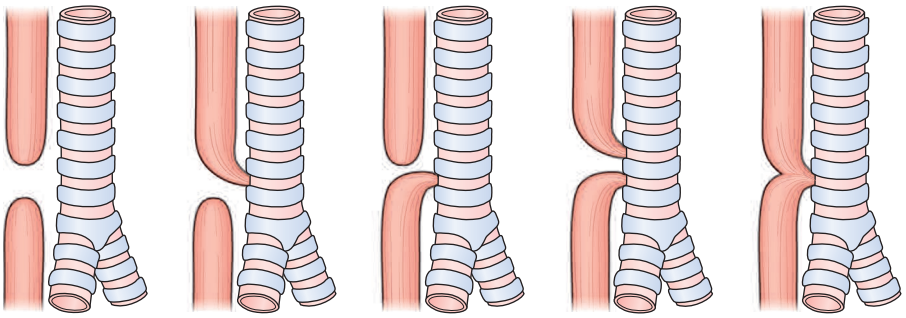
The basic embryology of the foregut and the variants of esophageal atresia and TEF are not understood completely.[46,47] Esophageal atresia may be associated with genetic syndromes such as Down syndrome, Edwards syndrome, CHARGE syndrome (coloboma of the eye, heart defects, atresia of the choanae, retardation of growth and development, and ear abnormalities and deafness), Feingold syndrome, and Fanconi anemia.[48] However, rather than any specific syndrome, esophageal atresia is most often accompanied by additional anomalies in the absence of a known genetic defect or syndrome. These additional anomalies are common in the distribution of VACTERL association (vertebral defects, anorectal malformations, cardiac defects, tracheoesophageal anomalies, renal anomalies, and limb abnormalities).[49] Among 2689 children with esophageal atresia in the United States, 59.1% were found to have associated cardiac defects (most commonly atrial septal defect 46.6% and ventricular septal defect 21.2%). Vertebral, spine, or rib defects were seen in 25.4% of children. Additionally, renal anomalies were associated in 21.8% of children, anorectal malformations in 11.6%, and limb deformities in 6.4%. Approximately one-third of patients had three defects including esophageal atresia, thus qualifying them for a formal VACTERL diagnosis. Duodenal atresia, while not part of VACTERL, was seen in 4.7% of children with esophageal atresia in one study.[50]

Classification

Esophageal atresias with tracheoesophageal malformations occur as a spectrum of anomalies. Several classification systems have been proposed to name the various arrangements[51,52]; however, in a clinical setting it is best to simply describe the anatomic abnormalities. The most important variants are seen in Fig. 61.2.

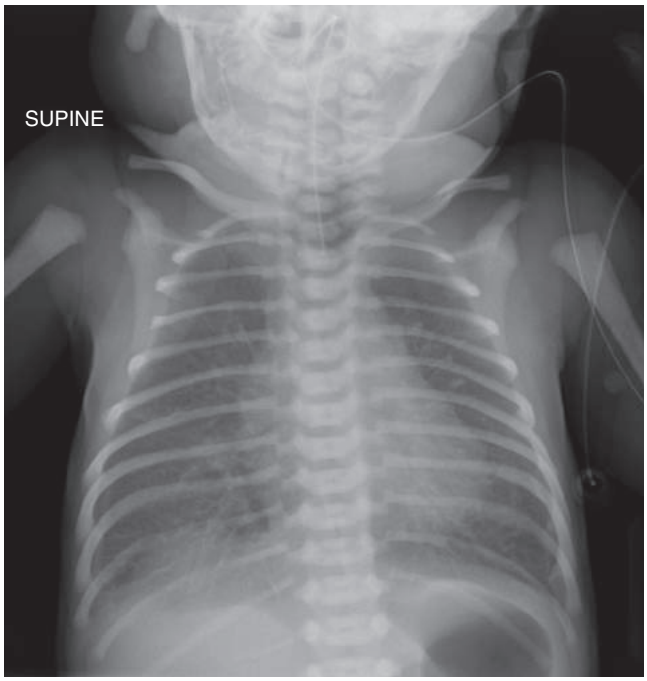
Diagnosis

Although the diagnosis of esophageal atresia may be suspected prenatally, the diagnosis is usually made after birth. Prenatal ultrasound showing polyhydramnios and an abnormally small stomach may suggest esophageal atresia; however, both findings are nonspecific.[53] If, in addition to polyhydramnios and a small stomach, a dilated esophagus is seen in the neck, a diagnosis of esophageal atresia is more likely.[54] Fetal MRI should be considered if prenatal ultrasound suggests esophageal atresia.[55] Prenatal diagnosis allows an opportunity to counsel parents about the diagnosis, postnatal management, and prognosis.[56]

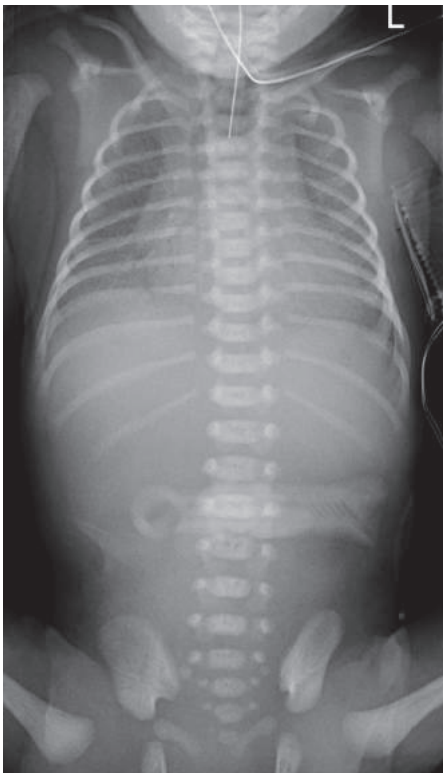


Gross	A	B	C	D	E
Vogt	II	III	IIIb	IIIa	H-type
Frequency	7%	2%	86%	1%	4%

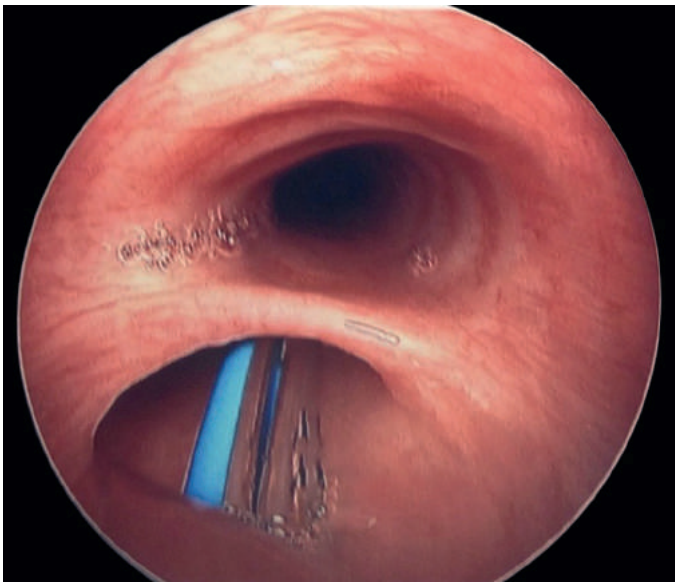
• **Fig. 61.2** Esophageal atresia with and without tracheoesophageal fistula, classified by Vogt and Gross Classification and Associated Frequency (Vogt, 1929; Gross, 1953; Spitz, 2007).



• **Fig. 61.3** Radiograph of Esophageal Atresia With Distal Tracheoesophageal Fistula.



• **Fig. 61.4** Radiograph of Pure Esophageal Atresia Without Fistula.



• **Fig. 61.5** Bronchoscopy of H-Type Tracheoesophageal Fistula.

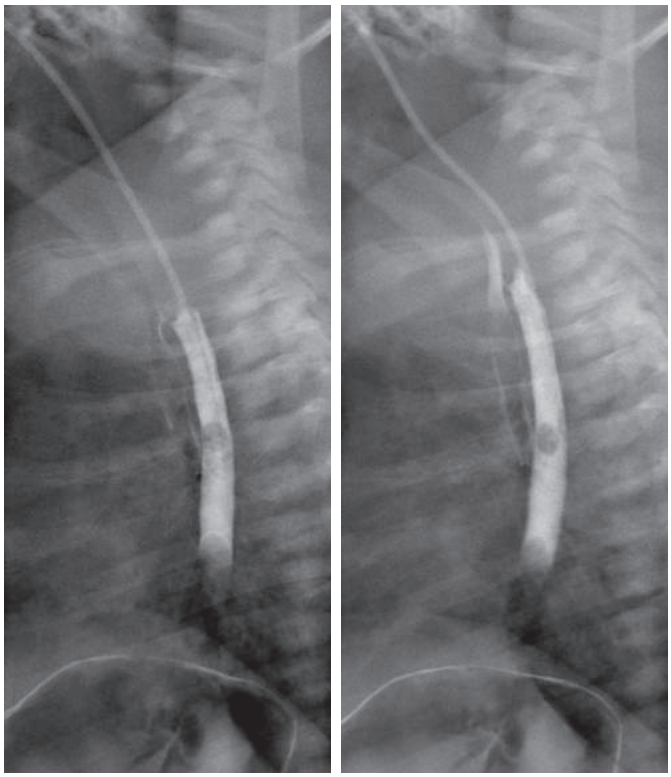
When a neonate is suspected to have esophageal atresia, because of prenatal findings or postnatal clinical symptoms such as excessive drooling, choking, or coughing with feeding, the best way to rule out the diagnosis is to pass a tube into the stomach. A nasogastric (NG) tube that does not pass easily into the stomach, and by x-ray stops or coils in the mid to upper thorax at the 2nd to 4th vertebral body level, is diagnostic of esophageal atresia (Fig. 61.3). A lateral film may demonstrate the tube posterior to the airway, and an air-filled TEF may sometimes be seen. A formal contrast study to outline the pouch is rarely necessary and carries a risk of aspiration.

When esophageal atresia is found, the presence of a distal TEF is confirmed by air in the stomach and intestinal tract (see Fig. 61.3). Absence of air in the abdomen indicates a pure esophageal atresia or an esophageal atresia with an isolated upper pouch TEF (Fig. 61.4). The diagnosis of an isolated TEF without esophageal atresia (i.e., H-type) is often not made at birth because presentation may be subtle. Symptoms can include choking and coughing with feeds and recurrent respiratory infections (caused by aspiration). The abdomen may also appear distended because of excessive air entering the GI tract from the fistula.[57] Since the esophagus is intact, a tube will pass normally into the stomach. The diagnosis may be made by bronchoscopy (Fig. 61.5) or specialized imaging studies such as a "pull-back esophagram" (Fig. 61.6) in which contrast is injected as the NG is slowly withdrawn from the stomach to better demonstrate the fistula.[57]

Management

When esophageal atresia is diagnosed or suspected, the infant should be promptly evaluated by a pediatric surgeon. In the most common situation, when a TEF to the distal esophagus is present, respiratory distress may result from aspiration of contents of the proximal pouch, aspiration of gastric contents into the trachea, loss of ventilation through the TEF, and compression of the lungs by a distended abdomen. Therefore, positive pressure ventilation should be avoided if possible, the patient positioned with the head elevated, and the proximal pouch contents evacuated with a nasoesophageal (NE) tube.

Most commonly, the infant is stable, and there is time before surgery to confirm the diagnosis and to evaluate for associated VACTERL anomalies. The most important investigation is an echocardiogram looking for cardiac defects or great vessel anomalies, which are present in 18% to 35% of cases and can influence the surgical approach.[56,58] The remainder of the VACTERL work-up including renal ultrasound and spinal imaging may be



• **Fig. 61.6** Pull-Back Esophagram.

performed after the operation. Occasionally, the inability to ventilate the infant due to leakage of tracheal air via the fistula into the GI tract forces an urgent operation to ligate the fistula or decompress the abdomen.

The primary goal of the initial operation is to divide and close the TEF. Simple ligation (tying off) of the TEF is not recommended because of the high risk of recurrence. Once the TEF is divided an end-to-end esophageal anastomosis should be performed. In unstable or premature infants, it is often safer to perform a staged repair by performing the esophageal anastomosis later, after the patient has stabilized and grown.[59] A long distance between the proximal and distal esophageal segments may also preclude anastomosis at the initial operation, although a variety of surgical approaches have been utilized in this situation.[60] If no anastomosis is performed, a gastrostomy tube should be placed if possible, at the initial operation for feeding access, until the atresia can be repaired. A chest tube is often placed to identify and evacuate any leakage from the esophageal anastomosis.

The infant should be extubated as soon as safely possible after surgery to reduce the risk of endotracheal tube and positive pressure trauma to the tracheal suture line. Similarly, deep suctioning should not be done, to avoid injury to the tracheal and esophageal repairs. Reintubation, if needed, should be performed with extreme care avoiding significant positive pressure ventilation prior to intubation. Enteral feedings may be given by gastrostomy or by an NG tube if placed across the anastomosis during surgery, or they may be delayed until the anastomosis is judged to be healed and ready for oral feeding or tube placement. A contrast study of the esophagus 7 days postoperatively is commonly performed with removal of a chest tube and commencement of oral feeds; however, practices vary.[56,61,62]

Outcomes

Overall survival from TEF is reported to be 93%, with low birth weight and the presence of major cardiac disease the most important predictors of mortality.[63] Ongoing improvements in neonatal critical care have led to greatly improved outcomes in even the smallest premature infants.[64,65]

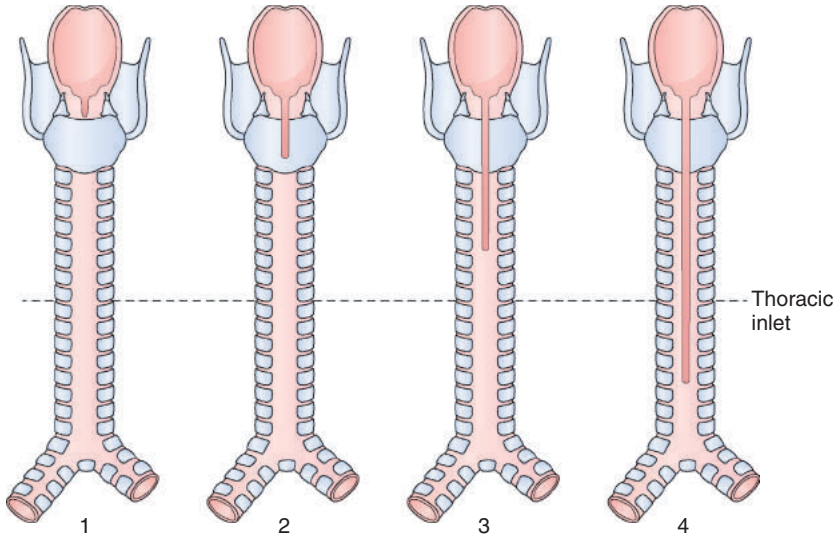
Early complications after repair of esophageal atresia include anastomotic leak and stricture. Leaks occur in up to 20% of cases although most are small, contained, and heal without another operation.[66] Leaks may be detected by observing saliva in the chest drain but are usually discovered in routine postoperative contrast studies. Stricture at the anastomosis is seen in up to one-third of cases and is more common when the anastomosis was created under tension or was complicated by a postoperative leak.[67] Clinically significant strictures usually manifest with feeding difficulty and can often be successfully treated with serial dilations.[68,69]

Patients who have esophageal atresia commonly suffer from gastroesophageal reflux (GER) and tracheomalacia.[70] GER may be more problematic in patients with esophageal atresia because of inherent dysmotility in the distal esophagus.[71] Routine use of gastric acid blockade is recommended to improve symptoms and to decrease the risk of stricture formation because of acid exposure. Many patients go on to require surgical treatment for reflux with fundoplication.[72]

Tracheomalacia is a focal or generalized structural weakness of the trachea leading to airway narrowing or collapse with increasing intrathoracic pressure (e.g., expiration). Patients may have expiratory stridor, a "barking cough," recurrent respiratory illnesses, or acute life-threatening events. The diagnosis of tracheomalacia is made by bronchoscopy, which shows airway collapse during spontaneous breathing. In severe cases, an aortopexy can effectively alleviate the condition by suspending the aorta (and thus the attached anterior wall of the trachea) to the sternum and thus pulling it away from the posterior wall of the trachea.[73,74] Similarly, posterior tracheopexy, a technique to hold and keep the posterior floppy portion of the trachea from collapsing anteriorly, has been used with success.[75]

Laryngotracheoesophageal Cleft

A laryngotracheoesophageal cleft (LC) is a rare congenital connection of variable length between the posterior larynx and trachea and the anterior esophagus resulting from failed midline fusion during development. The cleft starts proximally at the larynx and extends distally. The length of the communication can be as short as a superficial mucosal defect between the arytenoid cartilages or as long as the entire length of the trachea and even into the mainstem bronchi (Fig. 61.7).[76,77] An LC can be associated with Pallister–Hall syndrome, CHARGE syndrome, Opitz G syndrome, or VACTERL association.[78] Approximately 12% of infants with LC also have a more distal TEF[79]; however, among patients with EA/TEF, up to 20% are diagnosed with an LC.[80] Symptoms are related to the length of the connection between the esophagus and trachea and can include cough, stridor, recurrent aspiration and respiratory tract infections, reflux, swallowing difficulty, and even choking and cyanosis with feeds.[81] In long defects, there may an inability to ventilate with severe respiratory distress. It can be difficult to treat the respiratory distress because the endotracheal tube can migrate ("fall") into the esophagus after placement through the vocal cords. Diagnosis is best made by rigid bronchoscopy, which may also assist in placing the endotracheal



• **Fig. 61.7** Diagram of Laryngotracheoesophageal Cleft.

tube beyond the distal aspect of the cleft. Minor defects are often successfully treated with thickened feeds alone though may be repaired endoscopically.[82] Repairs of long defects are major, complex operations of the chest and neck that may require cardiopulmonary bypass.

Congenital Esophageal Stenosis

Congenital esophageal stenosis (CES) is rare, with an incidence of 1 in 25,000 to 1 in 50,000, and is caused by a malformation in the esophageal wall architecture.[83] The malformation may include fibromuscular thickening (53.8%), cartilage from ectopic tracheobronchial remnants in the esophageal wall (29.9%), and/or a membranous web (16.2%).[84] The majority of defects are found in the distal esophagus, most commonly 1 to 2 cm above the gastroesophageal junction. CES is classified by the Ramesh classification: type I is isolated CES, segmental type; type II is isolated CES, diaphragm type; and type III is a combined lesion.[84] CES is usually associated with other anomalies including trisomy 21, duodenal atresia, anorectal malformation, esophageal hiatal hernia, and congenital heart disease.[84] Between 25% and 50% will also have esophageal atresia.[84,85] More than half of cases are diagnosed after the neonatal period. Dysphagia and recurrent vomiting are common symptoms. Diagnosis can be reliably made with a contrast study and confirmed by endoscopy. Diagnosis is also possible in cases associated with esophageal atresia by evaluation of the distal esophagus during and after surgical repair.[83,86] Treatment is dilation or surgical resection.

Esophageal Duplication Cyst

Duplications are congenital malformations of unknown etiology that may form anywhere along the length of the developing GI tract but are most common in the small intestine. Duplications may be cystic or tubular. Esophageal duplication cysts, also known as foregut duplication cysts, are typically covered by a layer of smooth muscle and contain an epithelial lining that can comprise a hybrid histology with elements of respiratory and alimentary tissue within the same lesion.[87] The lumen of the cyst typically does not communicate with the normal foregut lumen.[86]

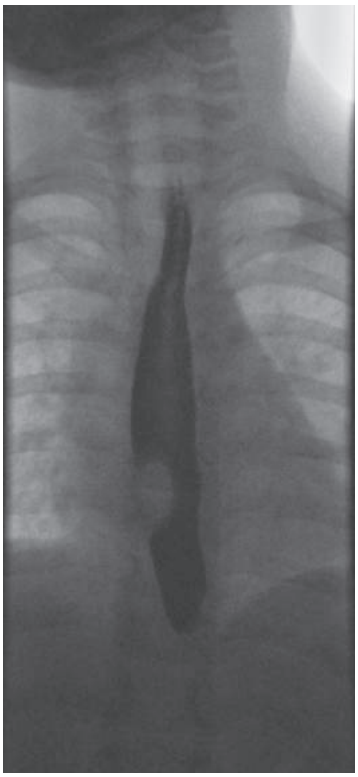
Clinical presentation is often due to compression of adjacent structures in the chest, and symptoms may include cough, wheezing, and dysphagia. Diagnosis is often made on prenatal ultrasound, although can also be made later in childhood.[84,88] Most cases have an abnormal chest x-ray because of mass effect, and contrast studies will demonstrate compression on the esophageal lumen. CT often offers better visualization of the lesions (Fig. 61.8).[89] Tc-99m pertechnetate scintigraphy can identify gastric mucosa in these lesions, and esophagram and endoscopy with or without ultrasound can also be a helpful adjunct to better delineate the lesion.[84] Treatment is via surgical resection.[90]

Esophageal Perforation

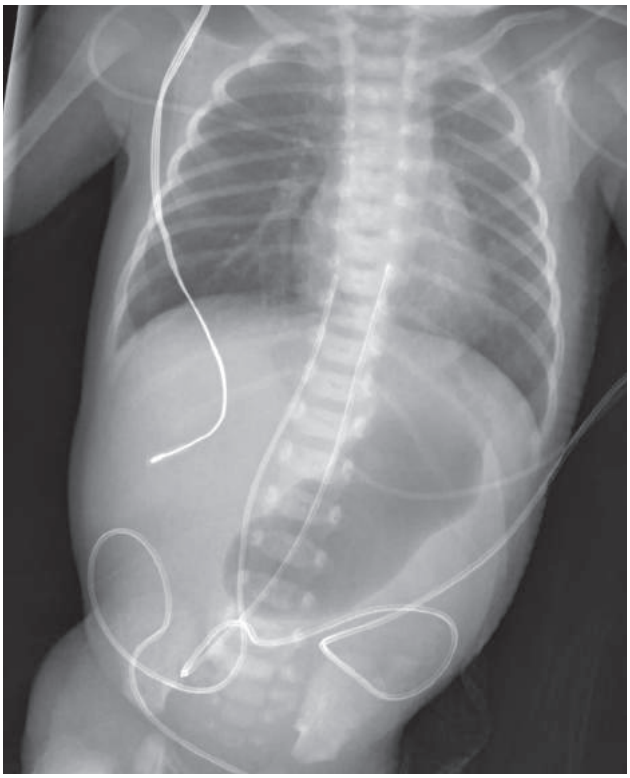
Low birth weight neonates are at higher risk for iatrogenic perforation of the esophagus.[91] In a newborn, the presentation of esophageal perforation can mimic esophageal atresia; in both, there is an inability to pass an orogastric or NG tube into the stomach.[92] When a tube passes beyond the level of the carina but does not enter the stomach, it should raise the possibility of perforation because atresias are almost always at or above the carina (see Fig. 61.8). Treatment depends on the location and severity of perforation. When the perforation is small and the leakage of esophageal contents is contained by surrounding structures in the mediastinum, it may heal without surgical repair. A follow-up contrast study to document healing is important before the initiation of feeds. When the perforation and leak of esophageal contents is larger, especially with contamination of the pleural space, then urgent surgical intervention is indicated.[93]

Disorders of the Stomach

The two congenital malformations of the stomach discussed here, pyloric atresia and gastric duplication, are quite rare and present with vomiting because of partial or complete obstruction. An acquired gastric obstruction, pyloric stenosis, is much more common and also presents with vomiting but not at birth. The last condition reviewed, neonatal gastric perforation, is an acquired condition that presents with respiratory distress, peritonitis, and sepsis.



• **Fig. 61.8** Esophagram of Esophageal Duplication Cyst.

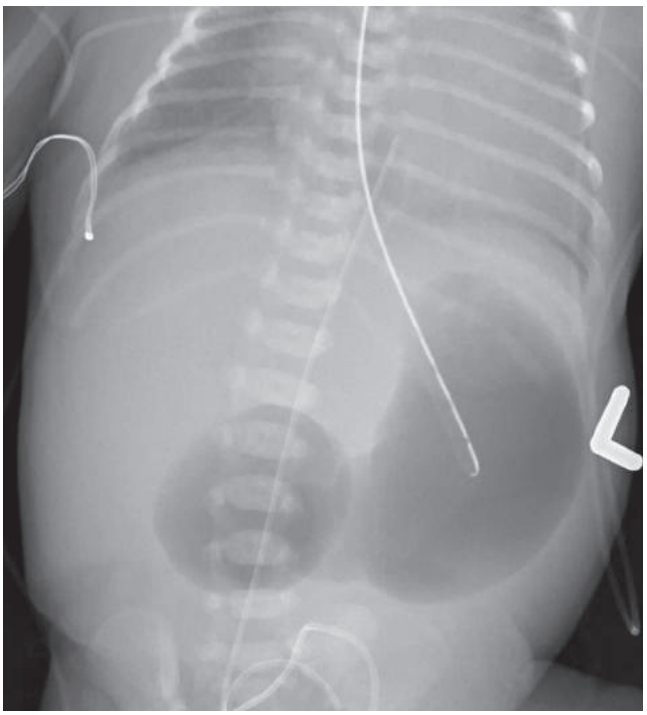


• **Fig. 61.9** Radiograph of Pyloric Atresia in the Setting of Epidermolysis Bullosa.

Pyloric Atresia

Pyloric atresia is a congenital, intrinsic, complete obstruction of the pylorus. It is rare, occurring in 1 in 100,000 live births. There are three distinct anatomic variants of pyloric atresia: (1) a simple membrane (57% of cases), (2) replacement by solid tissue (34% of cases), and (3) a complete separation of the stomach and duodenum (9% of cases).[94]

Prenatally, pyloric atresia leads to polyhydramnios and a dilated stomach, thus prenatal diagnosis is common. Nonbilious vomiting occurs with feeds after birth. Diagnosis is confirmed by abdominal radiographs showing air in a dilated stomach but no air in the GI tract distal to the stomach (Fig. 61.9). The lack of a dilated proximal duodenum on imaging studies distinguishes pyloric atresia from the more common duodenal atresia (Fig. 61.10). Babies with pyloric atresia should be evaluated for epidermolysis bullosa (EB) because of the common association between these two rare conditions.[95] The prognosis of patients with EB and pyloric atresia is poor, although survivors have been reported.[96] Surgical treatment for all forms of pyloric atresia consists of either removing or bypassing the obstruction.



• **Fig. 61.10** Radiograph of Duodenal Atresia Presenting With a "Double Bubble" Sign.

Gastric Duplication

Gastric duplications are similar to esophageal duplication cysts in that they are surrounded by smooth muscle and usually do not communicate with the lumen of the stomach. They are uncommon and comprise only 5% of abdominal duplications.

Gastric duplications are usually located in the distal stomach, and as they enlarge, they cause symptoms of progressively severe gastric outlet obstruction, with vomiting that is usually nonbilious.[88] Approximately one-third of gastric duplications are found in the neonatal period, though they are now also commonly being diagnosed on prenatal ultrasound. The diagnosis may be suspected by palpating an upper abdominal mass, though they are often asymptomatic. They can cause extrinsic compression of the distal

stomach and then be seen on a gastrointestinal series (UGI). Postnatal ultrasounds or CTs are also utilized for diagnosis. Treatment is surgical excision and is recommended even in cases discovered incidentally since most will eventually cause symptoms of gastric outlet obstruction.[88]

Pyloric Stenosis

Pyloric stenosis (or hypertrophic pyloric stenosis [HPS]) is an acquired disorder of hypertrophy of the pyloric muscle at the distal end of the stomach. This hypertrophy narrows the gastric outlet and leads to progressively severe, nonbilious vomiting.

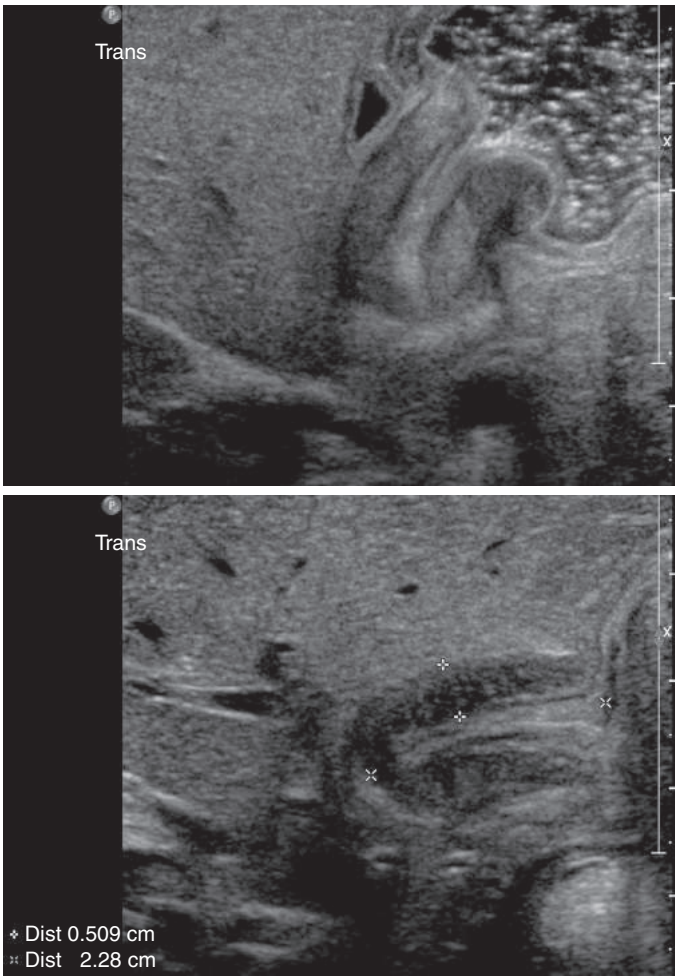
Pyloric stenosis is one of the most common structural problems of the GI tract in infants, with an incidence of 2 to 5 in 1000 live births in the Western population. It occurs almost exclusively between weeks 3 to 8 of life. It is four to five times more common in males than in females, and white infants are more commonly affected than Black or Asian infants. There is a decline in risk with increasing birth order.[97]

The etiology of HPS is unknown. Genetic factors must have a role since there is an increased risk within families: for example, if a baby has pyloric stenosis then the identical twin has an almost 25% chance of having the condition while a nontwin sibling has a 5.8% chance.[97] HPS also occurs more frequently than expected in infants with certain syndromes.[98] Exposure to erythromycin and prostaglandins may also increase the risk of developing HPS.[99] In addition, cesarean section delivery, preterm birth, and bottle-feeding have been found to be associated HPS.[100]

Infants with HPS present with a history of vomiting feedings and gastric contents, similar to patients with reflux or milk protein allergy. Because the pyloric hypertrophy and degree of gastric outlet obstruction are progressive, the vomiting is also progressively more severe. Unlike intestinal obstructions that are distal to the bile duct insertion in the duodenum, the vomiting in HPS is nonbilious, and the loss of gastric contents undiluted by bile and pancreatic secretions leads to a hypochloremic, hypokalemic metabolic alkalosis. The metabolic alkalosis and hypochloremia is due to a loss of hydrogen and chloride ions (HCl) from vomiting gastric contents. The emesis also results in a decrease in extracellular volume, causing contraction alkalosis which stimulates the renin angiotensin aldosterone pathway to reabsorb sodium in exchange for potassium, thereby causing hypokalemia.

The physical examination of a baby with pyloric stenosis may be notable for dehydration, and in some cases, the hypertrophied pylorus may be palpable as a firm, mobile upper abdominal mass known as the pyloric "olive." The diagnosis is usually confirmed with imaging, most commonly an abdominal ultrasound. Ultrasound findings of pyloric stenosis include a pyloric muscle thickness greater than 3 mm and a length greater than 15 mm (Fig. 61.11).[101] Upper GI contrast radiographs can also confirm the diagnosis of pyloric stenosis if ultrasound is not available or uncertain.

After diagnosis, the first step in treatment is to correct intravascular volume depletion as this will permit the correction of any associated hypokalemia and alkalosis. The metabolic alkalosis should be corrected before surgery to prevent alkalosis-induced apnea after general anesthesia. Preoperative protocols for resuscitation and normalization of electrolytes have been published.[102] It may take a day or more to optimize intravascular volume, acid-base balance, and serum electrolytes. To limit the risk of aspiration, the stomach may be evacuated with an NG tube from the time of admission and with a larger red rubber catheter immediately before induction of anesthesia.



• **Fig. 61.11** Ultrasound of Hypertrophic Pyloric Stenosis.

The operative treatment of pyloric stenosis is to incise and split the pyloric muscle layer completely so that the submucosa is exposed from the duodenum to the gastric antrum. This pyloromyotomy can be achieved by an open or laparoscopic approach. The operation is remarkably effective so that feedings can be resumed soon after surgery, and discharge is usually possible within a day or two. Atropine as an alternative to pyloromyotomy has been described but is less effective.[103]

The outcomes of modern management of pyloric stenosis are excellent. Mortality is extremely rare, and there is only a small risk of surgical site infection or wound problems. Other uncommon problems after pyloromyotomy are (1) peritonitis and sepsis from an unrecognized full-thickness mucosal injury and (2) an incomplete myotomy, which can lead to recurrent vomiting (diagnosed by UGI as repeat ultrasound will show postoperative changes). After pyloromyotomy, the pylorus eventually heals, remodels, and appears normal. There are no functional abnormalities of the stomach in the long term.

Gastric Perforation

Similar to esophageal perforations, low birth weight infants are thought to be at higher risk for catheter-associated gastric perforations than term babies. In the absence of obvious trauma, usually from placement of a gastric tube, rupture of the stomach is called "spontaneous," although possible risk factors such as

prematurity, duodenal atresia, mechanical ventilation, or neonatal hypoxia are often identified.[104,105] The mechanism of spontaneous gastric perforation is not known, but it appears to be distinct from necrotizing enterocolitis (NEC) as NEC typically spares the stomach, and the gross and microscopic appearance seen in NEC and spontaneous intestinal perforation are not seen in spontaneous perforations of the stomach.[106] Gastric perforations usually present with large amounts of free air on abdominal x-rays. While decreasing over time, the associated mortality with this condition is still significant.[107] Emergent surgical repair is indicated.

Disorders of the Intestine

Disorders of the intestine reviewed here include intestinal malrotation and midgut volvulus, intestinal atresias, enteric duplications, intussusception, and meconium ileus. These conditions present with intestinal obstruction that may be complicated by intestinal ischemia, necrosis, bleeding, perforation, and/or sepsis. The clinical presentations of these conditions and many other GI tract disorders are usually nonspecific and consist of some combination of signs and symptoms such as pain, feeding intolerance, vomiting, lack of stooling, tenderness, abdominal distention, or mass.

A careful history and physical examination often provide clues to the diagnosis. Pain and tenderness may be caused by visceral distention and/or peritoneal irritation. Typically, nonbilious vomiting is seen with obstruction proximal to the ampulla of Vater (the site of bile drainage into the duodenum), while bilious vomiting results from a more distal obstruction. Abdominal distention in infants is usually the result of diffusely dilated loops of bowel caused by either a functional ileus (intestinal stasis with the accumulation of swallowed air, feedings, and GI secretions) or a true distal obstruction. More-proximal obstructions of the stomach and duodenum usually do not cause significant abdominal distention. Failure to pass meconium in the first 24 hours of life should raise concern for distal GI obstruction.

A clinical concern for intestinal obstruction requires simultaneous supportive care and diagnostic evaluation. Feedings should be stopped, and if there is abdominal distention or signs of intestinal distention on imaging studies, then gastric decompression via an NG or orogastric tube should be done. Intravenous fluids should be given to meet maintenance needs, replace any deficits, and account for any ongoing losses. Antibiotics should be administered when there is a concern for infection.

A directed abdominal examination should be performed to evaluate for distention, tenderness, localized abdominal edema and erythema, hernias, and masses. Abdominal radiographs can help with diagnosis but are rarely definitive. The characteristic imaging findings of specific disorders will be reviewed below. If there are concerns for intestinal obstruction, then a surgical consultation is advised.

Malrotation and Volvulus

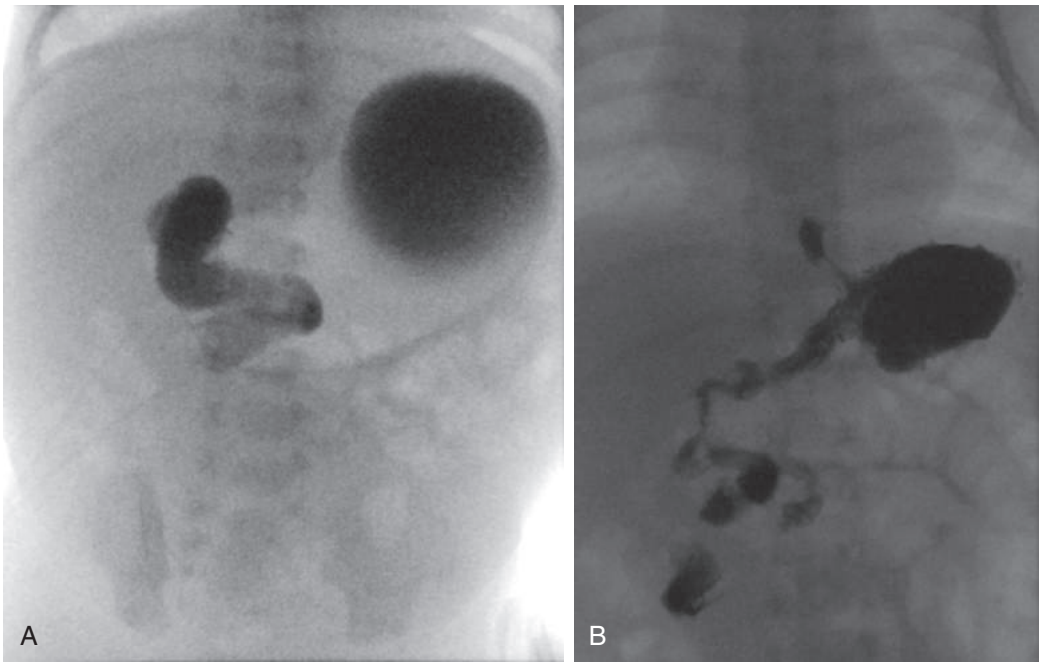
Normal intestinal rotation occurs when the midgut that is herniated outside the abdominal cavity into the umbilical cord early in development returns to the abdominal cavity during weeks 10 to 12 of gestation and fixes to the retroperitoneum in a precise pattern. Normal intestinal rotation results in (1) the duodenum being fixed in the retroperitoneum around the pancreas and transitioning to the intraperitoneal jejunum (visible as the ligament of Treitz) at a point to the left of the spine and above the level of the duodenal bulb, (2) all the small bowel loops floating free in the abdomen, (3) the cecum and ascending colon being attached to the right-lateral posterior body wall up to the hepatic flexure, (4) the descending colon attached to the left-lateral body wall from the splenic flexure to the sigmoid colon, and (5) the blood supply to the entire midgut (jejunum, ileum, and right and transverse colon) attached to the retroperitoneum in a wide pedicle from the ligament of Treitz in the left upper quadrant of the abdomen to the cecum in the right lower quadrant. While a wide spectrum of anatomic abnormalities exists (as a group known as intestinal rotation anomalies),[108] we will focus on classic intestinal malrotation where the duodenum does not cross over the spine, and the other normal attachments to the retroperitoneum do not form, resulting in a narrow mesenteric vascular pedicle. This narrow vascular pedicle supports most of the small and large bowel and is prone to twist which then obstructs blood flow and is called midgut volvulus. Intestinal malrotation may also be accompanied by abnormal retroperitoneal attachments, known as Ladd bands, which can cross the second portion of the duodenum and cause points of obstruction on their own in the absence of volvulus.

The true prevalence of malrotation is difficult to determine for several reasons including symptomatic presentation throughout life and a few children (and even adults) discovered to have asymptomatic variants during imaging studies or operations. Presumably, the more severe anatomic abnormalities are more prone to volvulus and present earlier in life, although life-threatening volvulus can occur in adults.[109] It is believed most malrotation cases, however, present in infancy. Malrotation may occur alone, but it is a regular component of gastroschisis, omphalocele, and congenital diaphragmatic hernia and may accompany intestinal atresia, intussusception (i.e., Waugh syndrome), heterotaxy, and various cardiac defects.

Newborns and infants with midgut volvulus initially present with bilious vomiting, since the twist and resulting intestinal obstruction occur distal to the ampulla of Vater. Early on, there may be abdominal pain, but abdominal tenderness and distention may also be absent. As the twist of the mesentery progresses, the blood supply to the bowel is compromised, and intestinal ischemia and eventually intestinal necrosis and peritonitis set in with resulting pain, tenderness, guarding, abdominal distention, and, eventually, signs of sepsis. Abdominal x-rays may show a gasless abdomen or distended bowel loops, but may also appear normal. A normal appearing x-ray does not rule out volvulus. Furthermore, laboratory studies may also be normal until intestinal ischemia peritonitis and sepsis develop.

Since the entire midgut is potentially at risk for vascular compromise with volvulus, it is essential to make the diagnosis at the onset of symptoms and signs of obstruction before symptoms and signs of intestinal ischemia develop. Midgut volvulus with prolonged ischemia can be a catastrophe either resulting in patient death or leaving a patient with insufficient bowel length to grow without supplemental parental nutrition (i.e., short bowel syndrome). Since early diagnosis is critical, any newborn or infant with bilious vomiting should be considered to have malrotation with volvulus until proven otherwise, and an urgent upper GI series should be obtained. In malrotation, the ligament of Treitz will fail to cross midline to its normal position at the left-sided vertebral body pedicle and will be lower (in the cephalad–caudal axis) than the level of the duodenal bulb (Fig. 61.12). The duodenum should follow a typical path toward the retroperitoneum prior to the transition to the jejunum.

Other imaging findings suggesting malrotation and volvulus include an abnormal position of the cecum by contrast enema



• **Fig. 61.12** (A and B) Upper gastrointestinal series showing malrotation with and without obstruction/volvulus.

and abnormal relationship of the mesenteric vessels by ultrasound. Malrotation can also be diagnosed by CT scan, but CT is rarely indicated in newborns. It is important to emphasize that volvulus in this setting can be associated with a relatively normal-appearing plain film of the abdomen, and thus a high degree of suspicion must be maintained when clinical findings are suggestive.

Midgut volvulus is a surgical emergency. While laparoscopic approaches have been described and may be useful for the evaluation of asymptomatic malrotation, in the setting of acute symptoms an open laparotomy is indicated.[110] The operation of choice is a Ladd procedure that consists of (1) untwisting the volvulized bowel, (2) dividing abnormal bands, (3) broadening of the mesentery, and (4) arranging the bowel so that the midgut mesentery is flat in the retroperitoneum, with the proximal small bowel on the right side of the abdomen and the distal ileum and cecum in the left upper quadrant. The appendix is usually removed since the operation renders its final location unpredictable and diagnosis of appendicitis later in life more difficult (in the developed world with access to modern imaging, the utility of this step is debated). When there is intestinal necrosis, the nonviable bowel is excised. When bowel viability is questionable, "second look" procedures may be performed after a temporary abdominal closure. In neonates with congenital heart defects and asymptomatic malrotation, the American Pediatric Surgery Association (APSA) recommends either observation or postponing the Ladd procedure until after palliation of the congenital heart defect.[108]

Intestinal Atresia

Intestinal atresia is a complete congenital obstruction of the bowel lumen. Atresia is an intrinsic malformation of the bowel and is not a result of extrinsic compression. Esophageal and pyloric atresias are discussed in other sections of this chapter. Duodenal atresia and anorectal atresia are considered separately since each has unique embryology, risk factors, and associated conditions. Jejunal, ileal, and colon atresias, however, have many common features and are considered together here. The process leading to atresia may be incomplete and, in this case, is more correctly called intestinal "stenosis," but the presentation, evaluation, and management are similar, so we will consolidate the discussion of atresias and stenosis together.

Duodenal Atresia

There are several anatomic variations of duodenal atresia including membranous webs, simple occlusions, complete separations of proximal and distal segments, and obstruction associated with annular pancreas, in which abnormal fusion of the ventral and dorsal pancreas lead to the encirclement of the second portion of the duodenum and complete or incomplete obstruction. The exact nature of the developmental abnormality in duodenal atresia is unknown but may be due to a failure of recanalization of the lumen.[111]

The prevalence of duodenal atresia is approximately 1 in 5000 births; however, many studies evaluate all intestinal atresias rather than duodenal atresia specifically.[112] Duodenal atresia is frequently associated with other anomalies including trisomy 21, VACTERL association, cardiac anomalies, intestinal malrotation, anorectal malformations, and biliary tract anomalies, which have important implications for evaluation and management.[113]

Duodenal atresia may be suspected prenatally when other associated anomalies or polyhydramnios are present and by an ultrasound showing a "double bubble." [114] Symptomatic newborns present with vomiting. The vomiting is usually, but not always, bilious since the obstruction is typically distal to the ampulla of Vater. An abdominal x-ray showing a double bubble with no air in the distal GI tract confirms the diagnosis of duodenal atresia (see Fig. 61.10). Duodenal atresia has a characteristic radiographic appearance with a bulbous rounded appearance. Intestinal malrotation and volvulus with complete proximal obstruction in a

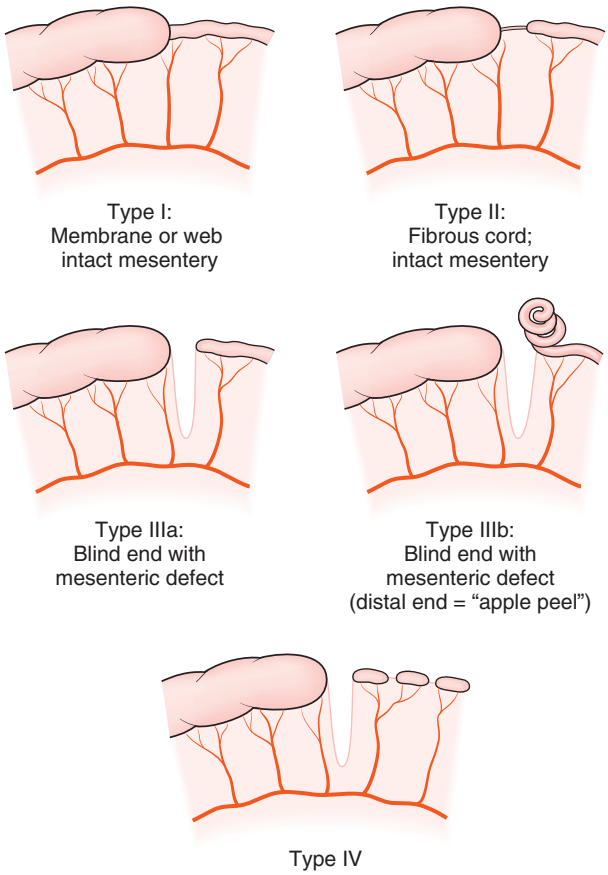
newborn may have an x-ray showing obstruction but usually without typical rounded features of duodenal atresia. If unsure, urgent surgical exploration is warranted. An x-ray with a double bubble sign and air present in the distal GI tract indicates the infant may have duodenal stenosis, but malrotation and volvulus must remain a concern. An upper GI study may be helpful in such cases.

After the diagnosis, initial management includes gastric decompression, fluid resuscitation, and correction of electrolyte abnormalities. Surgical treatment is a bypass of the obstruction with a duodenoduodenostomy. Laparoscopic and open repairs have both been shown to have excellent outcomes; however, gestational age and weight play a role in patient selection.[115] While once a concern, there is now known to be a low incidence of associated intestinal atresias and it is not necessary to "rule out" a distal atresia at the time of laparoscopic repair.[116] Early complications include anastomotic leak and stricture. Potential long-term problems include poor motility of the stomach and duodenum with or without associated megaduodenum.[117]

Jejunoileal and Colonic Atresia

Jejunoileal and colonic atresias are classified based on the anatomic abnormality (Fig. 61.13). The etiology of jejunoileal and colon atresia is thought to be due to a prenatal event associated with mesenteric vascular compromise, for example in utero volvulus. Genetic factors and defective fibroblast growth factor signaling may also play a role in some cases.[111]

The prevalence of small bowel intestinal atresias is approximately 1.6 per 10,000 live births.[118] About one-third of patients with jejunoileal atresia are premature and associated anomalies are less likely, especially compared with patients with duodenal atresia.[119]



• **Fig. 61.13** Types of Intestinal Atresias.

Prenatal ultrasound may show dilated loops of bowel with other loops decompressed. After birth, neonates with jejunoileal and colon atresia present with bilious vomiting, abdominal distention, and failure or delayed passage of meconium; the more proximal the atresia, the earlier the expected onset of signs and symptoms. Abdominal radiographs show dilated loops of bowel with air-fluid levels and an absence of gas in the rectum. After abdominal x-rays, the next step in the evaluation of possible intestinal atresia is typically a contrast enema, which will help differentiate intestinal atresia from meconium ileus, Hirschsprung disease (HD), and small left colon syndrome.[119] A limited upper GI series is sometimes performed as well to confirm normal intestinal rotation and rule out occult volvulus. Contrast enema is helpful to demonstrate the anatomy and patency of the colon, which is important for intraoperative decision-making during which the small bowel is more easily visualized and assessed than the colon.

With atresia, initial management is gastric decompression and fluid resuscitation. An operation is then performed to repair the atresia. The proximal limb of an atresia is typically more dilated than the distal limb, and sometimes significantly so. This creates a technical problem for an end-to-end anastomosis of the bowel in which one end is significantly larger than the other, but also a concern that this chronically dilated segment of bowel may never regain adequate function, leading to stasis and other problems of motility. If the intestine can be spared, it may be removed. When there is a concern for remaining bowel length, the dilated segment may be tapered rather than resected. The distal bowel may be of sufficient length or significantly compromised, raising the possibility for intestinal failure even after correction of the atresia. Early results and long-term outcomes of the operative repair of jejunoileal and colon atresia are good; however, they depend heavily on the total remaining bowel length.[120] Significant loss of bowel can result in short bowel syndrome and in long-term dependence on parenteral nutrition.

Newborns with colonic atresia present similarly to those with more proximal atresia; however, because the obstruction is more distal in the GI tract, the presentation is often delayed until the second or third day of life. Abdominal radiographs show dilated distal bowel and may show a very dilated colon if the ileocecal valve prevents air from refluxing back into the distal ileum. Treatment is similar to jejunoileal atresia, with laparotomy, resection of the dilated colon, and anastomosis. Timely diagnosis and treatment are essential and are associated with a generally excellent outcome, while late diagnosis and treatment (over 72 hours from birth) increase the risk of morbidity and mortality.[121] Colonic atresia can be associated with HD and this should be considered in all cases.[122]

Meconium Ileus

Meconium ileus is an intraluminal obstruction of the terminal ileum by abnormal meconium. Cystic fibrosis (CF) accounts for 90% of patients presenting with meconium ileus, and up to 20% of patients with CF present as newborns with meconium ileus. CF is caused by mutations in the CF transmembrane regulator (CFTR) that alter bicarbonate and chloride transport. These intraluminal fluid changes result in characteristic thickened meconium that adheres to the bowel wall and causes bowel obstruction. Meconium ileus may be "uncomplicated" and result only in bowel obstruction, or it may be "complicated" when the obstruction

leads to twisting and ischemia that may cause ileal atresia and/or intestinal perforation.[123] The term "meconium peritonitis" is a general description of the result of any in utero perforation with meconium spillage into the peritoneal cavity, whether that perforation is caused by meconium ileus or another problem.

Prenatal diagnosis of CF may be suspected when there is a family history of CF or when the parents are carriers of CFTR mutations. CF diagnosis can be confirmed by fetal DNA analysis and, while neither sensitive nor specific, prenatal ultrasound findings of hyperechoic bowel, bowel dilation, and inability to identify the gallbladder have all been associated with meconium ileus.[124]

The newborn clinical presentation of meconium ileus is similar to a jejunoileal atresia with abdominal distention 12 to 24 hours after birth, vomiting, and failure to pass meconium.[125] Abdominal x-rays show dilated loops of bowel suggesting distal intestinal obstruction; however, the appearance is often distinct from other types of bowel obstruction, showing "soap bubbles" in the bowel rather than air-fluid levels. If intrauterine perforation has occurred, abdominal x-rays may show intraabdominal calcifications. When a perforation results in a contained meconium cyst, abdominal distention is often present at the time of birth (rather than several hours later). Abdominal distention at birth should raise immediate concern for this possibility.

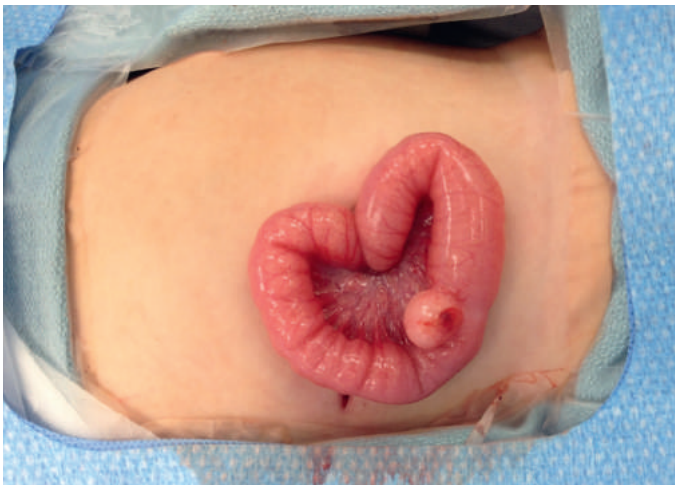
Initial management of babies with meconium ileus is the same as any neonate with bowel obstruction and includes intravenous fluids, correction of electrolyte abnormalities, and gastric decompression. A contrast enema can confirm the diagnosis and offers the chance of nonoperative management of uncomplicated meconium ileus. Instilling water-soluble contrast around and into the abnormal meconium softens it and encourages it to pass out per rectum, thus relieving the obstruction.[124] This can be repeated multiple times in order to relieve the obstruction if the patient is stable and progress is being made.[126] Hyperosmolar solutions have been advocated to draw fluid into the bowel lumen, but they carry the risk of potentially dangerous intravascular fluid shifts. The administration of N-acetylcysteine into the proximal or distal bowel lumen has also been used to help break up the abnormal meconium and relieve the obstruction. Failure of nonoperative measures or the presence of a complicated meconium ileus are indications for surgical intervention.

Operative management of uncomplicated meconium ileus involves laparotomy, opening the small bowel proximal to the obstruction, and irrigation with saline or N-acetylcysteine until the obstructing meconium is cleared. The bowel can often be closed primarily, although sometimes tubes are placed to allow additional irrigation after surgery or ostomies for decompression and potential for distal irrigation. By definition, complicated meconium ileus requires an operation. The goals of surgery are resection of compromised bowel and repair of atresias, often by primary anastomosis, although at times temporary diverting stomas are used.

Long-term GI outcomes after nonoperative or operative management of uncomplicated meconium ileus are generally quite good, although pancreatic insufficiency due to CF, if present, requires lifelong medical management. Any patient diagnosed with meconium ileus must undergo work-up and testing for CF.

Enteric Duplication Cysts

Enteric duplication cysts, often referred to simply as duplications, can occur anywhere in the alimentary tract from mouth to anus.[90,127] Duplications can be cystic or tubular and consist of an inner lining of GI epithelium and an outer layer of smooth muscle. Most duplications occur in the small intestine, especially the distal ileum, and are often on the mesenteric side of the lumen (Fig. 61.14).[128]



• **Fig. 61.14** Intraoperative Photo of a Duplication Cyst.

The overall prevalence of duplications is poorly defined, but a cumulative incidence for all sites and all types is probably around 1 in 4000 to 5000, although any specific duplication type or location is rare. Various theories exist to explain the embryologic etiology for enteric duplications; however, the split notochord theory is generally accepted.[129]

The clinical presentation of an enteric duplication depends on three factors: (1) its location, (2) its relative mass effect, and (3) complications relating to secretions of the involved epithelium.[88] Esophageal and gastric duplication cysts are described in other sections. Only about 4% to 5% of enteric duplications are in the duodenum.[130,131] Duodenal duplications may be connected to biliary or pancreatic ducts. Clinical presentations include asymptomatic masses, GI obstruction, and pancreatitis.[131,132] Jejunal and ileal duplications often present with bowel obstruction or intussusception, sometimes related to segmental volvulus from the weight of the cyst. Colonic and rectal duplications are rare but may present with obstruction or volvulus.[90]

Diagnosis of enteric duplication cysts is made with ultrasound and/or contrast studies, which may show a filling defect. Ultrasound can be used prenatally, leading to early treatment postnatally.[133] CT and MRI may be used to define the location and size of the duplication. Treatment is typically surgical excision; however, the specific location and length of involvement may require an individualized approach.

Intussusception

Intussusception is the telescoping of one portion of bowel into another, usually involving the distal ileum and ascending colon, with a lead point (mesenteric lymph node, Meckel diverticulum, etc.) dragging more proximal bowel into distal bowel. This is a significant source of obstruction and abdominal pain in older infants and toddlers, but it is also a rare cause of intestinal obstruction in neonates.[134] The presenting symptoms of feeding intolerance, vomiting, abdominal distention, and blood in the stool are nonspecific and can delay diagnosis and treatment.[135]

The diagnosis may be made by ultrasound or contrast enema or may be made at the time of operation for intestinal obstruction. Treatment is surgical reduction with resection of bowel if needed, though radiologic enema reduction may be considered in select patients as is standard therapy in older patients.

Disorders of the Colon and Anus

Neonatal Appendicitis

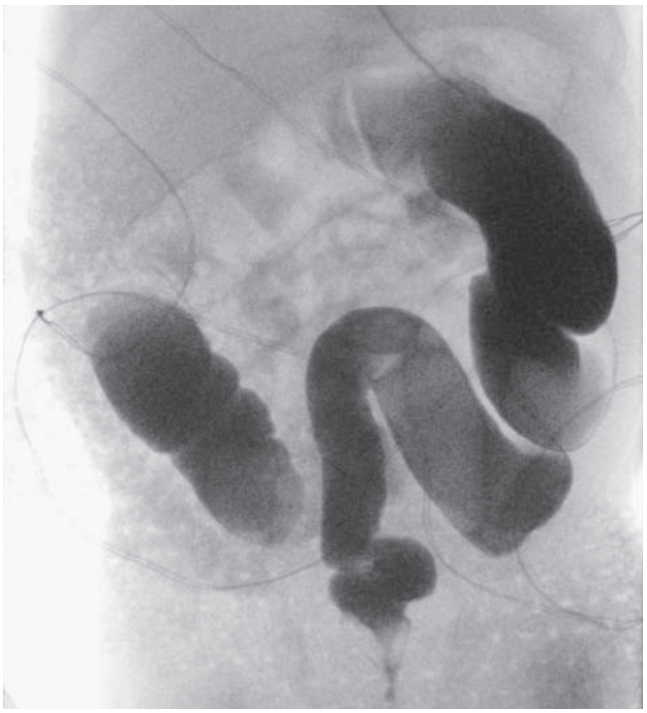
While one of the most common surgical conditions in children, neonatal appendicitis is rare.[136] The clinical presentation usually includes vomiting, abdominal distention and tenderness, and fever.[137] It is unusual to make the diagnosis in the neonate before an operation is performed for peritonitis because of appendiceal perforation. A possible association with HD has been discussed in the literature, and consideration should be given to a suction rectal biopsy in neonates who present with appendicitis.[138] The appendix itself is not a good marker for ganglion cells, and the presence of abnormal ganglion cells or an aganglionic appendix is not diagnostic of HD by itself.

Hirschsprung Disease

HD is characterized by the absence of intrinsic parasympathetic ganglion cells in the submucosal and myenteric plexuses. The condition is caused by premature arrest of the normal craniocaudal migration of neural crest cells in the bowel wall. A segment of abnormal, aganglionic bowel thus exists, extending from the anal canal proximally to the point of arrested migration. HD most commonly involves the rectum and sigmoid colon; however, up to 10% of cases involve the entire colon (i.e., total colon aganglionosis) even extending into the small bowel.[139,140] Aganglionic colon has impaired motility and results in a functional distal bowel obstruction of variable severity. Often depending on the length of involvement, it may present as an intestinal obstruction in the newborn or as intractable constipation in older infants and children if the segment is short. HD may be complicated by enterocolitis, which can cause a clinical picture of marked abdominal distention, pain, tenderness, and systemic manifestations of sepsis. The cause of enterocolitis is unknown; however, it can occur before or after HD is surgically corrected.

The prevalence of HD is estimated to be 1 per 5000 live births. Males are more frequently affected than females (4:1) and Asians, African Americans, and Caucasians (2.8, 2.1, and 1.5 per 10,000 live births, respectively) are more commonly affected than Hispanics (1.0 per 10,000 live births). HD may be an isolated problem; however, almost 20% of cases have chromosomal abnormalities. Although, abnormalities of many genes have been implicated in HD, in most patients the genetic abnormalities have not been defined.[139]

When HD presents in a newborn, the clinical picture is usually of distal bowel obstruction with feeding intolerance, vomiting, and abdominal distention. The diagnosis may be suspected when no meconium is passed in the first 24 hours after birth. An abdominal radiograph may show dilated loops of bowel and a paucity of rectal gas. A characteristic contrast enema will show an abnormally small rectum relative to a larger sigmoid colon, with a calculated rectosigmoid ratio <1 (Fig. 61.15). Diagnosis is confirmed by suction rectal biopsies, which show hypertrophic nerve trunks and a lack of ganglion cells.[141] Suction rectal biopsies cause minimal discomfort and may be performed without sedation at the bedside in the NICU.



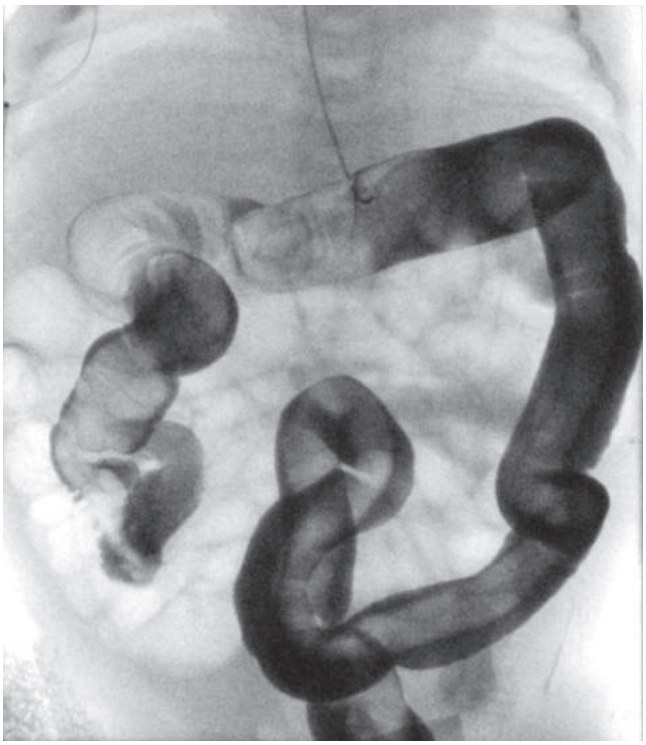
• **Fig. 61.15** Contrast Enema of Hirschsprung Disease.

The surgical treatment of HD involves excision of the abnormally innervated distal colon and a "pull-through" of the normally innervated proximal bowel that is connected to the preserved distal rectum, just above the anal canal. Surgery may be done in multiple stages, with an initial ostomy and then a definitive pull-through done later, or the procedure may be done in a single stage. Surgical approaches include laparoscopic, open, completely via a transanal approach, or with some combination of approaches.

Potential operative complications include anastomotic leak or stricture or damage to nearby structures, including components of the anal sphincter. If the dysfunctional bowel is not completely excised, obstructive symptoms may persist. The functional outcome for any one patient will not be known for years when the child reaches the age of toilet training, but in general, most patients can be expected to achieve social continence and have a good quality of life.[142] It is important to remember that HD is not cured by surgery (every operation leaves behind an abnormally innervated anal sphincter), but a well-done operation can provide excellent palliation, especially with long-term follow-up and management by appropriate specialists.

Meconium Plug

Meconium plug syndrome is the intraluminal obstruction of the colon by abnormal meconium. This contrasts with the intraluminal obstruction of the terminal ileum in meconium ileus. Infants with meconium plug present with delayed passage of meconium, abdominal distention, and perhaps vomiting.[143] Meconium plug is diagnosed by recognition of the passage of abnormal meconium after rectal stimulation, irrigation, or contrast enema (Fig. 61.16). Contrast enema usually resolves the obstruction, and few infants need surgical intervention. Neonates with meconium



• **Fig. 61.16** Contrast Enema of Meconium Plug.

plug syndrome require further evaluation for possible underlying CF or HD.

Anorectal Malformations

Anorectal malformations (ARMs) compromise a spectrum of congenital anomalies in which the anus is either absent or abnormally located outside the normal sphincter muscles. ARMs are often described as "high" or "low" based on where the rectum ends or is suspected to end (e.g., perineum, urethra, or bladder) and relative to the levator muscle component of the anal sphincter muscle complex. However, describing the precise anatomical abnormalities is more valuable and predictive of clinical outcomes (e.g., anorectal malformation with rectoprostatic urethral fistula).[144]

The embryology of ARMs is incompletely understood but results from a disturbance of the complex coordination of the distal GI tract (the hindgut), distal genitourinary structures (especially the bladder, urethra, and vagina), and the musculoskeletal components of the pelvis, spine, and perineum.[145]

The prevalence of ARMs is about 1 in 5000 live births. Associated anomalies are common. Most notable are those of the VACTERL association. In a large multicenter review, of the 4962 children who underwent ARM repair, 31% had vertebral anomalies, 40% had congenital heart disease, 7% had esophageal atresia (EA)/TEF, 34% had genitourinary anomalies, and 7% had limb defects. Thirty-six percent had three or more defined anomalies and thus met the criteria for VACTERL diagnosis.[50] ARMs can also be associated with malrotation, HD, and trisomy 21.[146,147]

Prenatal diagnosis of ARMs is uncommon. After birth, the diagnosis of an ARM is first and foremost dependent upon a good physical examination. Imaging studies are not necessary for initial diagnosis; however, they can assist in determining the level of defect and the existence of fistula. The normal anus is typically halfway between the coccyx and the scrotum or vaginal orifice, and a perineal fistula is typically located anterior to the normal position of the anus and partially or completely outside the sphincter muscle complex. If no anal opening is seen (anal atresia), there may be a blind-ending rectum without a fistula, or the rectum may connect to the urethra, bladder, or inside the vestibule of the vagina (with true rectovaginal fistulae being rare).

The initial management of patients with ARMs includes supportive care for bowel obstruction, evaluation for possible associated anomalies in the VACTERL spectrum, and relief of the obstruction either with dilation of an existing opening or through an operation to create a colostomy. Gastric decompression and intravenous fluids are needed until the bowel obstruction is relieved and enteral feedings are possible. The evaluation for associated anomalies includes a detailed physical examination and imaging studies including echocardiogram, spine radiographs, and renal and spinal ultrasound.[148]

If no anal opening of fistula is present to allow passage of meconium, newborns require surgery for the creation of a colostomy in the first 48 hours of life. Once a colostomy is created, surgical correction of the malformation is often planned for several months later, with the colostomy closed in a staged fashion after this reconstruction has healed. The goal of surgical repair of ARMs is to separate the rectum from other structures and place it into the middle of the muscle sphincter complex. The posterior sagittal anorectoplasty, first described by De Vries and Pena in 1982, is the most common reconstructive procedure performed.[149]

The outcomes of ARMs are related to the severity of the anatomic abnormality and to the presence and severity of associated anomalies. Similar to HD, the surgery for ARMs is more palliative than curative, and functional outcomes take years to accurately assess. Prognosis is suggested by the type of malformation and associated anomalies. A neonate with a perineal fistula and normal sacrum and spine is more likely to achieve voluntary continence than a patient with a rectourethral fistula with sacral agenesis. However, children with even the most severe malformations can still achieve social continence with the help of a long-term bowel management program.

Acknowledgment

This chapter is dedicated to Daniel J. Ledbetter, MD, greatly missed mentor, teacher, and friend.

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62

Abdominal Wall Defects

SHILPI CHABRA, JAMIE E. ANDERSON, AND PATRICK J. JAVID

KEY POINTS

- Gastroschisis is a congenital abdominal wall defect located just to the right of the umbilicus. The bowel that herniates out of the abdomen is not covered by a membrane.
- Fetuses and newborns with a gastroschisis have a low risk of associated anomalies, except for a 10% to 25% risk of intestinal atresia.
- The clinical outcomes of patients with gastroschisis are mainly determined by their intestinal function.
- Surgical options for gastroschisis closure include primary repair via surgical or sutureless bedside approach or delayed repair with temporary silo placement.
- Omphalocele is a congenital midline abdominal wall defect of the umbilical region. The bowel, liver, and other organs that herniate out of the abdomen are covered by a membrane. The umbilical cord inserts into the omphalocele membrane.
- Fetuses and newborns with an omphalocele have a high risk of associated anomalies, especially chromosomal abnormalities and cardiac malformations.
- The clinical outcomes of patients with omphalocele are mainly determined by their associated anomalies.
- Surgical options for omphalocele repair include primary repair or a delayed "paint-and-wait" approach with topical agents that epithelialize the membrane, followed by fascial closure months or years later.

Abdominal Wall Problems

This chapter discusses many of the congenital and acquired abdominal wall problems seen in newborns. It concentrates on gastroschisis and omphalocele, the two most common congenital abdominal wall defects seen in the neonatal intensive care unit (NICU), followed by a review of umbilical cord abnormalities, abdominal wall hernias, and less common congenital abdominal wall defects.

Abdominal Wall Defects

Gastroschisis and omphalocele are the two most common congenital abdominal wall defects that present with herniation of abdominal contents outside the body, requiring reduction of the organs into the abdominal cavity and closure of the abdominal wall defect. However, gastroschisis and omphalocele are distinct conditions with important differences in anatomy and associated conditions that account for their unique management and outcomes (Fig. 62.1).

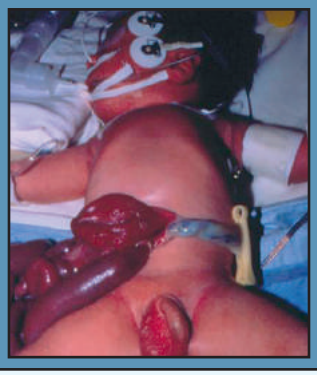
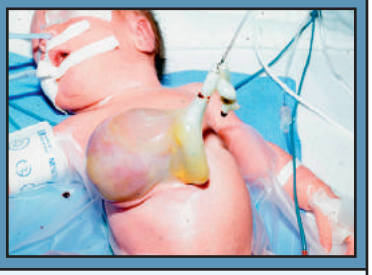
Gastroschisis

Gastroschisis is a congenital periumbilical abdominal wall defect that is typically located to the right of a normally inserted umbilical cord. The defect results in evisceration of a variable amount of intestine and other abdominal organs outside the abdominal cavity. There is no covering membrane or peritoneum, and the intestine is exposed directly to amniotic fluid prenatally and to the environment after birth (Fig. 62.2). Due to the exposure to amniotic fluid and potential ischemic constriction, there may be secondary bowel damage with thickening and edema (matted bowel) which is covered with fibrinous exudate, often termed the "peel" or "rind." It is unusual for a significant amount of liver to herniate out of a gastroschisis defect. Rarely, the gastroschisis defect is in a mirror image position on the left side of the umbilicus. Left-side gastroschisis has a worse prognosis and is associated with intestinal atresia and extra-intestinal anomalies such as situs inversus, cardiac defects, cerebral arterio-venous malformations, macrocephaly, and scoliosis.[1]

Gastroschisis may be classified as either simple or complicated/complex based on the condition of the bowel and association with intestinal pathologies (atresia, perforation, necrosis, or volvulus). Gastroschisis is considered "simple" when the bowel appears healthy with no intestinal complications and the herniated contents can be easily reduced into the abdominal cavity without underlying anatomic concerns and little post-closure complications. "Complicated" or "complex" gastroschisis includes cases in which there is an associated intestinal atresia, segmental or midgut volvulus, ischemic bowel, intestinal perforation, or the subsequent development of necrotizing enterocolitis.

Complicated gastroschisis has a 7.6× higher mortality than simple gastroschisis.[2] Associated atresias may not be identified initially as the matted bowel can be difficult to fully assess during initial closure. Volvulus, intestinal perforation, or necrotizing enterocolitis may also occur within a few weeks and sometimes up to a few months after closure. In this way, simple gastroschisis at birth can become complicated gastroschisis during the early infant period.

Vanishing or "closing" gastroschisis is a form of complex gastroschisis, in which the gastroschisis fascial defect closes in utero and strangulates the bowel (Fig. 62.3). Babies with vanishing gastroschisis may have little or no viable bowel outside the abdomen and can experience intestinal failure from short bowel syndrome. A new classification scheme for vanishing, or closing, gastroschisis identifies four subgroups[3]:

	GASTROSCHISIS	OMPHALOCELE
		
Location of abdominal wall defect	Right of umbilical cord	Midline, includes umbilical cord
Covering membrane	No	Yes
Umbilical cord insertion	Normal	Into omphalocele membrane
Herniated organs		
Bowel	Always	Common
Liver	Uncommon for more than an edge to be out	Common for large amount of liver to be out
Associated anomalies		
Chromosomal	Rare	Common
Syndromes	Rare	Common
Cardiac	Uncommon, 2%–5%	Common
Bowel atresia	10%–25%	Rare
Major determinant of clinical outcome	Condition and function of bowel	Associated anomalies

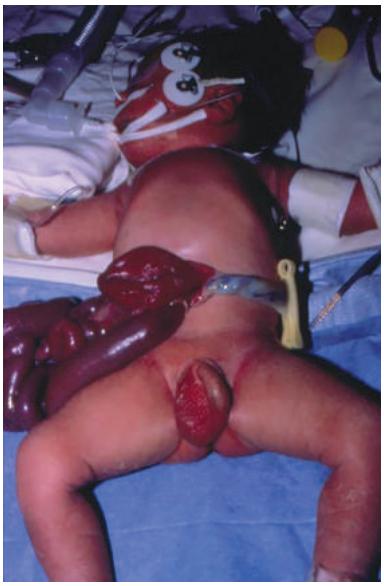
• **Fig. 62.1** Gastroschisis Versus Omphalocele.

- Type A (15%): ischemic bowel constricted at the ring but without atresia
- Type B (51%): intestinal atresia with a mass of ischemic, but viable, external bowel
- Type C (26%): closing ring with nonviable external bowel with or without atresia
- Type D (8%) completely closed defect with either a nubbin of exposed tissue or no external bowel.

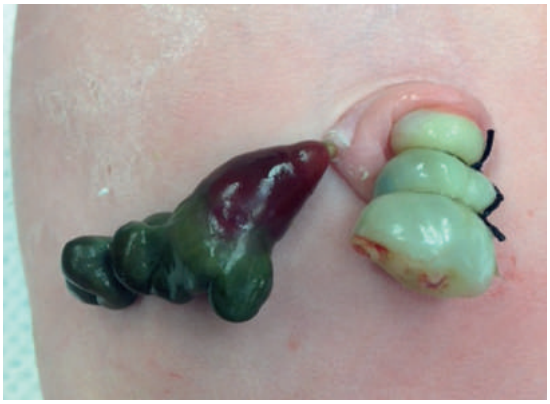
Epidemiology

The prevalence of gastroschisis in the United States is 4.3 per 10,000 live births.[4] However, in Europe it is reported to be lower at 2.0 per 10,000 births ranging from as low as 0.7 per 10,000 births in Italy to 5.5 per 10,000 births in England.[5] There is no gender predilection in gastroschisis, but the incidence is higher in Hispanic and non-Hispanic white families.[6] For unknown reasons, the incidence of gastroschisis has been reported to be increasing. In the United States, there was an increase in incidence from 4.49 per 10,000 live births in 2004 to 2006 to 5.12 per 10,000 live births in 2010 to 2014,[6] and another recent national inpatient study also reported rising incidence from 4.5 to 4.9/10,000 live births from 2010 to 2014.[7]

The most consistent risk factor for gastroschisis is young maternal age. Mothers under the age of 25 years have a several fold increased risk of carrying a baby with gastroschisis,[4,8–10] and teen fathers have double the risk compared to fathers over 25 years.[11] Numerous other maternal factors have been linked with gastroschisis, including short interpregnancy interval, prior pregnancy loss in mothers younger than age 20, illicit drug use (cocaine, amphetamines), alcohol consumption, prescription opioid use, low body mass index, cigarette smoking, anti-depressants, unmarried status, maternal nativity, and maternal genitourinary infections during gestation.[12–18] Population-based studies also show



• **Fig. 62.2** Gastroschisis Prior to Repair.



• **Fig. 62.3** Vanishing Gastroschisis. Note that the abdominal wall defect has closed.

a higher incidence of gastroschisis in areas where the common agricultural chemical atrazine is used and surface water concentrations of atrazine are elevated.[19,20] However, a more recent study found no correlation of increased risk of gastroschisis in agricultural counties.[21]

Pathophysiology

The embryology of gastroschisis is not completely understood. It occurs early in gestation and although there are rare family case reports, there is no known specific genetic etiology. Its association with young mothers is suggestive of a gene-environment interaction affecting fetuses with either a genetic predisposition or those exposed to unidentified exogenous factors. Pathologically, gastroschisis is a primary midline malformation involving the umbilical ring, with herniation of the intestines (without a covering sac) mostly to the right of the umbilical cord.[22–24] Whether this represents a failure of closure of the primordial umbilical ring before the physiological hernia returns to the abdomen or a rupture of the covering membrane at the ring's edge remains unclear. One theory is that gastroschisis occurs due to a ruptured physiologic hernia and the associated bowel injury is explained by a mesenteric insult.[25]

Unlike omphalocele, extraintestinal anomalies are not common in gastroschisis, although congenital heart disease was reported in 4% of babies with gastroschisis.[26] Anomalies of the intestine are the most common associated malformations in patients with gastroschisis. Nearly all babies with gastroschisis have intestinal malrotation since the bowel is not able to return to the abdominal cavity during fetal development and has no chance for normal rotation and fixation to the retroperitoneum. Intestinal atresia occurs in 10% to 25% of babies with gastroschisis.[27,28]

Clinical Presentation

With routine prenatal ultrasound, about 90% of gastroschisis cases are diagnosed early in pregnancy. Prenatal ultrasound shows extra-abdominal loops of bowel without a covering sac. The maternal alpha fetoprotein level is also elevated.[29] Currently there are no options for fetal intervention, but amnio exchange and fetal repair are being studied as possible future options.[30,31]

Fetuses with gastroschisis have a significant risk of intrauterine growth restriction (IUGR), spontaneous preterm labor, and intrauterine fetal death (IUFD).[32–37] IUGR is a frequent indication for preterm delivery in fetal gastroschisis; however, ultrasound has a low accuracy for diagnosing small for gestational age (SGA) status at birth[38] and the standard formula (that uses the abdominal circumference to calculate fetal growth) has been reported to underestimate the fetal weight.[39] The incidence of IUFD with fetal gastroschisis is approximately 4.5% to 5.2%, which is higher than the reported 2.8% in uncomplicated pregnancies. The mechanisms for sudden fetal death are unclear and possibly related to oligohydramnios, cord compression, vascular compromise, or volvulus.[40,41] Genetic testing of the fetus with gastroschisis is not routinely performed due to lack of specific genetic etiology.

Management

Babies with gastroschisis are best cared for by a multidisciplinary team of maternal fetal medicine specialists, neonatologists, and pediatric surgeons. Prenatal consultation with this group is strongly recommended. Rigorous prenatal surveillance with ultrasounds every 1 to 3 weeks is recommended starting at 24 weeks to evaluate the bowel anatomy, fetal growth, and amniotic fluid volume. Twice weekly nonstress testing (NST) is recommended starting at 32 weeks.

The timing of delivery in gastroschisis remains controversial. Planned preterm delivery is believed by some to reduce IUFD and postnatal complications, but there are no high-quality data to validate this approach, and retrospective studies have conflicting results.[42,43] Two randomized controlled trials failed to show any benefit of elective preterm delivery;[44,45] however, a systematic review reported benefits with decreased neonatal sepsis.[46] A study evaluating the risk of IUFD and neonatal death concluded that mortality can be minimized with delivery at 37 weeks,[41] while another study reported delivery at 38 weeks was associated with decreased risk of stillbirth and a minimal increase in neonatal respiratory morbidity.[47] A literature review concluded that elective delivery at less than 37 weeks is generally not indicated, and delivery should be scheduled between 37 and 38 weeks for uncomplicated gastroschisis.[43] The argument against planned preterm delivery is bolstered by contemporary outcome studies that

consistently demonstrate that preterm delivery is a major source of adverse neonatal outcomes in gastroschisis,[33,48–50] and IUFD did not increase after 35 weeks.[40] Neonates with gastroschisis delivered at less than 37 weeks had longer hospital stays;[51] however, there were no differences in neonatal outcomes between planned delivery at 36 to 37 weeks or at ≥38 weeks.[52] Many centers now advocate for delivery close to term.

There is a stronger consensus regarding vaginal delivery for patients with gastroschisis. Cesarean section seems to offer no benefit to the baby or the mother[53–55] and increases the risk of neonatal respiratory distress.[56] A recent metaanalysis found no difference with elective cesarean section in neonatal mortality or morbidity such as sepsis, short gut syndrome, and duration of hospital stay.[57] Cesarean section is thus not recommended solely for gastroschisis and the delivery mode should be on standard obstetrical indications.[43,58]

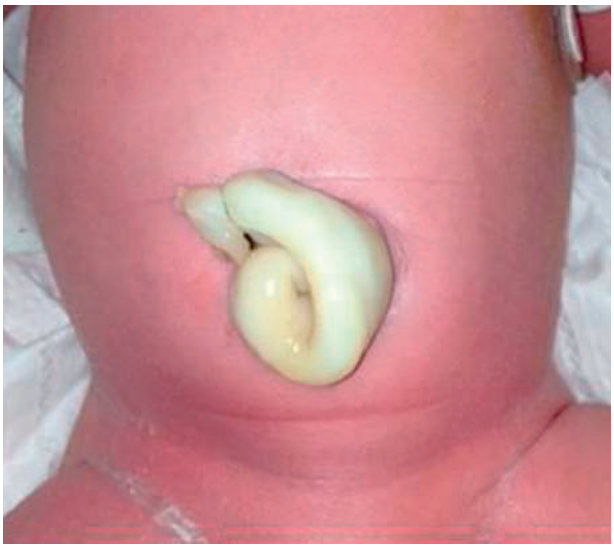
At birth, the umbilical cord should be kept long and clamped at least 30 cm from the baby to preserve the option of primary umbilical closure. Neonatal resuscitation should follow standard protocols, except for placing the baby's lower body, from the nipples to the feet, in a clear plastic bag as a temporary covering over the exposed bowel to minimize evaporative heat and fluid loss. After initial newborn resuscitation measures are performed, the perfusion of the herniated intestinal contents should be carefully evaluated. If bowel ischemia or infarction are suspected, bowel detorsion or emergency enlargement of the gastroschisis defect by a surgeon may need to be performed. An oro- or nasogastric tube is placed to suction to empty the stomach. The baby should be kept warm and dry.

After initial resuscitation and stabilization, the patient is urgently transported to a NICU with pediatric surgical consultation. In the NICU, a peripheral intravenous line is inserted to start intravenous fluids and broad-spectrum antibiotics. Endotracheal intubation is not required unless indicated for respiratory support. Noninvasive positive pressure ventilation is generally avoided to prevent distension of the eviscerated bowel.[59] The baby should be placed with the right side angled slightly down to prevent kinking of the mesentery and maximize blood flow to the bowel. The oro- or nasogastric tube should remain on suction. Bowel perfusion should be visually monitored through the clear plastic bag; opening the bowel bag should be avoided until surgical assessment to avoid further contamination of the bowel.

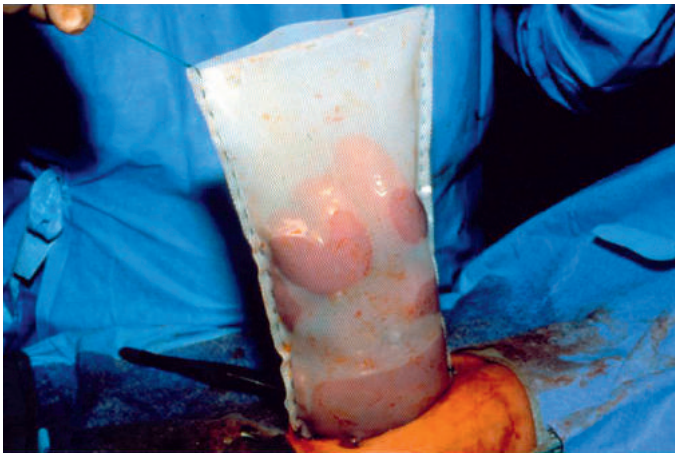
The goal of surgical repair in gastroschisis is safe reduction of the eviscerated contents and closure of the abdominal wall. Multiple surgical options exist to accomplish this goal, and both primary repair and staged repair are acceptable options.

Sutureless abdominal wall closure in gastroschisis uses the patient's own umbilical cord as a biologic dressing to seal the gastroschisis defect without attempting a primary fascial closure.[60,61] The cord is left long at delivery, and the eviscerated contents are reduced into the abdominal cavity while monitoring intra-abdominal pressure. The fascial defect is covered with the coiled umbilical cord that is secured into place with a bandage (Fig. 62.4). In most cases, the gastroschisis defect contracts and the skin heals beneath and around the cord within 14 days. Using sutureless closure, some children will have a persistent umbilical hernia that can be repaired electively at 3 to 4 years of age.[62]

Sutured abdominal wall closure of gastroschisis is now less commonly performed. Sutured closure involves closing the fascial defect with sutures in the operating room under general anesthesia. Intra-gastric or bladder pressure is monitored to ensure the closure does not cause abdominal compartment syndrome. In some cases, a prosthetic patch is required to close the defect. An umbilicoplasty is then performed.



• **Fig. 62.4** Gastroschisis Repair Using Sutureless Umbilical Cord Closure Prior to Application of Dressing.



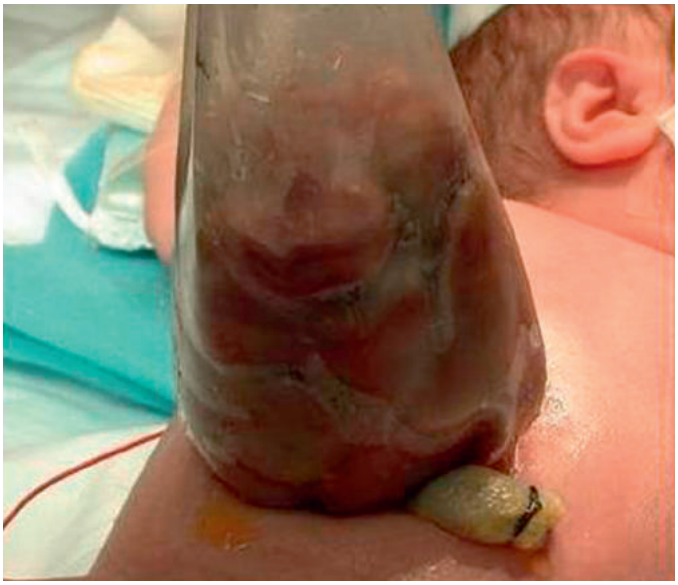
• **Fig. 62.5** Gastroschisis With Hand-Sewn Silo.

Staged repairs with initial silo coverage of gastroschisis are an accepted surgical strategy that entails delayed reduction of the herniated bowel and closure of the abdominal wall. Silos may be created from silastic or other plastic sheeting and sewn circumferentially to the fascial edges of the gastroschisis defect (Fig. 62.5). Another option is to use a pre-made, spring-loaded silastic silo that is manufactured in different diameters and can be inserted into the gastroschisis defect at the bedside (Fig. 62.6). Once a silo is placed, the bowel gradually reduces over several days into the abdominal cavity by either gravity alone or gentle external pressure. When the reduction takes more than 7 to 10 days, the risk of silo dehiscence from the abdominal wall increases. Delayed sutured abdominal wall closure is performed when the abdominal contents have almost completely reduced into the abdominal cavity. There is also the option of using the sutureless umbilical cord closure technique in a delayed fashion after the bowel has reduced in the silo if the cord is kept moist with dressings. The delayed approach requires close monitoring of the bowel appearance through the silo.

Despite many retrospective studies, database analyses, and meta-analyses, it is unclear which surgical approach is best for any individual baby with gastroschisis.[62–71] Many studies suffer from significant selection bias and do not separately analyze patients with complex gastroschisis, thereby making outcome comparisons difficult. Two randomized, prospective studies to evaluate primary versus delayed fascial closure have not found significant outcome differences between techniques.[72,73] A meta-analysis suggests that delayed primary repair may be associated with improved outcome measures when studies with selection bias are eliminated from the analysis.[74]

The choice of primary fascial closure versus delayed repair using a silo may be less relevant in the era of sutureless abdominal wall closure. Primary sutureless umbilical cord closure is successful in many babies with gastroschisis, may be performed at the bedside, does not always require general anesthesia, and reduces duration of mechanical ventilation.[66,75] In our experience, sutureless umbilical closure is associated with shorter NICU stays and shorter time to initiation of enteral nutrition,[76] as well as decreased hospital costs, ventilator days, and time to goal enteral feeding[77] compared to delayed closure with a silo. One retrospective case control study showed a decrease in mechanical ventilation of 2.8 days and reduction in need for general anesthesia in patients with sutureless versus sutured repair.[78] A multicenter cohort study by the Midwest Pediatric Surgery Consortium reported that sutureless closure was associated with less general anesthetics, antibiotic use, surgical site infections, and decreased ventilator time.[79] In contrast, a single-institution randomized controlled trial comparing sutureless versus sutured primary closure in simple gastroschisis found increased time to full feeds and discharge among patients with primary sutureless closure.[71] Multicenter randomized clinical trials are needed to determine the potential advantages of the sutureless approach.

The gastroschisis prognosis score (GPS) is a validated bedside visual bowel-injury-scoring tool performed after birth. It is helpful for distinguishing low from high morbidity groups and assists in initial counselling.[33] Babies with uncomplicated gastroschisis have average hospital stays of 4 to 6 weeks, which is largely due to their inability to tolerate full enteral feedings. Babies with complicated gastroschisis often have much longer hospital stays.



• **Fig. 62.6** Gastroschisis With Premanufactured Silo.

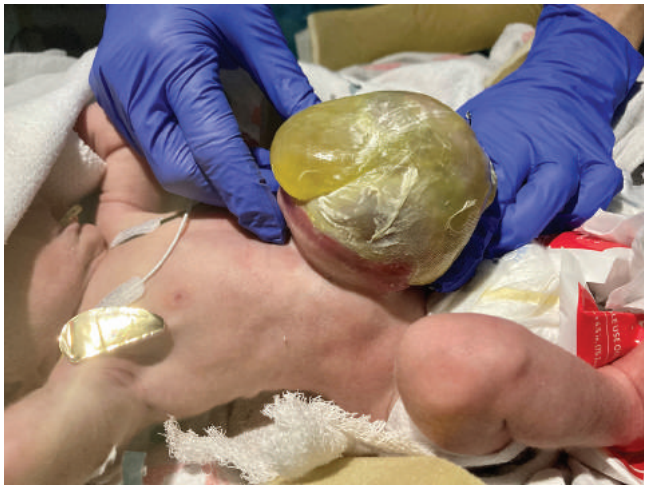
Outcomes

Outcomes for children born with gastroschisis in high-income countries are excellent. Survival of greater than 95% can be expected in cases of uncomplicated gastroschisis. Infants with complex gastroschisis are at higher risk for overall mortality (2%), need for assisted ventilation (95%), bowel resection (10%), necrotizing enterocolitis (5%), sepsis (9%) and need for home parenteral nutrition after discharge (2%).[3,80] While only a small minority of babies with gastroschisis will need extensive bowel resection, gastroschisis remains a leading etiology of short bowel syndrome in most series of children with intestinal failure.[81,82] In resource-limited settings, mortality remains very high, primarily due to lack of access to total parenteral nutrition, which is required when awaiting return of bowel function.[83] Targeted interventions such as improving prenatal diagnosis and management protocols for earlier enteral feeding, adequate vascular access, and fluid resuscitation have demonstrated improved outcomes in resource-limited settings.[84,85]

Long-term outcomes in gastroschisis are favorable. Most patients will have normal gastrointestinal function and neurodevelopmental outcomes.[86,87] Even though gastroschisis is almost always associated with intestinal malrotation, the risk of midgut volvulus later in life is low (reported at 1%), probably because intra-abdominal adhesions limit the ability of the bowel to twist.[88] These patients may develop hernias at the site of repair. Finally, although boys with gastroschisis commonly have undescended testicles, about 50% of patients will undergo spontaneous testicular descent and not require an operation.

Omphalocele

An omphalocele (known as exomphalos in the United Kingdom) is a midline abdominal wall defect with herniation of bowel and possibly liver and other organs outside the abdomen. Although there is no consensus definition of giant omphalocele, it is often described as omphaloceles with fascial defects greater than 5 cm in diameter or those containing large amounts of liver.[89] The herniated contents are covered with a membrane consisting of peritoneum on the inside, amnion on the outside, and Wharton's jelly between those two layers. The umbilical cord inserts into the membrane rather than the abdominal wall (Fig. 62.7). The



• **Fig. 62.7** Omphalocele.

omphalocele membrane is usually intact, but it occasionally ruptures, resulting in exposure of the herniated visceral contents.

A ruptured omphalocele can be difficult to differentiate from gastroschisis, especially during the prenatal period. Until recently, both omphalocele and gastroschisis shared the same International Classification of Diseases (ICD) code; however, they have since been separated when we submitted a proposal to change the two ICD codes.[90] Ultrasound findings that favor the diagnosis of gastroschisis include a relatively small abdominal wall defect (usually less than 4 cm in diameter), the absence of covering, no liver protruding outside the body wall, and an umbilical cord that is normally inserted into the body wall just to the left of the defect.

Some authors make a distinction between an omphalocele and a hernia of the umbilical cord. In a hernia of the umbilical cord, the umbilical ring is reportedly normal. Since there is only an open umbilical ring and no deficiency of abdominal wall, the surgical repair is much easier. More importantly, unlike newborns with omphaloceles, newborns with a hernia of the umbilical cord reportedly have a low risk of associated anomalies. The clinical differentiation can be difficult, and some physicians refer to a hernia of the umbilical cord as a small omphalocele.[91]

Epidemiology

The incidence of omphalocele is estimated to be 1.5 to 3 per 10,000 births.[4,5,92] The incidence of omphalocele has been stable over time, unlike the incidence of gastroschisis, which is increasing.[92,93] Most cases of omphalocele are sporadic and often associated with chromosomal anomalies, but there are rare familial occurrences.[94]

Risk factors for omphalocele include a maternal age less than 20 years or greater than 40 years, and maternal obesity.[4,95] In contrast to the relatively low risk of associated anomalies in babies with gastroschisis, babies with omphalocele are twice as likely as those with gastroschisis to present with other birth defects. These associated anomalies play a major role in how patients are managed and in their eventual outcome.[96] Chromosomal anomalies such as trisomy 13 and 18 are common, especially in fetuses diagnosed early in gestation.[92,97] In addition, other congenital malformations are frequent, especially congenital heart disease, which may occur in up to 50% of newborns with omphalocele.[93] Finally, omphalocele is a component of several syndromes of congenital malformations, including Beckwith-Wiedemann syndrome, which occurs in 6% of newborns with an omphalocele. Up to 10% to 20% of newborns thought to have an isolated omphalocele have Beckwith-Wiedemann syndrome on prenatal evaluation.[98]

Pathophysiology

Normal development of the abdomen involves the flat, three-layered embryonic disk folding from top, bottom, and both sides to meet at the umbilical ring to form the cylindrical torso during the fourth to fifth week of gestation. The gastrointestinal tract begins to grow very rapidly early in the first trimester, and due to lack of room in the abdominal cavity there is a physiologic bowel herniation through the umbilical ring into the umbilical cord. By 10 to 12 weeks, the bowel returns back into the abdomen in a precise pattern of rotation and fixation to its final position. The embryology of omphalocele is still not clear, and this malformation is thought to occur due to incomplete abdominal wall folding and failure of the intestinal tract to return from the umbilical cord.[99,100] Currently, omphalocele is thought to occur due to the embryonic dysplasia combined with malfunction of the ectodermal placodes.[101]

Clinical Presentation

The prenatal diagnosis of omphalocele may be suspected when maternal serum α-fetoprotein levels are elevated or when there is fetal aneuploidy. The definitive diagnosis is made by prenatal ultrasound. The prenatal ultrasound findings of omphalocele are abdominal organs herniated outside the abdominal cavity and covered with a membrane, and an abnormal insertion of the umbilical cord into the membrane rather than into the abdominal wall.[97] Prenatal ultrasound diagnosis of omphalocele can be reliably made after the first trimester when the bowel returns to the abdominal cavity. The finding of liver outside the abdomen potentially allows an earlier and more accurate diagnosis of omphalocele.[102] It is also important to note the presence or absence of liver within the omphalocele since the presence of liver is associated with a higher risk of other anomalies.[97]

Management

The prenatal management of omphalocele includes evaluation for associated anomalies with imaging for structural anomalies and monitoring of fetal growth because of the risk of IUGR. Because of the high risk of congenital heart disease, fetal echocardiography is indicated. Other specific evaluations for associated pulmonary hypoplasia and the size of the defect, such as fetal MRI, are recommended to provide improved prenatal counseling about the expected hospital course and the long-term prognosis.[103,104]

Obstetric care including the timing and method of delivery is usually determined by traditional maternal and fetal factors rather than by the presence of the omphalocele. Specifically, there is usually no benefit (and there is potential harm) with preterm delivery[105] due to lung immaturity in the setting of possible pulmonary hypoplasia. Cesarean section is indicated only for most fetuses with large defects containing liver (i.e., giant omphaloceles) and is commonly performed in this scenario[55,106] to avoid inadvertent rupture of the sac.

After delivery, the initial evaluation and resuscitation of a baby with an omphalocele follows the same priorities as for all newborns. During the initial resuscitation, a newborn with an omphalocele should be handled carefully to prevent the omphalocele membrane from tearing. After the initial resuscitation, the omphalocele should be inspected to confirm that it is intact and then covered with a nonadherent dressing or bowel bag to protect the sac.

Newborns with omphaloceles, especially large omphaloceles, may have respiratory insufficiency caused by pulmonary hypoplasia and pulmonary hypertension,[107] and they may require respiratory support.[108] In addition, those babies with associated Beckwith-Wiedemann syndrome may have hypoglycemia and require supplemental glucose. An early evaluation for possible associated anomalies, especially congenital heart disease, is required to diagnose conditions that may need further treatment.

The goal of operative treatment of omphalocele is to reduce the herniated organs back into abdomen and close the abdominal wall. Surgical closure of an omphalocele is not an emergency if the omphalocele membrane remains intact. This allows time for the evaluation of associated anomalies and supportive treatment of co-morbidities. If the omphalocele membrane ruptures, it can

sometimes be repaired using suture at the bedside.[109] When a ruptured omphalocele cannot be repaired, the care of the patient and the abdominal wall defect follows a pathway more similar to the care of newborns with a large gastroschisis, although surgical closure is usually more complicated, and both mortality and morbidity are high.[110]

Decisions regarding initial operative versus nonoperative management of the omphalocele depend on whether the membrane is ruptured, the size of the omphalocele including the eviscerated contents, and co-morbidities. When the omphalocele is relatively small and the baby is otherwise stable, an early operation with excision of the omphalocele membrane, reduction of the herniated organs, closure of the abdominal wall muscles, and umbilicoplasty is performed. When the defect is too large to safely reduce the viscera and close the abdominal wall, or if the baby has significant comorbidities, the repair can be staged using a "paint-and-wait" approach if the membrane is intact, or silo placement with delayed surgical repair if the membrane is ruptured.

The "paint-and-wait" delayed approach is accomplished by treating the intact omphalocele membrane with daily application of topical agents that allow the sac to epithelialize, followed by delayed surgical repair months later.[111] A variety of topical agents have been used to treat the omphalocele sac. The agents most commonly used in the United States are silver sulfadiazine cream and povidone iodine solution.[89,112–114] As the sac opacifies and eventually epithelializes, the baby continues to grow with adequate caloric intake. Caloric goals in neonates with omphalocele can generally be reached with enteral nutrition and oral feeds. Prolonged need for parenteral nutrition is rare with uncomplicated omphalocele. The growth of the abdominal cavity allows the herniated abdominal organs to reduce back into the abdomen with gravity alone or with mild external compression. Lung growth over time will also improve the baby's ability to tolerate an operation and an abdominal closure. The eventual repair of the abdominal wall can be done several months or even years later.[115] The "paint-and-wait" approach is especially useful in the management of giant omphaloceles when there is no reasonable chance for early reduction, and in the management of smaller omphalocele in patients with significant congenital cardiac or other disease that precludes an upfront surgical repair.

If the membrane is ruptured, unable to be repaired, and the defect is very large, a staged surgical repair can be performed. The staged surgical repair involves initial silo placement followed by serial reduction of the silo, and finally closure of the abdominal wall. This strategy is almost identical to the staged approach often used for gastroschisis.[111] The early, staged approach is not always successful if the abdominal defect is large and a significant amount of herniated contents need to be reduced into a small abdominal cavity. In addition, reducing the abdominal contents may impair ventilation which can be a significant problem in the setting of underlying pulmonary insufficiency.[116]

Outcomes

The survival and long-term outcomes of newborns with omphaloceles are mainly determined by the severity of associated anomalies.[92,117] After the repair of omphalocele, volvulus occurs in up to 4% of omphalocele cases.[88] Long-term morbidity includes feeding difficulties, failure to thrive, gastroesophageal reflux disease, inguinal hernias, chronic lung disease, and neurodevelopmental and motor delays.[118,119] Babies with giant omphaloceles have increased mortality and morbidity because of the large abdominal wall defect and associated pulmonary hypoplasia and pulmonary hypertension.[103,106,120] Overall survival for liveborn omphalocele infants has improved over the last few decades, and 1-year survival rates have been reported as high as 90% in cases of isolated omphalocele.[92]

Other Abdominal Wall Defects

Body Stalk Anomaly

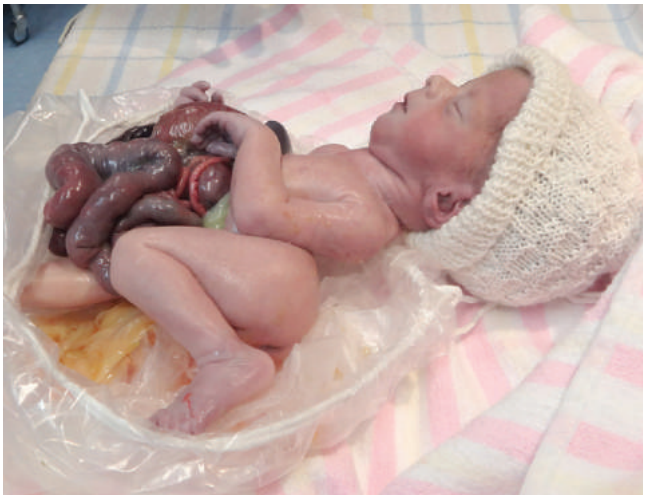
Body stalk anomaly (BSA), also known as the limb-body wall complex, is a rare, lethal, severe body wall defect of the abdomen and chest in which the abdominal wall does not develop and the peritoneal cavity is left open to the extraembryonic coelom (Fig. 62.8). Prenatal ultrasound can visualize the herniated organs from the chest and abdomen. In addition, a fetus with a body stalk anomaly will usually have scoliosis and other abnormalities including malrotation of the spine and incomplete closure of the pelvis with malrotated limbs and/or club feet. The umbilical cord is either short or absent. If the umbilical cord is absent there are direct vascular connections between the fetus and placenta. It is important to recognize BSA on prenatal imaging so that families can receive appropriate prenatal counseling.[121] Diagnostic criteria for BSA include two of the three anomalies:

1. Exencephaly/encephalocele with facial clefts
2. Thoraco- and abdominoschisis (midline defect)
3. Limb defect (i.e., club foot, polydactyly, oligodactyly, syndactyly, brachydactyly, amelia)

Body stalk anomalies are generally not associated with chromosomal anomalies.[122]

Bladder Exstrophy

Bladder exstrophy is an anomaly of the lower abdominal wall, pelvis, and pelvic organs in which an open bladder makes up the lower part of the anterior abdomen. The open bladder is



• **Fig. 62.8** Body Stalk Anomaly, Also Called Limb Body Wall Complex. (From Plakkal N, John J, Jacob SE, et al. Limb body wall complex in a still-born fetus: a case report. *Cases J*. 2008;1:86. Image under the terms of the Creative Commons Attribution License. https://creativecommons.org/licenses/by/2.0.)

accompanied by a separation of the pubic symphysis. It is a rare anomaly with an incidence of 3 to 5 cases in 100,000 births, and it occurs at least twice as often in boys compared to girls.[123] The etiology is unknown, and it is grouped into the exstrophy-epispadias complex of anomalies. Surgical treatment is required but is not an emergency, so patients can be transferred to specialized centers for definitive care. Early management consists of protecting the exposed bladder mucosa from injury by applying a nonadherent dressing. Evaluation for associated anomalies includes ultrasound for associated upper urinary tract anomalies and an abdominal radiograph to assess the degree of pelvic bone separation.

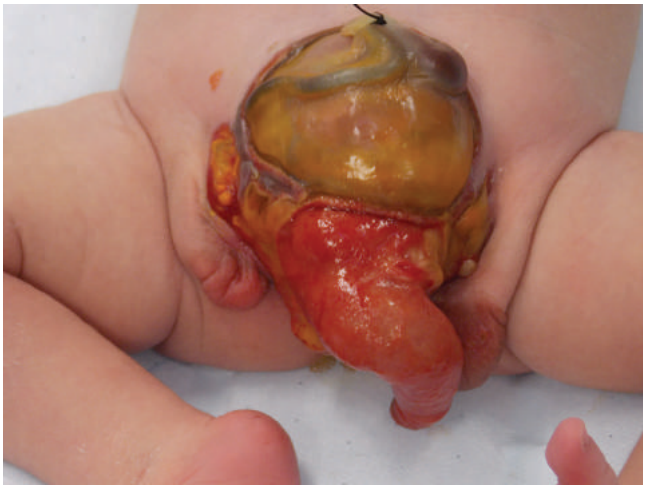
Cloacal Exstrophy

Cloacal exstrophy is another anomaly grouped into the exstrophy-epispadias complex of anomalies (Fig. 62.9). It is also known as OEIS complex—Omphalocele, Exstrophy, Imperforate anus, Spinal dysraphism—that describes its usual features. Cloacal exstrophy is rare, occurring in about 1 in 100,000 births, and is associated with the 1p36 deletion.[124] Most patients present with an abdominal wall defect that at its cephalic aspect consists of an omphalocele. The omphalocele is in continuity with a more caudal complex exstrophy of two lateral bladder halves joined to a central bowel exstrophy called a cecal plate. This exstrophy complex is accompanied by pubic symphysis diastasis (similar to that seen in bladder exstrophy) and imperforate anus. It may be associated with spinal dysraphism.

Bowel peristalsis and abdominal pressure often lead to prolapse of the terminal ileum and the distal colon/hindgut lumens that are normally attached to the cecal plate. Early management consists of a nonadherent bandage to the exposed bladder and bowel mucosa. Surgical reconstruction is complex and often done in stages. Overall survival is high, but there is substantial long-term morbidity including abnormal function of the anorectal and urinary tracts, and neurologic impairment.[125]

Prune Belly Syndrome

Prune belly syndrome (PBS), also known as Eagle-Barrett syndrome, is a rare condition notable for complete or partial lack of abdominal wall muscle and severe genitourinary abnormalities including bilateral intra-abdominal cryptorchidism, megalourethra, megacystis, hydroureteronephrosis, and renal dysplasia.[126] The cause of PBS is unclear; however, genetic etiology is supported due to 3% of cases being associated with a genomic HNF1β (hepatocyte nuclear factor) mutation, a transcription factor that regulates gene expression for mesodermal/endodermal development.[127] A significant cause of mortality in newborns with PBS is pulmonary hypoplasia, while a major component of long-term morbidity is urinary tract abnormalities, which often result in end-stage renal disease.[128,129] Treatment involves a multidisciplinary team to address urinary tract anomalies, correction of cryptorchidism, and repair of abdominal wall flaccidity. Since bladder dynamics and renal function can change over time, ongoing monitoring of the urinary tract remains crucial.[128] Several reconstruction procedures of the abdominal wall have been described to improve bladder and gastrointestinal function.[130]



• **Fig. 62.9** Cloacal Exstrophy.

Umbilical Abnormalities

Umbilical abnormalities seen in newborns usually present as an umbilical mass or umbilical drainage.

Umbilical Granulomas

The most common umbilical abnormality in infancy is acquired granulation tissue, also described as granuloma formation. Umbilical granulomas are the result of persistence and hypertrophy of the normal granulation tissue present at the base of the umbilicus after the cord separates away from the body wall. Histologically, granulomas are comprised of fibroblasts and capillaries. Granulomas usually present as small (less than 1 cm in diameter), moist, pink or red masses at the umbilicus. They may have serosanguinous drainage or fibrinous exudate that can be confused with the drainage of a soft tissue infection. Most small granulomas will spontaneously dry out and the surrounding skin will contract and heal around the umbilicus.[131,132] Sometimes skin will grow over the granuloma and, as the inflammatory tissue shrinks, eventually result in a skin tag. These skin tags usually become smaller over time. Silver nitrate cauterization of the granulation tissue will eliminate or decrease the size of the umbilical granuloma and allow for the skin to heal. Surgical excision or ligation is usually not necessary unless the lesion persists.[132]

Delayed Separation of the Umbilical Cord

After birth the umbilical cord usually dries out and falls off within a week, but there is considerable variability in the timing of this separation. Some of the variability in umbilical cord separation depends upon how the cord is cared for after birth since bacterial colonization may play an important role in the process. Cords that are treated with more intensive antiseptic regimens typically fall off later than cords that are simply kept clean and dry.[133]

There is no standard definition of delayed separation of the umbilical cord, but in the United States, if cord separation takes more than 2 to 3 weeks, it raises concerns for immune deficiency, urachal anomalies, or infection.[134] Leukocyte adhesion deficiency should be suspected when a newborn presents with delayed cord separation and soft tissue infection around the umbilical stalk.[135] However, most infections associated with the umbilical cord are not associated with immune deficiencies.

Umbilical and Periumbilical Infections

The lack of an epithelial barrier at the unhealed umbilicus, normal bacterial colonization of the skin, and potential contamination of the area during cord care make the umbilicus vulnerable to bacterial invasion. Newborns with unimmunized mothers and improper cord care are at risk for neonatal tetanus, which can be a significant problem in resource-limited countries.[134,136]

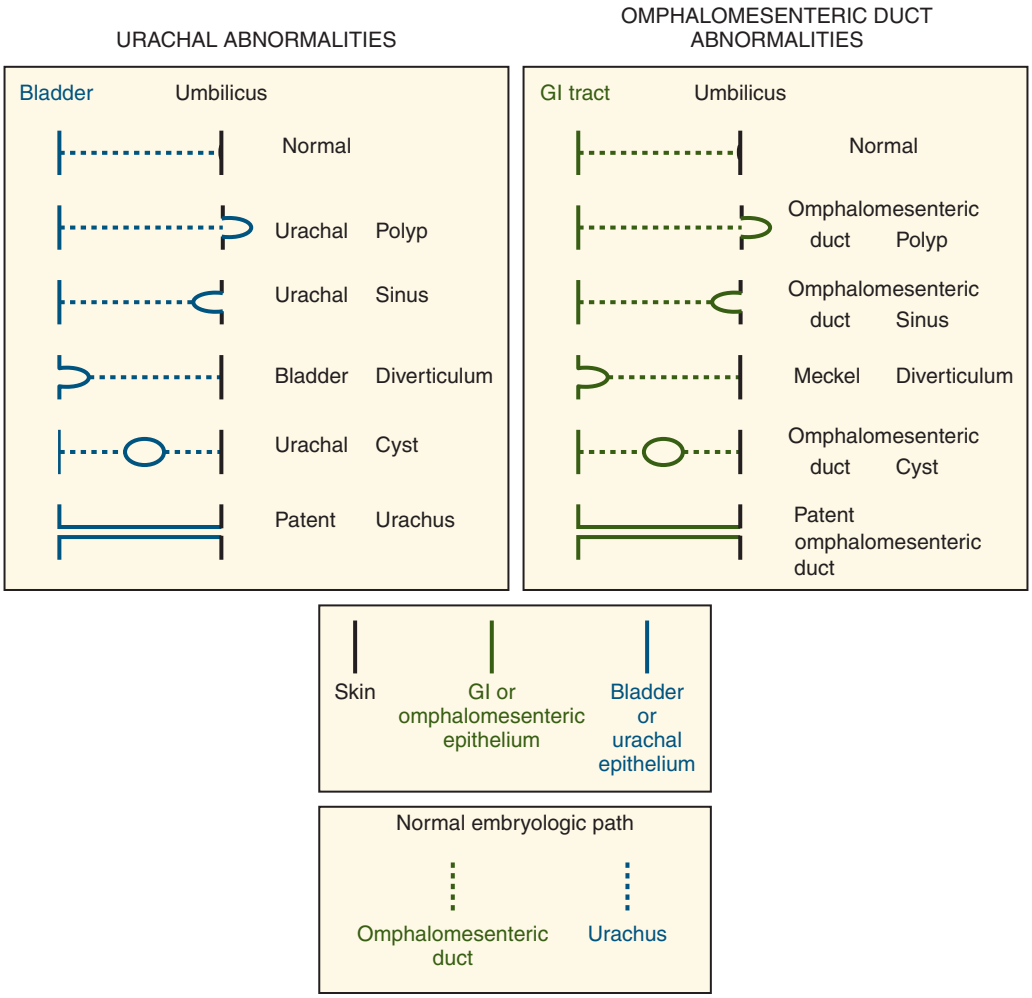
A more common bacterial soft tissue infection of the periumbilical region is omphalitis. Omphalitis ranges in severity from simple cellulitis to necrotizing soft tissue infection of the abdominal wall. The severity of infection depends on host defenses, the virulence of the infecting organism, and the depth of invasion. In high-income countries the incidence of omphalitis is reported at less than 1%, but in resource-limited countries the incidence is much higher.[137] Omphalitis is often a polymicrobial infection with staphylococcus, streptococcus, aerobic gram-negative rods, and anaerobes.[138] Treatment of omphalitis includes broad-spectrum antibiotics and, for invasive infections, surgical debridement of involved tissues. Omphalitis can be a serious infection causing life-threatening systemic sepsis. The infection can also spread up the umbilical vein and cause inflammation and thrombosis of the portal venous system that may evolve into symptomatic portal hypertension years later.[138]

Persistent Remnants of Urachus and Omphalomesenteric Duct

The omphalomesenteric duct and urachus are present during early development but normally regress or obliterate. Persistent remnants of the urachus and omphalomesenteric duct may present with drainage from the umbilicus with or without an umbilical or periumbilical mass. The size and location of the persistent remnant determines the clinical presentation more than the remnant's embryologic origin. The individual pathologic types of remnants are schematically represented in Fig. 62.10 and include polyps, sinuses, fistulae, cysts, and bands. These types may exist alone or in combination.

Umbilical polyps are much less common than umbilical granulomas as a cause of an umbilical mass or umbilical drainage. An umbilical polyp is an epithelial remnant of the omphalomesenteric duct or the urachus that persists at the skin level and presents as a moist, bright red, round mass. The serous exudate or mucous secretions of the epithelium account for the umbilical drainage. Unlike the granulation tissue of an umbilical granuloma, the epithelium of an umbilical polyp will not respond to silver nitrate cauterization, and surgical excision is required.

A partial, persistent remnant of the urachus or omphalomesenteric duct that is open to the skin at the umbilicus is known as



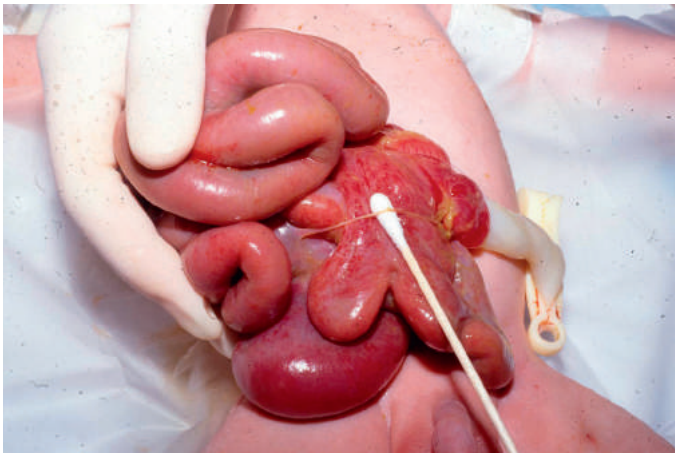
• **Fig. 62.10** Schematic of Urachal and Omphalomesenteric Duct Remnants.

a urachal sinus or an umbilical (or omphalomesenteric) sinus (see Fig. 62.10). Similar to umbilical polyps, sinuses present with umbilical drainage and require surgical excision. A partial, persistent remnant of the urachus or omphalomesenteric duct that is open to the lumen of the bladder or GI tract is known as a bladder diverticulum or Meckel's diverticulum, respectively (see Fig. 62.10). A partial, persistent remnant of the urachus or omphalomesenteric duct that does not connect to the skin or the underlying bladder or bowel lumen is known as a urachal or omphalomesenteric cyst, respectively (see Fig. 62.10), and presents as a midline mass in the abdominal wall at or near the umbilicus. Cysts may also present with increasing size, pain, tenderness, and fever if they become infected. Cysts require excision, although if infected they may first require incision and drainage, and then interval resection.

A complete persistent remnant of the urachus or omphalomesenteric duct results in a fistula between the umbilicus and bladder or bowel respectively (see Fig. 62.10). When the fistula is connected to the bladder it is known as a patent urachus and may drain urine. When the fistula is connected to the GI tract it is known as an omphalomesenteric (or vitelline) duct fistula and may drain stool. When this fistula is obliterated, it may also form an adhesive band, which could be a nidus for bowel obstruction in the future (Fig. 62.11). Treatment for fistulae includes surgical excision and closure of the bladder or bowel respectively using an open or laparoscopic technique.[132]

Abdominal Wall Hernias

A hernia exists when the contents of a body cavity extend through the normal wall of that cavity. Abdominal wall hernias are conditions in which intra-abdominal contents protrude through the normal muscle and fascial layers of the abdominal wall. The congenital abdominal wall defects discussed in the previous section are types of abdominal wall hernias. In contrast to those conditions that present prenatally or immediately after birth, this section will briefly discuss abdominal wall hernias that usually present after birth, although their anatomic cause may be due to an abnormality of development.



• **Fig. 62.11** Omphalomesenteric (or Vitelline) Duct Remnant in a Patient With Gastroschisis.

Inguinal Hernia and Hydrocele

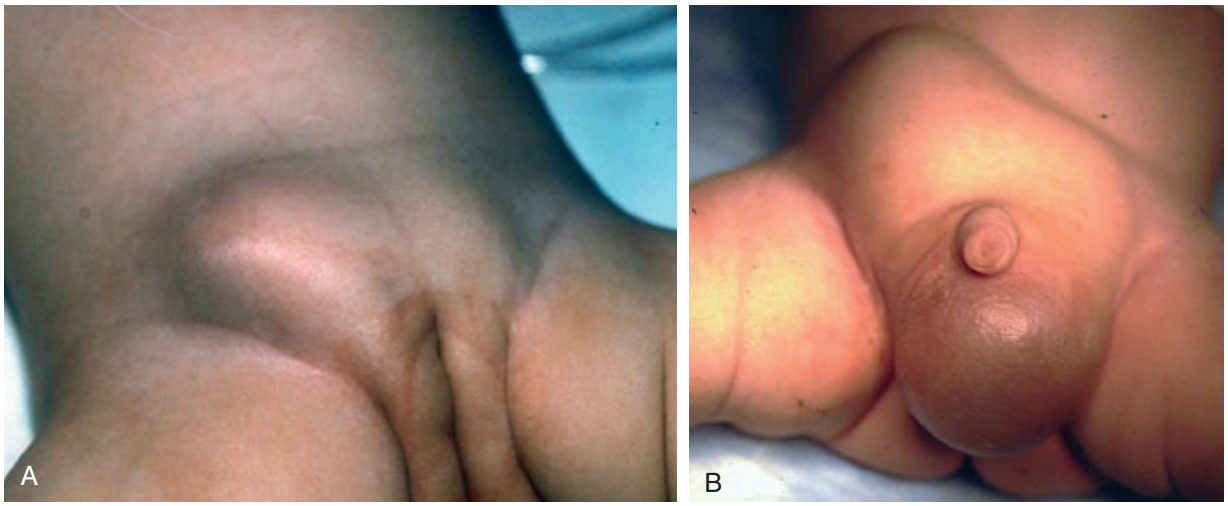
Inguinal hernias are the bulging of intra-abdominal contents through the abdominal wall in the groin (Fig. 62.12). In adults, this may be the result of abdominal wall muscular injury or weakness, but in infants and children the hernia is most commonly the result of a persistent congenital protrusion of the peritoneum through the internal and external ring openings of the abdominal wall muscles. This peritoneal protrusion is known as a patent processus vaginalis. As it crosses the abdominal wall, it is immediately adjacent to the spermatic cord structures in boys, and the round ligament of the uterus in girls. In boys, the processus vaginalis is the path of normal descent of the testis, and it normally closes except for a small pouch around the testis. When it remains open and is large enough, pressure in the abdomen may allow passage of intra-abdominal contents through the abdominal wall, perhaps all the way into the scrotum. When organs or parts of organs protrude it is called a hernia, and when only fluid goes into the processus vaginalis it is known as a hydrocele.[139]

Approximately 1% to 5% of children will have an inguinal hernia. Hernias are more common in boys compared to girls.[140] Hernias are also much more common in premature babies compared to term babies.[141] Unlike umbilical hernias, inguinal hernias do not spontaneously close and tend to enlarge over time. The major risk of an inguinal hernia is the possibility of strangulation of the hernia contents with life-threatening intestinal ischemia. An additional complication may be compression of blood supply to a gonad by the protruding hernia with subsequent loss of testicle or ovary. Strangulation only occurs when hernias are incarcerated; that is, when the contents are not reducible back into the abdomen. Therefore, a nonreducible or incarcerated inguinal hernia requires urgent surgical evaluation. In otherwise healthy infants, inguinal hernias are repaired electively, soon after their diagnosis, since the operation is well tolerated and the risk of anesthesia is low.[142] Premature infants are prone to apnea after general anesthesia, so the ideal timing of operation in this group is controversial.[143,144] A baby who has an inguinal hernia has an increased risk of developing a contralateral hernia later in life, but the risk may not be high enough to justify routine contralateral "prophylactic" hernia repair.[145]

Inguinal hernias present with groin bulges that may extend into the scrotum or labia (see Fig. 62.12). When fluid alone is present in a persistent processus vaginalis it is known as a hydrocele. Hydroceles may be simple collections present at birth in the normally present pouch around the testis, or they may develop after birth along any portion of the path of the processus vaginalis. Hydroceles present at birth may spontaneously resolve, although it may take up to a year. Hydroceles that have an open connection into the abdomen "communicate" across the internal ring will show size fluctuations. Communicating hydroceles are unlikely to resolve, so they are treated as inguinal hernias, although their repair is not urgent. Large hydroceles can be difficult to differentiate from inguinal hernias, especially when hydrocele fluid extends up into the inguinal canal.

Umbilical Hernia

The umbilical ring is the normal fascial layer that surrounds the umbilical cord going through the abdominal wall. It represents



• **Fig. 62.12** Right inguinal hernia in a female (A) and male (B).

the junction of superior, inferior, and lateral body wall folds. An umbilical hernia is a protrusion of bowel or omentum through an open umbilical ring, producing a bulge at the umbilicus. The umbilical ring normally closes after birth, but it may take months or years to become too small for contents to herniate through the abdominal wall.

Umbilical hernias are usually not present in the first few days of life but may become noticeable after a few weeks. They are more common in infants born prematurely and much more common in infants of African descent compared to Caucasian infants. Most umbilical hernias are sporadic, but some families have an increased risk. Umbilical hernias are more noticeable when the intra-abdominal pressure increases with crying or straining, but the hernias usually do not cause pain or distress. The great majority of umbilical hernias close spontaneously before school age, and symptoms including incarceration are uncommon early in life. Surgical repair of an umbilical hernia is usually delayed until 3 to 5 years of age or until symptoms develop.[139] Surgical repair of an umbilical hernia in a child is almost always performed with absorbable suture and mesh is not routinely used.

Epigastric Hernia

Epigastric hernias are small fascial defects in the linea alba, the midline fascia between the rectus abdominis muscles, through which preperitoneal fat can herniate. Because they are typically only a few millimeters in diameter, the fascial defects are often too small to palpate. However, preperitoneal fat can herniate out like a mushroom with only a small stalk traversing the defect and a larger cap in the subcutaneous tissue that may be visible and palpable.[146] Epigastric hernias are usually asymptomatic and surgical repair can be delayed until childhood. Common symptoms include pain at the site with exercise and growth of the hernia lump.

Diastasis Recti

Although diastasis recti is a normal variant and not a hernia, it is often mistaken for an abdominal wall hernia. The central section of a normal anterior abdominal wall consists of the two rectus abdominus muscles that extend from the ribs to the groin. Between the rectus muscles there is an extension of the rectus muscle fascia, known as the linea alba, that joins the two rectus muscles and creates a solid abdominal wall. A visible separation of the rectus muscles with an intact linea alba fascia is known as diastasis recti or rectus abdominis diastasis. The body wall musculofascial layer is intact, but the midline bulge of the linea alba fascia between the xiphoid process and the umbilicus when the abdominal muscles contract is often mistaken for a hernia. Imaging studies are not indicated. The condition is not pathologic and will typically be less noticeable after normal growth. Surgical treatments may be performed in adults for cosmetic reasons but are not indicated in infants and children.[147]

Umbilical Cord Abnormalities

As the direct connection between the placenta and the fetus, the umbilical cord normally consists of an umbilical vein and two umbilical arteries encased in a gelatinous matrix, known as Wharton's jelly, that is covered with a layer of amnion. Even when umbilical cord abnormalities are not problematic, they are often associated with other pathologic conditions that result in poor fetal and neonatal outcomes. Therefore, when abnormalities of the umbilical cord are found, it usually warrants further investigation for chromosomal abnormalities and structural malformations. In addition, many fetuses with umbilical cord abnormalities will need more frequent evaluations of fetal well-being.[148] Common umbilical cord abnormalities are summarized in Table 62.1, although many more have been described.

Acknowledgment

We would like to acknowledge the contributions of Dr. Daniel J. Ledbetter's work on this chapter in the last edition and his devotion to children with surgical problems over many years.

TABLE 62.1 Umbilical Cord Abnormalities

Cord Anomaly	Description	Incidence	Associations	References
Non-coiled or hypo-coiled cord	Umbilical coiling index is based on gestational age is used to quantify degree of umbilical cord coiling.	5%	IUGR, preterm labor, fetal distress during labor and delivery, meconium-stained amniotic fluid, stillbirth, chromosomal anomalies.	149–150
Single umbilical artery	One umbilical artery as opposed to the normal two.	0.5%–1%	Stillbirth, IUGR, multiple gestations, stillbirth, risk of genitourinary malformations and chromosomal anomalies.	131,152–154
Umbilical vessel dilations	Includes dilations of the umbilical vein (umbilical vein varices); dilations of umbilical artery (umbilical artery aneurysms).	Rare	Associated with fetal and chromosomal anomalies, increased risk of adverse fetal outcomes.	155,156
Umbilical cord cyst or pseudocyst	Cyst: Epithelial-lined remnants of vitelline (or omphalomesenteric) duct or allantois (which forms urachus). Pseudocyst: due to degeneration of Wharton's jelly, pooling of cord edema, or liquefaction of umbilical cord hematoma.		Large cysts may lead to compression of umbilical cord blood flow and may require aspiration. Pseudocysts that persist into second trimester are associated with fetal aneuploidy and malformations.	157–159

IUGR, Intrauterine growth restriction.

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63

Neonatal Gastroesophageal Reflux

ERIC C. EICHENWALD

KEY POINTS

- Gastroesophageal reflux (GER) is almost universal in neonates.
- It is a physiologic process secondary to frequent spontaneous transient lower esophageal sphincter relaxation, relatively large volume liquid diet, and age-specific body positioning.
- Preterm infants have relative immaturity of neural control of the gastrointestinal tract, leading to delayed gastric emptying and slow gut motility.
- The diagnosis of GER in the neonatal intensive care unit (NICU) is frequently made based on behavioral signs (irritability, back arching, discomfort with feedings) which most often are not temporally associated with documented reflux.
- Multichannel intraesophageal impedance monitoring combined with lower esophageal pH measurement is the best diagnostic test for GER in infancy.
- Treatment of GER involves both nonpharmacologic and pharmacologic management, but drug therapy of GER is associated with the potential for several adverse outcomes.

Gastroesophageal Reflux

Epidemiology

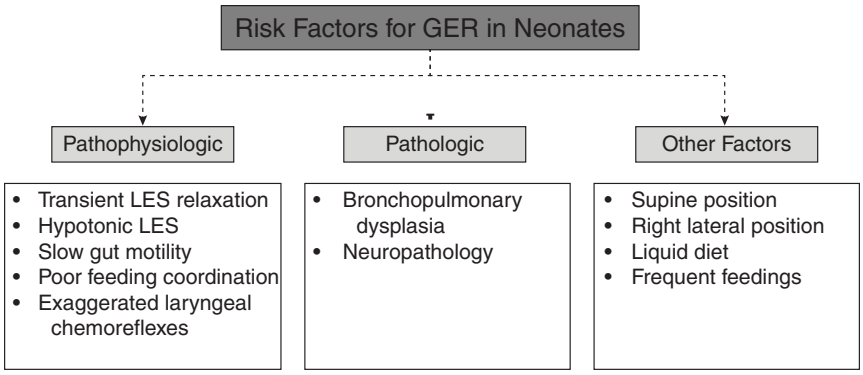
Gastroesophageal reflux (GER) is defined as the retrograde movement of gastric contents into the esophagus with or without regurgitation. Uncomplicated GER is a normal physiologic process in infants, in part due to relatively large liquid volumes ingested during feedings and supine positioning which places the gastroesophageal junction in a liquid environment. Preterm infants are at particular risk for reflux due to gastrointestinal dysmotility and delayed gastric emptying affected by immaturity. Uncomplicated GER should not prompt further investigation or treatment. However, when reflux of gastric contents is associated with troublesome clinical symptoms or signs, it is called gastroesophageal reflux disease (GERD). In clinical practice, since "troublesome signs" in neonatal patients are most often nonspecific and subjective, it is difficult to differentiate GER from GERD in the neonatal intensive care unit (NICU); as a result, these terms are frequently used interchangeably by medical professionals and parents alike.

The difficulty in making a diagnosis of GERD in neonatal patients can lead to its overdiagnosis and treatment, as well as significant practice variation. In one study of 33 academic children's hospital NICUs, there was a 13-fold variation (2% to 26%) in the diagnosis and treatment of GERD in NICU patients.[1] In this study, patients with a GERD diagnosis had longer hospital stays and higher costs than infants without this diagnosis. In addition, infants discharged from the NICU on antireflux medications frequently remain on treatment for many months after discharge, making it an important clinical entity in the NICU and beyond.[2] This chapter will discuss the physiology, diagnosis, and therapy of GER in nonsurgical patients in the NICU.

Pathophysiology

There are many pathophysiologic mechanisms which increase the risk of GER in the neonatal period (Fig. 63.1). The lower end of the esophagus is defined anatomically by the junction of the esophagus with the stomach, and functionally by the junction of the inferior border of the high-pressure area of the lower esophageal sphincter (LES) with the lower pressure in the stomach. The LES is comprised of smooth muscles organized in two layers, with circular and longitudinal muscle fibers separated by a myenteric plexus. The gastroesophageal junction is functionally augmented by the diaphragmatic crural fibers, the intra-abdominal esophagus, and sling fibers of the stomach, which together form the principal barrier to GER. Extrinsic innervation of the esophagus involves neurons in both the brainstem and spinal cord. The LES is innervated by both parasympathetic (vagus) and sympathetic (primarily splanchnic) nerves. The LES is tonically active and functionally closed. The vagal pathways are the central neural pathway for reflex relaxation of the LES. Parasympathetic vagal nerve pathways, coordinated through the dorsal motor nucleus and nucleus tractus solitarius, trigger periodic reflex relaxation induced by swallows and esophageal dilatation to facilitate bolus transit from the upper esophagus. These mechanisms of swallow-related relaxation of the LES and tonic activity of the LES to maintain esophagogastric competence are present even in extremely preterm infants.[3]

In addition to reflex relaxation of the LES related to swallowing, the LES also spontaneously relaxes without stimulation. These events are termed "transient lower esophageal sphincter relaxation" events, or TLESR. TLESR is an abrupt reflex decrease in LES pressure to levels at or below intragastric pressure unrelated to swallowing and is the primary mechanism for GER in infants. TLESR appears to use the same efferent neural pathway as the swallow reflex, whereas afferent pathways may originate in the pharynx, larynx, or stomach. In contrast to swallow-related LES relaxation, TLESRs are generally longer in duration and at lower LES pressures.



• **Fig. 63.1** Risk Factors for Gastroesophageal Reflux (GER) in Neonatal Intensive Care Unit (NICU) Patients. LES, Lower esophageal sphincter. (Adapted from Gulati IK, Jadcherla SR. Gastroesophageal reflux in the neonatal intensive care unit: who needs to be treated and what approach is beneficial? *Pediatr Clin N Am*. 2019; 66:461–473.)

The normal function of TLESR is to prevent gastric bloating by periodically releasing gas from the stomach. As such, gastric distention after a meal stimulates stretch receptors in smooth muscle in the stomach wall, stimulating a vasovagal response with resultant TLESR. The number of TLESR events per day in neonates appears to decrease with maturity, so that preterm infants have dozens of episodes per day, many of which are associated with GER.[3] GER and regurgitation is very common in neonates, induced by a reflex TLESR associated with gastric distention after a feeding. As such, physiologically, some degree of subclinical or clinically apparent reflux is a normal phenomenon in neonates. In neonates, GER is exacerbated by a pure liquid diet and age-specific body positioning.[4] Newborns spend most of their sleeping time in the supine position because of its known protective effect on the incidence of sudden infant death syndrome (SIDS).

Infants positioned supine and in the right-side-down lateral position have more episodes of TLESR and GER compared with the left-side-down lateral position, despite more rapid gastric emptying in the right lateral position.[5,6] Prone position decreased episodes of GER versus the supine position, likely due to a more optimal positioning of the LES relative to the liquid-filled stomach rather than a decrease in TLESR events.[5]

Impaired gastric motility and delayed emptying do not appear to play a contributory role in GER in infancy, in that infants with symptomatic GER do not have delayed gastric emptying compared with other infants.[7] However, the composition of gastric contents does affect the rate of gastric emptying, which may influence the frequency of GER. Increased caloric density feeding, especially with fats, frequently fed to preterm infants, slows gastric emptying, whereas expressed breast milk empties at almost double the rate of formula.[8]

Preterm infants, especially those with intrauterine growth restriction, are at a higher risk for gut dysmotility, causing feeding intolerance which may be assessed as a sign of GER.[9] The motility of the small intestine is less organized in preterm compared with term infants, due in part to intrinsic immaturity of the enteric nervous system that slows intestinal transit. In growth-restricted infants, prolonged periods of intrauterine hypoxia can produce injury to the bowel, which modulates the development of motor, secretory, and mucosal functions after birth, and may result in a higher risk for dysmotility and stasis.[10]

Mechanisms to protect the esophagus and airway from GER appear to be intact even in the most preterm infants. These include reflex antegrade peristalsis of the esophagus in response to esophageal dilatation with gastric contents, and closure of the upper esophageal sphincter to prevent refluxate from reaching the pharynx. Despite this, if refluxed material does reach the upper esophagus, the upper esophageal sphincter will reflexively open to allow the material into the pharynx to protect the laryngeal structures, resulting in episodes of "spitting" or emesis frequently seen in infants.

Evaluation

Several different methods to diagnose reflux in infants are currently utilized and are comprehensively reviewed in a joint clinical practice guideline published by the North American and European Societies of Gastroenterology.[11] These include contrast fluoroscopy, lower esophageal pH monitoring, direct laryngoscopy and bronchoscopy to assess airway inflammation, and multichannel impedance (MII) monitoring combined with pH measurement.

Contrast fluoroscopy will demonstrate if reflux occurs and can determine the height in the esophagus reached by the refluxate, but it cannot differentiate between clinically significant and insignificant GER. As such, it is not generally recommended as a sole methodology to diagnose GER.[11]

Continuous monitoring of pH over several hours using a probe placed in the lower esophagus has been used to diagnose GER in older children and adults, since reflux of acidic stomach contents will result in transient periods of acidic pH in the lower esophagus. A scoring system developed by gastroenterologists may be used to determine if a study is "normal" or "abnormal." Measurements include the total number of reflux episodes during the recording period, the longest duration of acidification recorded, and a "reflux index (RI)" defined as the percentage of the total recording time with an esophageal pH < 4. In pH studies in older children and adults, an RI $> 7\%$ is considered abnormal, an RI $<3\%$ normal, and an RI between 3% and 7% indeterminant.[11,12] However, the measurement of esophageal pH is not a sensitive method to detect GER in newborns and in early infancy because of a higher baseline stomach pH in part due to frequent milk feedings and the fact that the stomach pH is rarely below 4.[12]

A potentially less invasive diagnostic technique investigated in infants is to measure salivary pepsin levels as an extra-esophageal biomarker of GER.[13] In one small study, 44.5% of infants with suspected GER had salivary pepsin detected, as compared to 29% of control infants.[13] However, this measurement has limited sensitivity and specificity in part due to a lack of normative values for salivary pepsin in infants and is not routinely used outside of research studies.[11]

Otolaryngologists have developed a "reflux finding score" for airway inflammation suspected to be caused by GER, which is assessed by direct laryngoscopy and bronchoscopy. It is frequently used to recommend antireflux medical treatment.[14] However, in one prospective cross-sectional cohort study of children with reflux-like symptoms, there was no correlation between the reflux finding score and other confirmatory tests of reflux such as pH and MII measurements.[14] The authors concluded that airway examination in children could not predict GERD and should not be used as a basis for prescribing therapies. Although not systematically studied, this is likely to be true in infancy as well.

The most accurate way to detect GER in infants is multichannel esophageal impedance (MII) monitoring, combined with simultaneous measurement of pH.[11] As used in infants, MII tracks the movement of fluids and air in the esophagus through measurement of changes in electrical impedance between electrodes spaced along an esophageal catheter. It can detect whether a fluid bolus in the esophagus is antegrade, as in a swallow, or retrograde, as in a reflux episode, as well as determine the height of the reflux event. When combined with a pH probe, it can also determine if the episode of GER is acidic, mildly acidic, or alkaline. Normative data collected by MII suggest that healthy preterm infants mostly have mildly acidic reflux events which occur more frequently after a feeding.[15] Although somewhat invasive and technically difficult, MII/pH measurement is the recommended evidence-based way to correlate presumptive symptoms and signs of GER with actual measured reflux events during infancy.[11]

TABLE 63.1 Signs and Symptoms Frequently Attributed to Gastroesophageal Reflux in the Neonatal Intensive Care Unit

Gastrointestinal

- Regurgitation
- Spitting
- Abdominal distention
- Nipple aversion

Aerodigestive

- Swallowing problems
- Feeding intolerance
- Choking
- Coughing
- Stridor

Cardiorespiratory

- Apnea
- Bradycardia
- Desaturation events
- Respiratory distress

Behavioral

- Irritability
- Back arching
- Crying
- Grimace

Adapted from Gulati IK, Jadcherla SR. Gastroesophageal reflux in the neonatal intensive care unit: who needs to be treated and what approach is beneficial? *Pediatr Clin N Am.* 2019;66:461–473.

Clinical Presentation

Many clinicians continue to diagnose GER, particularly in preterm infants, based on nonspecific behavioral signs (Table 63.1). Other clinical signs often attributed to GER include feeding intolerance (i.e., abdominal distention, spitting), poor growth, frequent apnea, and desaturation episodes, as well as worsening lung disease in infants with evolving bronchopulmonary dysplasia (BPD).[16]

Behavioral and Feeding Issues

Infants in the NICU frequently display nonspecific behavioral signs which clinicians often ascribe to GER. These behavioral signs include arching, irritability, and apparent discomfort associated with feedings, as well as other feeding difficulties, such as frequent spitting and nipple aversion. Data to support the relationship between these signs and GER are sparse in otherwise healthy infants as well as those who are clinically suspected as having GER. Most studies which examined the correlation of the timing of these behavioral signs with documented reflux as measured by MII/pH show no temporal relationship, and infants frequently display behaviors ascribed to GER unrelated to documented GER episodes.[17–19]

Apnea, Bradycardia, and Desaturation

There are several physiological reasons to suspect that GER may precipitate apnea, bradycardia, and desaturation events in preterm infants. Because preterm infants possess a hyperactive laryngeal chemoreflex response to liquid stimulation which induces apnea as an airway protective mechanism, some clinicians believe that apnea spells in preterm infants may be caused or worsened by the frequent GER events.[16] However, studies which examined the timing of reflux episodes in relation to apnea, bradycardia, or desaturation events have found that they are rarely temporally related,[20,21] and the measured GER does not prolong or worsen apneic events.[22] In contrast, it does appear that spontaneous apneic events can result in a reflex relaxation of the LES (and resultant GER), presumably due to vagal stimulation.[23] Other studies of empiric treatment of clinically diagnosed GER with agents which decrease gastric acidity or promote faster gastric emptying have no effect on the incidence and severity of apnea or bradycardia in preterm infants.[24,25]

Respiratory Disease

It remains unclear whether GER causes clinically silent microaspiration in ventilated infants that worsens lung disease, particularly in infants with developing or established BPD. Infants with BPD may be at a higher risk for GER due to greater respiratory symptoms causing increased intra-abdominal pressure swings and use of medications which could influence LES tone, such as caffeine and albuterol.[26] Small studies have reported a high frequency of the recovery of measurable levels of pepsin in tracheal aspirates of ventilated preterm infants, higher levels of pepsin in tracheal aspirates of infants who developed BPD,

and an association of higher concentrations of pepsin in tracheal aspirates with the severity of BPD, leading to speculation that chronic microaspiration may contribute to the development and severity of BPD.[27,28] However, these results have not been replicated in larger studies, and there remain questions whether the measured pepsin is of gastrointestinal origin. Other studies have examined whether infants with BPD have a higher frequency or severity of GER events compared with infants without BPD using esophageal pH only, or MII/pH monitoring with negative results.[18,29] Infants with BPD also are frequently assessed to have behavioral signs ascribed to GER, but similar to other preterm infants, these behavioral signs are infrequently temporally associated with either acidic or nonacidic reflux as measured by MII/pH monitoring.[18] Despite the lack of evidence that GER is a worse pathologic problem in infants with BPD, infants with BPD are more likely to be clinically diagnosed and receive pharmacologic therapy for GER.[30]

Management

Infants in the NICU frequently receive both nonpharmacologic and pharmacologic therapies for clinically diagnosed GER, though there remains little data about the effect of treatment on either symptom burden or long-term outcomes.[2,31] The lack of randomized placebo-controlled trials of GER therapies in infants in the NICU also makes it impossible to assess the effectiveness of therapy versus the natural history of GER improving with maturity.[24]

Nonpharmacologic Approaches

Changes in body positioning are widely used as a management strategy in infants clinically diagnosed with GER. These include placing babies in a head-up angle, using the left lateral position after feeding, and prone versus supine positioning which may enhance gastric emptying and reduce episodes of TLESR. These maneuvers, while perhaps benign, have not been shown to reduce behavioral manifestations of presumptive reflux, such as frequent crying or irritability.[32]

Changes in feeding strategy and approach are also frequently prescribed for infants with suspected GER. If GER is precipitated in part by gastric distention with large bolus feedings, smaller volume, more frequent feeding might in theory decrease GER. Some evidence supports this hypothesis, although whether a decreased incidence of GER has a beneficial effect on other outcomes is unknown.[33,34] Continuous transpyloric feedings as opposed to intragastric are also prescribed in infants, particularly those with BPD.[35] However, in one small cohort of infants with severe BPD, trans-pyloric feedings were associated with an increased frequency of intermittent hypoxemia events when compared to gastric feedings in an *N* of 1 crossover trial.[36]

Another feeding strategy used is to thicken feedings with several different agents including xanthan gum, starch, or rice cereal.[37] Commercially available formula products that thicken upon acidification in the stomach are also available. Neither of these approaches is recommended for use in preterm infants. One systematic review of randomized-controlled trials of thickened formulas in term infants with GER showed that these agents reduced episodes of spitting and regurgitation but did not reduce the frequency of acidic GER.[38] Elemental or extensively hydrolyzed protein formulas increase gastric emptying and reduce gastrointestinal transit time, and may reduce symptoms of GER in term infants.[39]

Pharmacologic Management

Pharmacologic management of GER in infants includes prokinetic agents, and medications which decrease gastric acid production. Prokinetic agents used in infants include metoclopramide and erythromycin. These drugs work to improve gastric emptying and appear to enhance LES tone and are occasionally used for other functional motility disorders in preterm infants, including delayed gastric emptying causing frequent regurgitation, and slow gut motility which may prevent feeding volume advancement in extremely premature infants. Erythromycin has been studied as both a prophylactic as well as rescue drug for feeding intolerance in preterm infants.[40,41] Erythromycin is a competitive motilin agonist, which mimics the effects of motilin on the proximal gastrointestinal tract enhancing contraction of gut smooth muscle that produces forward propulsion of nutrients in the small bowel. The timing of erythromycin's use, its dose (high or low dose), administration (intravenous or enteral), and the most appropriate patient population remain poorly studied.[40,41] Metoclopramide has not been shown to reduce GER symptoms in preterm infants.[24,42] Caution should be exercised in their use, since they carry a potential for significant adverse effects, including a higher risk of infantile pyloric stenosis (erythromycin), cardiac arrythmia (erythromycin), and neurologic side effects (metoclopramide).

Acid-reducing agents include histamine$_2$ (H_2) receptor blockers and proton pump inhibitors (PPI). H_2 receptor blockers (e.g., ranitidine, famotidine) compete with histamine for the H_2 receptor in parietal cells in the stomach, thereby decreasing hydrochloric acid secretion and increasing gastric pH. PPIs (e.g., omeprazole, lansoprazole) block the gastric proton pump, with a resultant decrease in both basal and stimulated parietal cell acid secretion. Since clinicians often equate the suspected clinical signs of GER (irritability, crying, and discomfort after feeding) with acidic irritation of the lower esophagus, these agents were frequently prescribed to infants with a clinical diagnosis of GER.[2,16] However, no studies have assessed the efficacy of acid blockade on the symptom profile of infants with clinically suspected GER. Acid-reducing agents may be associated with a higher rate of adverse events, especially when used long term, including a higher risk of infection and pneumonia.[43,44] Because of an increased recognition for the potential for serious complications associated with their use, prescriptions of acid blockade agents have become less commonplace in NICUs.[45–47]

Suggested Readings

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Necrotizing Enterocolitis and Short Bowel Syndrome

GREGORY KEEFE, TOM JAKSIC, AND JOSEF NEU

KEY POINTS

- What we have termed "necrotizing enterocolitis" (NEC) is not a discrete entity but, rather, a manifestation of different forms of intestinal injury that can lead to intestinal necrosis. To make progress in terms of prevention, a better delineation of these forms of injury is critical.
- The pathophysiology of the classical form of NEC involves an interaction of factors that include an immature bowel and a dysbiotic microbiota that induces intestinal mucosal inflammation, leading to intestinal necrosis.
- Medical treatments are not evidence based but include the use of broad-spectrum antibiotics, bowel rest, close observation, and frequent diagnostic testing.
- Surgical interventions include placement of primary peritoneal drainage or laparotomy with direct inspection and resection. The decision as to when and which procedure to use is not based on clear criteria.
- Future studies should focus on delineation of the different entities under the umbrella of NEC, including an evaluation of multiomics and predictive analytics for biomarker development used to guide prevention and treatment.
- NEC is the most common cause of pediatric short bowel syndrome (SBS). Outcomes in SBS have dramatically improved in the past 20 years. Hallmarks of successful management include preservation of maximal intestinal length, hepatoprotective parenteral nutrition, and multidisciplinary intestinal rehabilitation programs.

Necrotizing Enterocolitis

Epidemiology

In the past several decades, a pattern of intestinal injuries has been referred to as necrotizing enterocolitis or "NEC." This set of entities affects approximately 2000 to 4000 newborns in the United States each year.[1–5] Of these, 10% to 50% die, approximating the childhood death rate from leukemia or meningitis. Long-term consequences often include short bowel syndrome (SBS), discussed later in this chapter, and neurodevelopmental disorders.[6]

To understand NEC, a brief historical perspective is in order. Prior to the emergence of neonatal intensive care in the 1960s, an entity affecting preterm infants that caused intestinal necrosis in neonates was rarely mentioned in the literature. Sparse references are made to a disease resembling NEC in the 19th and early 20th centuries.[7,8] In the 1960s and early 1970s issues of Schaffer and Avery's *Diseases of the Newborn*, very little mention is made of NEC. During these same years, with the precipitous emergence of neonatal intensive care and survival of less mature infants, the high prevalence of NEC also materialized. Thus, even though this is a disease of progress (in terms of greater survival of low birthweight infants), not much progress has been made in its prevention or treatment in the past 50 years. One of the most distressing issues is that we have yet to "define" this disease, which is difficult because what we are referring to likely represents more than one entity.[9]

This chapter will present some of the major issues surrounding NEC and why it has become such an enigma in terms of prevention and treatment. We will provide some of the current thinking about the different entities that have been termed NEC and will discuss epidemiology, diagnosis, pathophysiology, treatment, and prevention. There will also be a discussion of how we might proceed with future studies to improve our understanding of the processes that have been placed under the umbrella of NEC, how we differentiate them, and target our preventative and therapeutic measures with greater precision. In this chapter, we will also emphasize what is currently thought of as the "classic" form of NEC, its diagnosis, treatment (medical and surgical), sequelae in terms of SBS, and intestinal rehabilitation methods.

Defining Necrotizing Enterocolitis: A Conundrum

It is evident that what we have termed NEC is not a discrete entity. This lack of a clear phenotypic and pathophysiologic description thwarts progress. The underlying reasons are multifactorial and include:

1. Greater emphasis and support for the smallest, most preterm infants who have the highest propensity to develop intestinal injury.
2. Intestinal injury seen in the smallest, most immature infants occurs at a different time after birth than in more mature infants.
3. Many different forms of intestinal injury in the neonate are consolidated under the all-encompassing term NEC; however, preventative and therapeutic procedures are not specifically aimed at these subentities of intestinal injury.
4. Animal models used in studies of pathophysiology and preclinical preventative and treatment strategies do not accurately reflect the disease in preterm infants.

5. Multifactorial pathophysiology of these individual-specific injuries placed under the umbrella of NEC has not been clearly elucidated.

If progress is to be made in the prevention and treatment of the various forms of intestinal injuries termed NEC,[9] a better classification system is in order. Numerous retrospective observational studies have suffered from classifying different pathophysiologic entities as NEC. Because of this, databases include infants with spontaneous intestinal perforations (SIPs), ischemic bowel disease secondary to cardiac anomalies, congenital intestinal abnormalities, and food protein intolerance enterocolitis syndrome (FPIES). These all have different pathophysiologic features. Furthermore, the staging system for NEC, developed about five decades ago, has less current utility due to the greater degree of immature infants seen in today's intensive care setting.

NEC Stages

Variants of a staging system originally developed in the 1970s by Dr. Martin Bell have been relied upon to guide the management of NEC.[10] These have been incorporated into databases such as the Vermont Oxford Network (VON), but it is becoming clear that this system has numerous pitfalls and may cause considerable confusion and not be as useful as intended.[11] Numerous previous chapters have listed these stages, but we will not because they lead to confusion and, in the authors' opinion, should no longer be used. For example, in stage 1 NEC, intestinal necrosis is implied but not validated. Many of the symptoms and signs in this stage are seen in extremely low birthweight (ELBW) infants without any clear bowel pathology. Stage 2 is largely dependent on radiographic diagnosis, which provides no clear evidence for necrosis of the bowel and can be misread. Stage 3 largely depends on stronger evidence of bowel injury, which is optimally diagnosed by direct examination of the bowel by intraoperative inspection and/or histologically. However, when reliance for the diagnosis of stage 3 NEC is placed on radiographic diagnosis of pneumoperitoneum and is treated with surgical drain placement without direct visualization, the differentiation between true bowel necrosis and SIP, a distinct entity, is not possible. This matters in terms of most appropriate treatment.[12,13]

Newly emerging artificial intelligence technologies such as machine learning may help us better differentiate these entities and provide a more accurate definition of NEC.[1,2]

Pathology/Pathophysiology

The pathologic findings of NEC have been described by examination from severely affected infants. Gross anatomic exam reveals predominantly terminal ileum and proximal colon involvement. However, in severe cases ("NEC totalis"), the entire bowel from the stomach to the rectum may be involved. Histology reveals mucosal edema, hemorrhage, coagulation necrosis, and mucosal ulceration. The pathophysiology of NEC is discussed as follows.

Animal Models

In the early years of NEC research, hypothermia, severe hypoxia, and infection were considered to be stressors that led to NEC.[14] These stressors were associated with difficult birth. However, studies have shown that low Apgar scores, when corrected for prematurity, are not associated with NEC.[15] Furthermore, in the most preterm infants, in whom NEC occurs most frequently, the disease tends not to develop until several weeks after birth,[16] hence undermining hypoxia and ischemia as significant pathophysiologic triggers for classic NEC.[17] Despite these caveats, the most common animal model used to study NEC[18] involves some combination of inducing hypoxia, exposing the animal to a cold environment, infusing pathogenic bacteria or proinflammatory bacterial components such a lipopolysaccharide (LPS), and feeding the animals with a formula that is very different than the milk provided by the mother.[19] It is highly unlikely that this model is relevant to NEC seen in the human preterm baby.

Piglets have been used as models for NEC largely because the porcine gastrointestinal (GI) tract shares similarities to that of humans, but there are also important differences. In contrast to the human neonate, one critical difference is that piglets require mother's colostrum or infusion of immunoglobulin G (IgG) to prevent death from a sepsis-like syndrome.[20]

One rodent model uses a chemical inhibitor of intestinal Paneth cells and ingestion of pathogenic *Klebsiella* during the later stages of preweaning.[21] Whether this model expresses fidelity to the disease seen in humans is unlikely because it uses damage to a single cell type to induce disease, whereas it is more likely that true inflammatory NEC involves a multifactorial pathophysiologic cascade.[22]

"Classic" Necrotizing Enterocolitis Pathophysiology

As we discuss what is considered the most "classic" form of NEC, it is critical to remember that some of our knowledge is derived from patient databases that are diluted with "imposters" as well as animal and cell culture studies that may not accurately simulate the disease seen in human preterm infants. The focus on single pathophysiologic pathways may also be misleading because this disease involves multifactorial predispositions along with pathophysiologic cascades culminating in intestinal necrosis that may best be understood using rapidly developing systems network-based illustrations.

One of the forms of intestinal injury that has been termed NEC involves acute antecedent inflammation but is also associated with several other factors.[12,16–19] Factors that are associated with this inflammatory injury include characteristics associated with intestinal immaturity: intestinal barrier immaturity, immature immune response, and an immature regulation of intestinal blood flow.

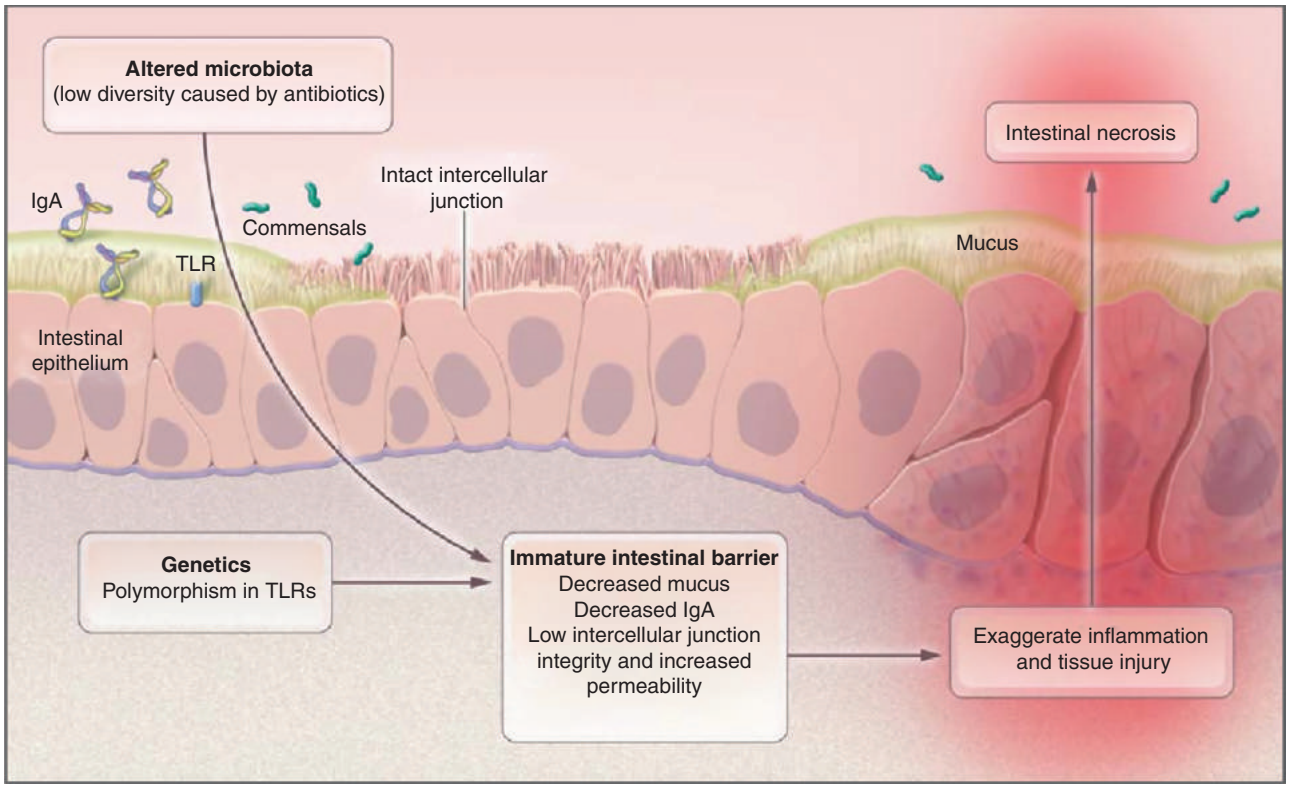
Based on observations of concordance in twins, there is suggestion that there is a genetic component to the pathogenesis of this form of intestinal injury. However, genome-wide association studies (GWAS) and exome-sequencing based studies are limited by the lack of adequately powered replication cohorts to validate the accuracy of these discoveries.[23]

Intestinal Microbiota, Mucosal Immune System, and Vascular Immaturities

The environment affects the taxonomy and function of intestinal microbiota. Although a precise pathophysiologic cascade has not been based on solid mechanistic evidence, an overall likely scenario has been envisioned (Fig. 64.1).

Intestinal Mucosal Immune System

An in-depth review of intestinal mucosal immune system development is beyond the scope of this chapter. The reader is referred to more comprehensive reviews of this topic.[24] Here we will summarize some of the main components as they are related to development of NEC.



• **Fig. 64.1** Conceptualization of Intestinal Injury, Which Could Be Termed "Classic Necrotizing Enterocolitis (NEC)." Etiopathogenesis of inflammatory intestinal injury in the preterm neonate. Acting in concert with host immaturities, a dysbiosis disposes to disruption of the intestinal mucosal surface via increased permeability of tight junctions between epithelial cells. This in turn allows for greater interaction of the intraluminal microbes and antigens that incite an inflammatory response that can lead to a cycle of further intestinal injury. (From Neu J, Walker WA. Necrotizing enterocolitis. *N Engl J Med*. 2011;364:255-264.)

Although most attention is paid to the developing intestinal immune system after birth, it is becoming obvious that there is considerable development that occurs prior to parturition in the fetus, and this is a largely unexplored area. The development of the intestinal mucosal immune system is programmed to develop in different phases. These phases can be disrupted by various environmental circumstances, which we will discuss after our brief summary of some of the main components of the intestinal mucosal immune system.

The first layer of defense encountered by a pathogen or foreign antigen is the acidic environment of the stomach.[25,26] Although primarily designed for digestive processes, these enzymes also are able to destroy many pathogens and immunogenic proteins. On the small intestinal surface, there are additional defense mechanisms. These defense mechanisms include production of mucus by goblet cells, which hinders microbial adherence in concert with polymeric secretory immunoglobulin A (sIgA) that binds luminal antigens. Peristalsis is key because it keeps the antigen-antibody complexes moving aborally to be subsequently eliminated in the stool.[27] Paneth cells from the bottom of the crypts secrete antimicrobial peptides (AMPs).[28] Interepithelial junctions contain various intercellular proteins that maintain junctional integrity and keep the epithelial cells in close proximity to prevent paracellular passage.[29,30] Cell types such as dendritic cells, undifferentiated and differentiated T cells, and plasma cells interact in a manner that depends on the type of stimulatory milieu present in the intestine for the incitement of proinflammatory and antiinflammatory responses, as well as providing a homeostatic balance between these processes. Dendritic cells are of the macrophage-monocyte lineage that capture luminal antigens, process inflammatory signals, and then migrate to secondary intestinal lymphoid organs to interact with T-cell lymphocytes.[24]

Intestinal epithelial cells, macrophages, and other intestinal cells carry both cell membrane and intracellular receptors involved in inflammatory responses; the best known of these being the Toll-like receptors (TLRs). TLRs recognize and bind to highly conserved and specific pathogen-associated molecular patterns (PAMPs) or microbe-associated molecular patterns (MAMPs). TLR4, one of the receptors that has been provided considerable attention in the pathogenesis of NEC,[31] is preferentially expressed by enterocytes within crypts. The intracellular pathway commonly associated with TLR4 and some of the other TLRs includes activation of nuclear factor kappa B (NF-κB), which is a critical transcription factor inciting inflammatory responses in the developing intestine.[32]

The fetus and preterm infant usually respond to luminal microbial stimuli with inflammation.[33] This response can be beneficial.[34] However, an abnormal intestinal microbial profile associated with an excessive immature inflammatory response is a critical pathophysiologic component of the "classic" form of NEC (see Fig. 64.1). When in proper balance, they contribute to innate immunity by maintaining a healthy epithelial barrier.[24] However, imbalances may occur. Specific microbial components such as LPS stimulate the production of proinflammatory cytokines and

initiate a downstream signaling cascade that may upset the balance of proinflammatory and tolerizing mechanisms. These intestinal mucosal interactions are complex and require additional investigation to attain clinical diagnostic and therapeutic relevance. Antibodies in the intestinal tract are also of importance. Studies of secretory IgA appear to play a role in prevention of intestinal injury in both humans and animal models.[35,36]

Microbial Colonization and Dysbiosis

It is becoming increasingly recognized that the microbial environment of the intestine plays an important role in inflammatory bowel injury in preterm infants.[37] Microbes harbor various agonists that interact with the aforementioned intestinal receptors, which incite an inflammatory response. Of interest, these same inflammatory agonists can also induce tolerance and produce various metabolites that may be either injurious or protective to the intestine depending on dose and presence of other mediators.[24]

Over the past two decades, research stemming from the Human Microbiome Project has allowed for molecular identification of many microbes that are difficult to culture when using conventional techniques. Evaluation of fecal microbiota from infants in whom NEC was diagnosed and compared with control samples suggests that NEC is associated with altered intestinal microbial taxa prior to the development of the disease.[16] From these studies, an imbalance in microbes with differing inflammatory and metabolic potentials is associated with pathogenesis. The microbial ecology of the intestine appears to be an important feature that predisposes to intestinal inflammatory injury such as that seen in NEC. However, to develop preventative approaches, we need a better understanding of how these microbes and their metabolites interact with the developing intestine. Such studies should use newly emerging multiomic approaches integrated with prospective randomized studies exploring different environmental conditions, as will be discussed next.[38]

Antibiotics

Antibiotics are routinely prescribed in very low birthweight (VLBW) neonates after birth due to fear that preterm delivery is secondary to infection. This is despite the low incidence of culture-positive, early-onset sepsis. There is considerable evidence showing that antibiotics in the early perinatal and neonatal period, even when provided for short periods, are associated with intestinal dysbiosis,[39–43] which has been linked to short-term adverse outcomes that include bronchopulmonary dysplasia, late-onset sepsis, NEC, and retinopathy of prematurity.[16,44–56] On the other hand, one large observational study suggests a lower incidence of NEC after early antibiotic use.[57] Whether or not such routine antibiotic use is warranted can be at least partially addressed with a prospective, randomized, multicenter, appropriately powered trial.[58] Such a trial should take into account not only clinical outcomes but also multiomic features, which will provide an improved mechanistic framework for pathogenesis and prevention.

Human Milk

It is very difficult to perform controlled randomized studies between human milk (especially that derived directly from the infant's own mother) versus formula. Nevertheless, studies in human infants comparing the relation of type of diet to intestinal barrier function have demonstrated increased intestinal permeability in those receiving formula. This suggests either a compromise in intestinal barrier function[59] from the cows' milk or intestinal protection from the human milk. Infants fed predominantly breast milk also have a reduced incidence of infections[60] and are less likely to develop NEC compared with infants fed formula.[61] The numerous bioactive factors present in fresh breast milk include live microbes, immunoglobulins, cells, enzymes, growth factors, oligosaccharides, and IgA, to name a few, all of which may play a protective role against NEC and other intestinal injuries.

Clinical Presentation

The most typical initial signs and symptoms of NEC in a preterm infant include feeding intolerance, bloody and/or mucus-containing stools, periumbilical erythema, and abdominal distention (Fig. 64.2), usually occurring at a peak of 29 to 32 weeks' corrected gestational age.

Evaluation

Ultrasound and Radiographs

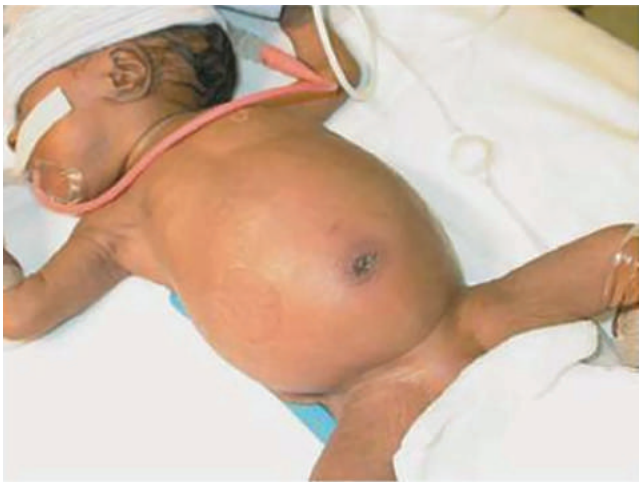
Bowel ultrasound is a modality that is becoming of major interest in the evaluation of bowel injury in neonates. Fig. 64.3 shows the appearance of NEC on abdominal radiographs, and Fig. 64.4 shows some of the salient features on ultrasound in preterm infants diagnosed with NEC.

Laboratory Findings

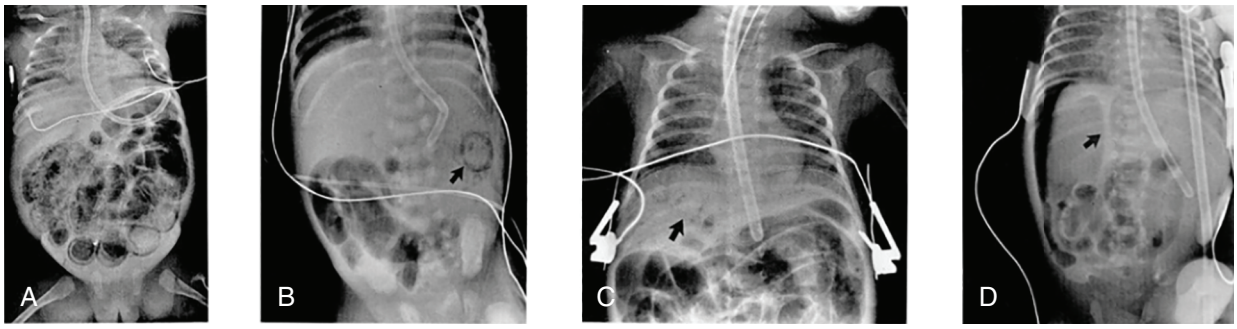
Several biomarkers, including C-reactive protein, white blood cell, and platelet counts, are often used to help establish the diagnosis of NEC but of themselves are not of sufficient sensitivity, specificity, or accuracy to be of high diagnostic value. We need better biomarkers that accurately predict the full expression of NEC.[62]

Differential Diagnosis

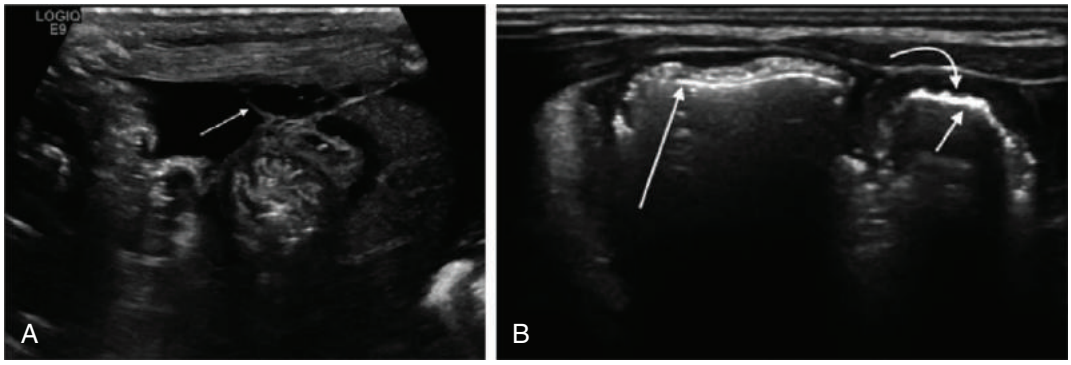
As seen in Table 64.1, there are several forms of intestinal injury in the neonate ("imposters") that have been called NEC. There have been recent strides to establish a more accurate definition for NEC based on the fact that these are different pathophysiologic entities.[9,63] Here we discuss the other forms of intestinal injury that are sometimes labeled an "NEC."



• **Fig. 64.2** Preterm Infant with Abdominal Distension and Necrotizing Enterocolitis. (Courtesy Dr. David Kays, Department of Pediatric Surgery, University of Florida.)



• **Fig. 64.3** (A–D) Radiologic Findings of Necrotizing Enterocolitis (NEC). Findings used to diagnose NEC using abdominal radiography are pneumatosis intestinalis, portal venous gas, or both. Extraluminal air ("free air") outside the bowel can be a sign of advanced NEC or spontaneous intestinal perforation. (A) Several dilated air-filled loops of bowel in the right lower quadrant indicate a focal ileus. The arrow points to submucosal air in the splenic flexure. (B) Film of the chest and abdomen shows massive air-distended bowel with diffuse intramural air. (C) Air in the portal system *(arrow)*. (D) Left lateral decubitus film of the abdomen shows free intraperitoneal air from a bowel perforation. Free air is shown on both sides of the falciform ligament *(arrow)*. (From Neu J. Necrotizing enterocolitis: the search for a unifying pathogenic theory leading to prevention. *Pediatr Clin North Am*. 1996;43(2):409–32.)



• **Fig. 64.4** (A, B) Ultrasound Findings of Necrotizing Enterocolitis (NEC). Focal fluid collection with septations *(arrow)* indicating bowel perforation. (From Alexander KM, Chan SS, Opfer E, et al. Implementation of bowel ultrasound practice for the diagnosis and management of necrotising enterocolitis *Arch Dis Childhood Fetal Neonatal Edition* 2021;106:96–103.)

TABLE 64.1 Stages of Necrotizing Enterocolitis and Some Examples of "Imposters" of Necrotizing Enterocolitis

Name of Entity	Major Diagnostic Criteria	Treatment	Pitfall
Stage 1 NEC	Clinical signs in baby: feeding intolerance, vomiting, abdominal distension, gastric residuals.	Observation, but often NPO, antibiotics, frequent blood monitoring, radiographs.	Seen very commonly in extremely preterm infants, those on nasal or mask ventilation, being monitored for gastric residuals.
Stage 2 NEC	Clinical signs can be similar to those above but also include radiographic criteria such as pneumatosis intestinalis and/or hepatic portal venous gas.	NPO, gastric suction, antibiotics for 7–10 days, frequent lab and radiographic monitoring.	Benefits of antibiotics and length of antibiotic treatment unknown. Radiographic signs can be misleading, especially a bubbly appearance in the bowel where a radiographic reading is "cannot rule out NEC."
Stage 3 NEC	Abdominal distension, pneumoperitoneum on radiograph, necrosis diagnosed at laparotomy or autopsy.	Usually surgical emergency, with drain placement and/or exploratory laparotomy with resection of diseased bowel.	May be readily confused with spontaneous intestinal perforation or congenital bowel disease if bowel is not directly visualized. This is common when only a drain is placed without direct examination of the bowel.
Spontaneous Intestinal Perforation (SIP)	As above with Stage 3 NEC but minimal bowel necrosis.	Surgical emergency with drain placement and/or exploratory laparotomy. Resection usually not required.	As above—often confused with surgical NEC, but treatment and prognosis are different. Pathophysiology is also different high level of inflammation.

Continued

TABLE 64.1 Stages of Necrotizing Enterocolitis and Some Examples of "Imposters" of Necrotizing Enterocolitis —cont'd

Name of Entity	Major Diagnostic Criteria	Treatment	Pitfall
Ischemic Bowel due to Cardiac Anomalies	Abdominal distension. Most commonly associated with low left ventricular output such as seen in hypoplastic left heart, aortic atresia, interrupted aortic arch.	As with Stage 2 or 3 NEC.	Complexities associated with heart disease may cause hesitation to do abdominal surgery.
Variants of Food Protein Intolerance Syndrome (FPIES)	Abdominal distension can be seen but not pathognomonic. Often presents with bloody stools, may have eosinophilia.	Responds well to highly elemental diet.	Does not have good biomarkers to differentiate from other intestinal injuries.

NEC, Necrotizing enterocolitis; *NPO*, nil per os.

Data from Lure AC, Du X, Black EW, et al. Using machine learning analysis to assist in differentiating between necrotizing enterocolitis and spontaneous intestinal perforation: a novel predictive analytic tool. *J Pediatr Surg*. 2021;56:1703–1710; Lueschow SR, Boly TJ, Jasper E, et al. A critical evaluation of current definitions of necrotizing enterocolitis. *Pediatr Res*. 2022;91:590–597.

Cardiogenic Intestinal Ischemia

One form of intestinal injury, sometimes incorrectly called "NEC," is secondary to ischemia of the intestine caused by a low blood flow state, most commonly associated with heart diseases in human infants. These cardiac defects include hypoplastic left ventricle, interrupted aortic arch, or other etiologies that lead to inadequate perfusion of the intestine, thereby not providing adequate oxygen delivery. This form of intestinal injury should be classified as a different entity and receive a name such as cardiogenic ischemic intestinal necrosis.[22]

Spontaneous Intestinal Perforation

SIP is associated with several risk factors, including severe prematurity, early postnatal steroid or indomethacin exposure, as well as a combination of the two.[64] Chorioamnionitis and the stress-related elevated cortisol that accompanies it are risk factors for SIP. Antenatal indomethacin may also be a risk factor when given close to the time of birth, especially when there is coexposure to antenatal steroids.[65,66] SIP is most often seen in extremely preterm, low birthweight infants within the first week after birth. The pathophysiology of SIP remains poorly understood. Histopathologic evaluation in SIP reveals focal perforations and necrosis of the muscularis externa without clear signs of ischemia.[64] The factors associated with the onset of SIP suggests attenuation of the muscularis in the ileum.[67] Whether this is causally related to antenatal steroids, indomethacin, or the combination remains unclear, but studies in rodent models suggest that steroids may induce intestinal atrophy.[68]

Food Protein Intolerance Enterocolitis Syndrome

FPIES is a hypersensitivity to food antigens that is non–IgE mediated. FPIES was previously thought to occur in infants only in the first months after birth outside of the neonatal period. However, a small case series of neonatal intensive care unit (NICU) patients with FPIES and clinical features comparable with typical NEC has been reported.[69] Signs and symptoms of FPIES are similar to NEC and include vomiting, diarrhea, hematochezia, or lethargy with exposure to a triggering antigen. The gold standard for FPIES diagnosis is a positive response to an oral food challenge. Studies suggest that certain laboratory trends are common to these patients but, by themselves, do not confirm the diagnosis. Leukocytosis with an eosinophil predominance, thrombocytosis, hemoccult positive stools, anemia, hypoalbuminemia, and radiographic findings of pneumatosis intestinalis may be seen with FPIES. Recent studies have shown that patients who subsequently develop FPIES have relatively high eosinophil counts in their cord blood.[70] More recent evidence suggests that the pathogenesis of FPIES relates to marked activation of innate immune and neuroendocrine pathways, but how these relate to specific recognition of antigens in foods remains poorly understood.[71] Clinically, differentiating FPIES from NEC is challenging. Eosinophilia is more likely to be present in FPIES, and babies with FPIES show tolerance to feeding with hydrolyzed or amino acid–based formulas.

Importantly, the treatment of FPIES and NEC is quite different. Intravenous antibiotics and bowel rest is recommended for NEC. However, for FPIES, a change in the diet to a protein hydrolysate is usually most appropriate.[72]

Medical Management

NEC is a very difficult disease to treat because no therapy or intervention definitively slows or stops the progression of the disease process. Usual therapy consists of immediate initiation of broad-spectrum antibiotics, cessation of feedings, and gastric decompression. Specific antibiotic regimens for treating NEC are not data driven but include broad-spectrum antibiotics that cover typical enteric flora, including gram-negative and anaerobic coverage. In patients who require surgical intervention, intraoperative cultures may help guide antibiotic therapy. The length of antibiotic therapy also is not data driven, but 7 to 10 days is a common length of treatment. Some centers may use inflammatory markers such as C-reactive protein to guide length of therapy.

Once these measures are incorporated, close observation of the patient's clinical status is used to guide therapy. If the patient is stable or improving with these measures, these medical measures are continued until there is improvement of the abdominal examination, radiographs, and/or ultrasound findings. Once the clinical course has stabilized for several days, antibiotics are discontinued, and if that baby no longer requires gastric decompression, enteral feedings are carefully resumed.

If the patient deviates from a stable clinical course with signs and symptoms such as hemodynamic instability, respiratory compromise from abdominal distention, thrombocytopenia, anemia, or disseminated intravascular coagulation, this should prompt strong consideration for surgical intervention.

Surgical Management

Multicenter cohort analyses show that approximately 50% of VLBW neonates diagnosed with NEC receive laparotomy, primary peritoneal drainage (PPD), or both.[73,74] Widely accepted indications for surgery are intestinal perforation or continued clinical deterioration despite maximal medical management.[75] The goals of surgical intervention include controlling enteric soilage and, if laparotomy is performed, the resection of frankly necrotic bowel.[76] NEC is the most common cause of pediatric SBS; hence it is important to preserve as much viable small intestine as possible.[77] In cases of extensive NEC, "second look" laparotomies, usually done within 24 to 48 hours of the initial procedure, may be necessary to better differentiate viable from nonviable bowel. If there is concern for abdominal compartment syndrome, a silo closure is a useful temporizing measure.

Laparotomy Versus Primary Peritoneal Drainage as Initial Therapy for NEC

Surgical therapy for NEC classically consists of exploratory laparotomy, resection of segments of necrotic bowel, and creation of ostomies.[78] In selected cases of minimal NEC, primary repair may also be effective.[79,80]

Bedside PPD under local anesthesia was first reported as a stabilizing modality for small or very ill neonates with severe NEC.[81,82] Interestingly, approximately 30% of surviving patients treated in this manner recovered intestinal function without the need for further surgery.[74,82] Over time there has been greater utilization of PPD for the treatment of NEC.[83] A multicenter analysis found that the use of PPD as the initial surgical therapy for NEC in VLBW neonates born in the United States has increased in frequency from 23% to more than 45% between 2006 and 2017.[73]

A Cochrane review of two relatively small randomized controlled trials[84,85] failed to show a statistically significant difference in 90-day mortality between initial PPD versus initial laparotomy treated neonates with NEC.[86] Due to sample size restrictions, no definitive recommendations were possible. A large cohort study confirmed that the frequency of subsequent laparotomy in initially PPD treated neonates with NEC approaches 50%.[74]

One recent randomized control trial compared outcomes of neonates with NEC or SIP treated with initial laparotomy vs. PPD and found no difference in mortality or neurological impairment at 18-22 months' corrected age. However, on Bayesian analysis, it appears that preoperative diagnosis of NEC vs. SIP modifies the effect of initial treatment. Overall, there is need for further investigation to fully elucidate the differences between PPD and laparotomy as initial surgical interventions for NEC.[85a]

Outcomes

Mortality

In VLBW neonates, the mortality of medical NEC declines linearly as birthweight increases.[74] Surgical NEC mortality is significantly higher than medical NEC for each birthweight range and tends to maintain a plateau of 30% even in patients with birthweights from 1000 to 1500 g.[74] The overall mortality of laparotomy-confirmed NEC in VLBW neonates is 38% as compared with a significantly reduced mortality of 19% for laparotomy-confirmed SIP.[12] The ischemic bowel, with or without pneumatosis, that is sometimes loosely referred to as "NEC" in term babies with severe congenital heart disease has an associated mortality of 23% if abdominal surgery is required and only 8% if the bowel injury can be managed medically.[87] In 5% of gastroschisis patients, pneumatosis is demonstrated, and it is often, somewhat confusingly, also termed NEC, although the pathogenesis is likely quite different than that found in VLBW neonates. The mortality of gastroschisis-associated "NEC" is 5%, whereas the baseline mortality of gastroschisis without "NEC" is 2%.[88]

The VON has recently completed a 12-year multicenter cohort analysis examining changes in the mortality of NEC in VLBW infants. That study found that mortality decreased significantly year over year during the study period, from 20.6% to 16.5% for medical NEC and from 36% to 31% for surgical NEC.[73] Of note, this improved survival was observed despite a concomitant decline in median birth weights. Interestingly, the decrease in surgical mortality was similar for both the initial PPD and initial laparotomy groups.[73]

Necrotizing Enterocolitis-Associated Strictures

The development of intestinal strictures is the most common delayed surgical complication of NEC patients. Clinically significant strictures usually manifest with feeding intolerance and abdominal distension 4 to 8 weeks after the initiation of feeds. Their presence is confirmed with contrast studies, and the majority require an operation to resolve the obstruction.[89] It is estimated that the incidence of medical NEC-related strictures is upwards of 15%, with the colon being the most common site.[89–91] In the absence of obstructive symptoms, routine contrast studies are not needed. Asymptomatic strictures incidentally found on diagnostic imaging do not require surgical intervention.

Neurodevelopmental Disability

There is little question that surgical NEC is associated with an increase in adverse neurologic outcomes. A VON follow-up of ELBW neonates indicated that 38% of those with surgical NEC had severe neurodevelopmental disability at approximately 2 years of age, which was significantly higher than baseline.[92] Early descriptive studies comparing PPD to laparotomy suggested possibly improved neurodevelopmental outcomes in patients undergoing initial laparotomy versus PPD.[93–95] Unfortunately, all of these studies were confounded by selection bias, with a tendency for PPD to be reserved for ELBW and severely ill neonates. In contradistinction, a VON cohort of ELBW neonates did not show any difference in neurologic outcomes between initial PPD versus initial laparotomy, and with risk adjustments there was, in fact, an indication that the PPD group may have had less neurologic impairment.[96]

Prevention

Currently, there are very few preventative strategies for NEC, but provision of baby's own mother's milk appears to be protective. Beyond the evidence for protection by human milk, there are several notions not based on clear evidence regarding the prevention of NEC. These include very slow feeding advancement, holding feedings during transfusion, and not feeding infants treated with certain vasoactive drugs. None of these is substantiated with clear evidence.

Prebiotics, probiotics, postbiotics, and synbiotics are proposed for the prevention of NEC. Metaanalyses of numerous studies suggest benefit to the routine use of probiotics as a group. However, in the United States, probiotics are categorized as nutritional supplements and thus are not regulated by the US Food and Drug

Administration (FDA). Hence no commercially available probiotics have been approved by the FDA.[97]

Not all probiotics are the same, and there are no adequately powered randomized controlled prospective trials that show both safety and benefit in the prevention of NEC. Thus little guidance is available in terms of which probiotics should be used, their dosage, which age group should be most appropriately targeted, and what kind of feedings should be used as an adjunct (prebiotics). If used routinely, any such agent should undergo rigorous prospective evaluation for safely and efficacy along with regulatory guidance.[98] In a 2021 Clinical Report from the American Academy of Pediatrics (AAP), the AAP Committee on Fetus and Newborn[99] stated that, given the lack of FDA-regulated pharmaceutical-grade products, conflicting data on safety and efficacy, and potential for harm in a highly vulnerable population, the current evidence does not support the routine administration of probiotics to preterm infants, particularly those with a birthweight of less than 1000 g.

The Future of Necrotizing Enterocolitis

To make progress in the field of NEC, it is important to understand that prevention is the best strategy. We first need to have a better definition and understanding of the different entities that are called NEC, and they need to be clearly differentiated in the various databases that are analyzed for research in this field. In addition, there are likely different pathophysiologies and treatments for these entities. Using multiomic and artificial intelligence technologies, we are on the verge of considerably more accurate predictive analytics which will guide our therapies.[38,100]

Short Bowel Syndrome

Epidemiology

Pediatric intestinal failure (IF) may be defined as a reduction in functional gut capacity with an inability to meet nutrient, fluid, and electrolyte requirements, ultimately manifesting as growth failure. This is an umbrella diagnosis that includes actual bowel loss which is termed SBS, motility disorders (i.e., pseudo-obstruction), and congenital mucosal disorders (i.e., microvillus inclusion disease). SBS is the most common form of IF in children, and NEC is the most frequent cause of SBS in the pediatric population.[101] Other frequent etiologies of childhood SBS include complicated gastroschisis, intestinal atresia, and midgut volvulus.

Animal models use an 80% to 90% small bowel resection model to create SBS.[102] However, a simple functional definition of SBS in children is intestinal loss resulting in a parenteral nutrition (PN) requirement of at least 90 days.[103,104] The achievement of independence from PN or "enteral autonomy" is a strong predictor of survival in SBS.[105,106] Therefore the overarching goal in the management of SBS is to attain enteral autonomy, and if that is not possible, to maintain normal growth using both enteral and intravenous means while mitigating the negative effects of long-term PN.

Management

The optimal management of SBS requires coordinated interdisciplinary care and has resulted in the formation of regional intestinal rehabilitation centers. These programs integrate the work of pediatric surgeons, pediatric gastroenterologists, neonatologists, dieticians, nurses, pharmacists, social workers, and feeding team specialists. Such efforts have resulted in a substantive survival benefit for children with SBS.[107,108]

Enteral Autonomy

There are various factors that can influence a patient's ability to attain enteral autonomy. First, the intestine has an innate ability to adapt and maximize its absorptive potential. Various mechanical and humoral factors have been implicated in intestinal adaptation, which often results in some bowel dilation as well as increased villous height and crypt depth.[101,109–111] In SBS patients, persistently dilated bowel seen on plain abdominal x-rays is sometimes mistaken as a sign of a distal obstruction or stricture. Another major factor that aids in achieving PN independence is that neonatal protein and energy needs markedly decrease on a per kilogram basis as patients age.[101] Quantitatively, there is a four- to fivefold reduction in macronutrient requirements from the neonatal period to late adolescence. Other considerations associated with a higher likelihood of achieving enteral autonomy include greater residual small bowel length, the presence of an intact colon, and the absence of intestinal dysmotility. The presence of an ileocecal valve is not consistently associated with enhanced weaning from PN, and this may be secondary to variable amounts of terminal ileum associated with a preserved valve. The terminal ileum has unique functional properties including the absorption of fat-soluble vitamins, zinc, and vitamin B_{12}. The underlying diagnosis causing pediatric SBS also affects the likelihood of attaining enteral autonomy with NEC patients having the highest success rate of weaning from PN.[105,112]

Parenteral Nutrition

PN is a lifesaving therapy for SBS patients and is necessary for adequate growth. Although vital, PN is associated with complications including intestinal failure–associated liver disease (IFALD), central line–associated bloodstream infections (CLABSIs), and deep vein thrombosis (DVT).[101]

The development of IFALD is likely multifactorial, including PN composition, lack of enteral stimulation, the presence of prematurity, and recurrent sepsis. The replacement of the standard soybean-based lipid emulsion in PN with fish oil–based lipids, which are omega-3 rich and low in phytosterols, leads to improvement of IFALD.[113,114] In addition, restriction of intravenous soy-based lipid to a total allotment of 1 g/kg per day can both prevent and reverse IFALD in pediatric patients with SBS.[115] Many centers now routinely treat infants who have significant direct hyperbilirubinemia with lipid emulsions low in soy or lipid restriction to avoid the complications of IFALD. A balancing factor in neonates treated with relatively low lipid allotments is the need for high glucose infusion rates.

Enteral Nutrition

The goal of intestinal rehabilitation is to wean PN support while maximizing enteral intake. Enteral nutrition effectively improves IFALD[116] and eventually eliminates the need for central venous access. Even SBS children with established cirrhosis have a 95% long-term transplant-free survival if they are successfully transitioned to full enteral nutrition.[106]

The prompt initiation of enteral feeding after bowel resection has a favorable impact on achieving enteral autonomy.[103] Utilizing breast milk appears to augment intestinal adaptation in neonatal SBS and is linked to a decrease in the number of PN days required.[117] If human milk is not available, amino acid–based formulations are often favored as they manifest enhanced enteral tolerance compared with hydrolyzed formulas.[118–120] Amino acid formulas also minimize the risk of allergic enteritis.

The theoretic advantage of bolus feeding compared with continuous feeding is its cyclical nature, which more closely simulates the normal release of GI hormones.[121] However, some studies have shown that continuous feeding is more easily tolerated and is associated with improved nutrient absorption and growth.[122–124] The usual progression of enteral nutrition in SBS consists of low-rate continuous feeds that are slowly increased with respect to volume while paying close attention to weight gain, stool output, and signs of feeding intolerance. Suitable patients are further transitioned to bolus feeds (ideally oral) during the day and receive continuous feeds overnight to help maximize absorption. Oral aversion is a common sequela of SBS. With this in mind, it is important to introduce oral feeding, even if it is nonnutritive, to help encourage oral-motor development.[118,125]

Medical Management

The prevalence of micronutrient deficiencies is high in pediatric SBS.[126] The segments of intestine that are missing as well as the absorptive capacity of the remaining bowel influence the micronutrient deficiency states that are present. It is important to carefully monitor micronutrient levels and provide appropriate supplementation. For patients with high stool output, antimotility agents such as loperamide are of benefit and help prolong transit time and absorption.

Children with gastric dysmotility may benefit from prokinetic agents such as erythromycin (motilin receptor agonist).[127] SBS patients with massive bowel resections have been shown to have gastric hypersecretion, for which histamine (H_2) blockers and proton pump inhibitors are helpful for amelioration. However, this increase in gastric output is transient, and the protracted administration of acid suppressive agents has been linked to an elevated risk of infections and bacterial overgrowth.[101,128]

In general, patients with SBS have an increased capacity for developing small bowel bacterial overgrowth (SBBO). This is, in part, due to the altered intestinal microbiome, lack of enteral stimulation, as well as the presence of dilated and dysmotile segments of intestine that can foster bacterial colonization. Symptoms of SBBO include poor growth, decreased enteral tolerance, and occasional D-lactic acidosis. SBBO can be treated with empiric enteral antibiotic regimens, and if this is unsuccessful, endoscopically obtained duodenal aspirate cultures can more precisely direct therapy.[129] The use of probiotics tends to be avoided in SBS patients with central venous catheters in situ. This is due to the risk of CLABSIs from probiotic seeding of the line or translocation of gut bacteria.[130]

Glucagon-like peptide 2 (GLP-2) is a hormone with a significant effect on intestinal adaptation. It stimulates proliferation of the small bowel epithelium and delays gastric emptying.[131] Teduglutide, a substituted GLP-2 analogue with a prolonged half-life, has been shown to increase villous height and crypt depth, thereby increasing intestinal absorptive capacity.[132] As found in adults, a recent randomized control trial of teduglutide administration in pediatric patients with SBS demonstrated a reduction in PN support, increase in enteral nutrition, and higher serum citrulline levels (a biomarker of intestinal mucosal mass).[133]

Studies examining the safety and efficacy of teduglutide are restricted to patients older than 1 year of age and greater than 10 kg. Hence, at this time, teduglutide is not an intervention recommended for neonates. Long-term teduglutide therapy does potentially increase the risk of developing GI neoplasms and may cause bowel obstructions, so careful follow-up is required.

Surgical Management

The hallmark of the surgical management of SBS is the preservation of as much viable small intestine as possible. Colonic salvage is also important to promote fluid homeostasis and enhance the absorption of some nutrients.[134] In addition, the establishment of bowel continuity with prompt stoma closure optimizes intestinal absorptive surface area.

Surgical bowel lengthening and tapering operations are considered for select SBS patients with bowel dilation. Indications for surgery are failure to advance enteral feeding despite full medical management, severe SBBO resistant to enteral therapy, and neonatal bowel obstruction with very limited residual small bowel length. Techniques for bowel lengthening include the longitudinal intestinal lengthening and tapering (LILT) procedure[135] and now the more commonly used serial transverse enteroplasty (STEP).[136] The International STEP Data Registry reported that, after STEP, about half of children reached enteral autonomy and two-thirds demonstrated enhanced enteral tolerance.[137]

Transplantation is used as a salvage strategy for SBS patients with progressive IFALD, loss of venous access, recurrent life-threatening CLABSIs, and complete mesenteric thrombosis and in some individuals with extremely short residual bowel length resulting in a lifelong dependence on PN. The 5-year patient survival for isolated intestinal transplantation is 75% and 62% if both liver and intestine are transplanted.[138]

The long-term patient and graft survivals in pediatric intestinal transplantation have improved.[139] Coincident with development of multidisciplinary intestinal rehabilitation programs, there has been a reported 25% reduction in the number of intestinal transplants performed in the United States.[139]

Outcomes

Advances in the care of patients with SBS over the past two decades have led to multidisciplinary teams now reporting long-term transplant-free survivals of greater than 90%.[104] This improvement extends to children with "ultrashort" bowel, which is defined as having less than 20% of expected small bowel length. A study evaluating outcomes in patients with ultrashort bowel secondary to midgut volvulus found that long-term survival in the context of multidisciplinary IF care was 91%. Remarkably in this cohort, greater than 50% of patients who did not have concomitant gastroschisis were able to achieve enteral autonomy without transplantation.[140] Similarly, children discharged from the NICU with severe NEC, defined as having residual small bowel lengths of less than 30 cm, had a 93% survival with a median follow-up of 8 years in a multidisciplinary setting.[141] Those weaning completely from PN had a median small bowel length of only 22 cm. Given these findings, the dated term "NEC totalis" is perhaps best abandoned. A precise determination of residual small bowel length is more appropriate when assessing prognosis and goals of care in the neonatal setting.

Cycled home PN allows children with SBS to lead relatively normal lives where attending school, exercise, and outside activities are all possible. However, SBS can negatively impact the quality of life of both patients and their families.[142] There are ongoing investigations to quantify and improve the quality of life of these children. Furthermore, as patients with pediatric SBS are now routinely living to adulthood, additional longitudinal studies to evaluate pubertal development, childbearing potential, and neurodevelopmental sequelae are required.[104]

Suggested Readings

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Disorders of the Liver

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KEY POINTS

- Early onset (<24 hours of age) or new jaundice is never normal and should be investigated.
- Initial evaluation of a jaundiced infant should always include conjugated and unconjugated bilirubin levels.
- Infants presenting with jaundice secondary to conjugated hyperbilirubinemia should undergo expedient evaluation for potentially life-threatening and treatable causes of cholestasis and then for other causes.

Neonatal Liver Disease

The liver is the largest abdominal organ and serves as the body's main location of energy production, metabolism, protein synthesis, and detoxification. Embryologically, the liver is derived from an endodermal outgrowth from the foregut into the septum transversum. There are multiple cell types within the liver parenchyma, including hepatocytes, cholangiocytes, stellate cells, endothelial cells, and cells of the innate immune system. The key roles of the hepatocyte include protein synthesis, fatty acid synthesis and oxidation, formation of lipids, cholesterol and bile salts, bilirubin metabolism, gluconeogenesis, glycogen synthesis, urea cycle and production of ammonia, and detoxification. The hepatocyte is the only cell in the body that manufactures albumin, fibrinogen, and prothrombin clotting factors. In the setting of significant liver injury, loss of normal hepatocyte synthetic function often results in the development of coagulopathy and hypoalbuminemia.

Bile is primarily composed of bile acids, bilirubin, cholesterol, and phospholipids, which are manufactured in the hepatocyte, secreted into the canaliculus, transported into the biliary ducts, and, ultimately, secreted into the intestine or stored within the gallbladder. Bile is critical for solubilizing dietary fats and fat-soluble vitamins (A, D, E, and K) to make them available for absorption. Disruption of this process at any level results in cholestasis. Cholestasis refers to obstruction of the normal excretion of bile from the liver resulting in the abnormal accumulation of bile components within the liver and serum. While cholestasis is not synonymous with jaundice from conjugated hyperbilirubinemia, serum conjugated bilirubin level is the most clinically useful marker of cholestasis.

Like the lungs, the liver is unique in that it has a dual blood supply: nutrient-rich venous blood from the portal vein, and oxygen-rich arterial blood from the hepatic artery. Venous drainage of the liver occurs through the hepatic veins. In the fetus, the umbilical vein delivers oxygenated blood to the liver via the left portal vein and the ductus venosus, which then joins the left hepatic vein as it drains into the inferior vena cava. The ductus venosus closes spontaneously at birth. Congenital anomalies of the portal vein or thrombosis associated with umbilical vein catheter placement may lead to portal vein obstruction and portal hypertension. Portal hypertension describes a pathologic increase in venous pressure in the portal venous system and occurs when the portal pressure rises above 10 mmHg. Portal hypertension may be prehepatic (portal vein), intrahepatic (cirrhosis), or posthepatic (hepatic venous) in etiology. Signs and symptoms of portal hypertension include splenomegaly, ascites, and gastrointestinal bleeding secondary to bleeding varices.

Neonates with liver disease may present with an array of clinical signs ranging from asymptomatic jaundice to acute liver failure. Clinical evaluation and management differ depending on underlying etiology. Clinical signs suggestive of underlying liver disease include ascites, hepatomegaly with or without splenomegaly, coagulopathy, elevated transaminases, hyperammonemia, hypoglycemia, and cholestasis. The initial evaluation of suspected liver disease must include a careful physical examination, laboratory evaluation, and imaging. The initial laboratory evaluation for suspected liver disease includes an assessment of hepatocellular injury and function, including aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyl transpeptidase (GGT), conjugated and unconjugated bilirubin levels, prothrombin time/international normalized ratio (INR), and albumin levels. Elevations in serum ALT and AST indicate hepatocellular injury; however, they are not specific to the liver and may be found in other tissues such as red blood cells and skeletal muscle. An elevation in ALP and GGT may indicate biliary obstruction or inflammation; however, care must be taken in children, as elevated ALP is also indicative of normal bone growth and metabolism. ALP can be particularly elevated in preterm infants with osteopenia of prematurity. As stated previously, an elevation in transaminases (AST, ALT) indicates hepatocyte injury, whereas an elevation in prothrombin time/INR and hypoalbuminemia indicates a loss of normal hepatocyte function and most likely a greater degree of hepatocyte injury. The most appropriate initial imaging modality is abdominal ultrasound (US) with Doppler, which can detect anatomic or vascular anomalies, thrombosis, or suggest underlying portal hypertension.

Liver disease in the neonate frequently presents with new or persistent jaundice. Infants presenting with new jaundice deserve an urgent evaluation, as this is never normal. Term infants with jaundice persisting beyond 14 days and preterm infants with jaundice beyond 21 days of life deserve expedient evaluation.[1,2] In any jaundiced infant, it is necessary to determine whether jaundice

is due to conjugated versus unconjugated hyperbilirubinemia. Although the differential of potential causes will vary by the type of predominant bilirubin, it is prudent to prioritize the evaluation to identify conditions where early intervention is associated with improved outcome.

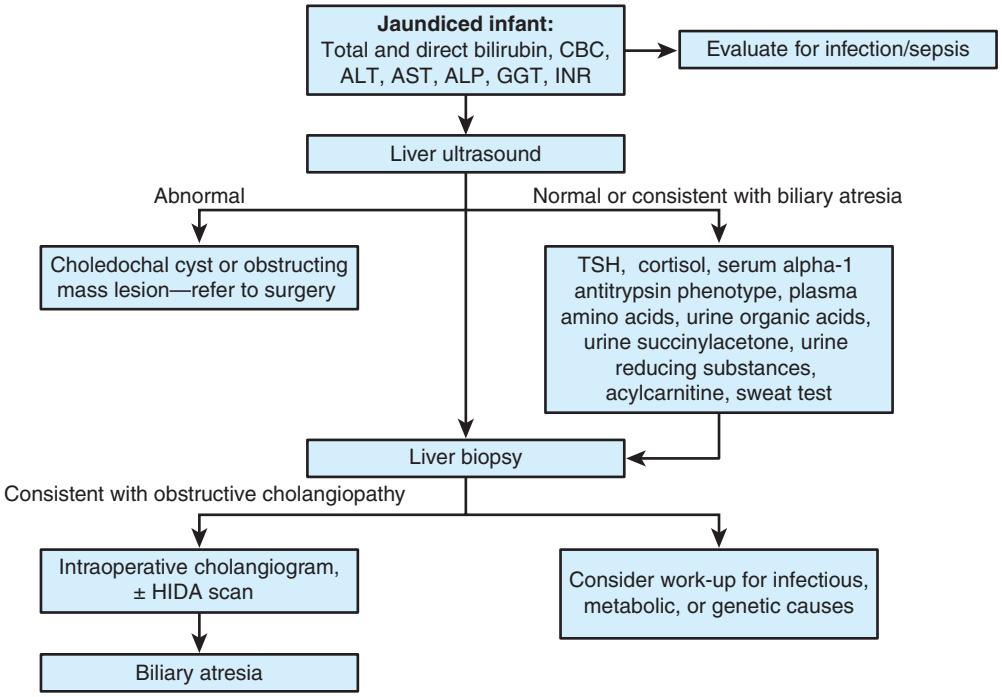
Elevated unconjugated hyperbilirubinemia is often seen secondary to breast milk jaundice, sepsis, hemolysis secondary to blood group incompatibility (ABO and rhesus) or red blood cell dyscrasia, and, more rarely, Crigler–Najjar syndrome. While physiologic jaundice and breast milk jaundice are common, jaundice secondary to unconjugated hyperbilirubinemia should still be investigated if it is of very early onset (within 24 hours of life), prolonged beyond 14 days, or at high levels. Breast milk jaundice is the most likely etiology of unconjugated hyperbilirubinemia if the serum level is downtrending; there is no evidence of hemolysis, infection, abnormal thyroid function, or elevated serum aminotransferases; and the child is clinically well. However, if unconjugated hyperbilirubinemia persists or is rising, or the child is ill, disorders influencing bilirubin conjugation and hemoglobin metabolism should be investigated.

The presence of conjugated hyperbilirubinemia (defined as conjugated bilirubin level ≥15% of the total) should raise concern for underlying liver disease. An initial approach to the evaluation of neonatal conjugated hyperbilirubinemia is shown in Fig. 65.1. Specific etiologies of neonatal cholestasis are reviewed below and summarized in Table 65.1. Idiopathic neonatal hepatitis is a term historically applied to infants presenting with neonatal cholestasis or hepatitis in whom no specific etiology can be identified. Liver biopsies in these infants often demonstrate nonspecific intrahepatic cholestasis and giant cell transformation of hepatocytes (Fig. 65.2).[3] Currently, it is recognized that multinucleated giant cells represent a stereotypical response by the immature liver to many etiologies of hepatocellular injury, including infection, biliary obstruction, and metabolic disease. Today, with advancements in next generation DNA sequencing, the number of identifiable etiologies of neonatal cholestasis and hepatitis has increased dramatically, further reducing the frequency and utility of the idiopathic neonatal hepatitis diagnosis.

Cholestatic Liver Disease

Cholestasis refers to obstruction of the normal excretion of bile from the liver, resulting in abnormal accumulation of bile salts, bilirubin, and lipids in the blood. In infants, cholestasis may present as asymptomatic jaundice, pruritus, unexplained fat-soluble vitamin deficiency, or acute liver failure. The presence of acholic stools suggests functional or anatomic biliary obstruction. The following sections review the most common etiologies of neonatal cholestasis and discuss the corresponding disease-specific evaluation and clinical management.

Nutritional management is critical and central to the care of infants with chronic cholestasis. Growth failure commonly occurs secondary to malabsorption from inadequate bile flow, intestinal congestion from portal hypertension, and increased caloric needs in the setting of chronic liver inflammation. The estimated daily caloric intake for infants with chronic cholestasis may approach 150% of that of healthy infants.[4] In addition, malabsorption of fat-soluble vitamins (A, D, E, and K) can result in progressive coagulopathy and pathologic fractures. While enteral nutrition is preferable, some infants go on to require nutritional optimization with total parenteral nutrition. The general principles of nutritional management in cholestatic infants include assurance of adequate absorbable calories and nutrients, monitoring levels of and supplementation with fat-soluble vitamins, and preparation to escalate the nutritional support and supplementation in infants who cannot sustain adequate intake for growth orally, with



• **Fig. 65.1** Algorithmic Approach to the Evaluation of an Infant With Conjugated Hyperbilirubinemia. *ALP*, Alkaline phosphatase; *ALT*, alanine aminotransferase; *AST*, aspartate aminotransferase; *CBC*, complete blood count; *GGT*, gamma-glutamyl transpeptidase; *HIDA*, hepatobiliary iminodiacetic acid scan; *INR*, international normalized ratio; *TSH*, thyroid-stimulating hormone.

TABLE 65.1 Causes of Neonatal Cholestasis

Infection

Herpes simplex virus
Cytomegalovirus
Adenovirus
Hepatitis B
Sepsis/urinary tract infection
Cholecystitis
Cholangitis

Endocrine

Hypothyroidism
Panhypopituitarism
Adrenal insufficiency

Metabolic/Genetic

Galactosemia
Tyrosinemia type 1
Dubin-Johnson syndrome
Rotor syndrome
Bile acid synthesis defects
α-1 Antitrypsin deficiency
Cystic fibrosis
Defects of bile transport (progressive familial intrahepatic cholestasis)
Peroxisome biogenesis disorders

Cardiovascular

Heart failure
Shock
Hepatic ischemia

Syndromic

Trisomy 21
Trisomy 13
Trisomy 18
Joubert syndrome
Ivemark syndrome
Beckwith-Wiedemann syndrome
Bardet-Biedl syndrome

Biliary

Biliary atresia
Choledochal cyst
Alagille syndrome
Choledocholithiasis
Neonatal sclerosing cholangitis
Caroli disease
Obstruction from mass or stricture

Nutritional

Total parenteral nutrition

supplemental enteral feeds via nasogastric tube, or initiation of parenteral nutrition in situations of failure to grow or gain weight adequately with maximal enteral nutrition. The selection of formula should consider medium-chain triglyceride (MCT) content, as this fat source is directly absorbed into the portal venous system and does not require emulsification by bile acids or active transport that is disrupted in cholestasis. Children with portal hypertension and ascites also benefit from sodium restriction.

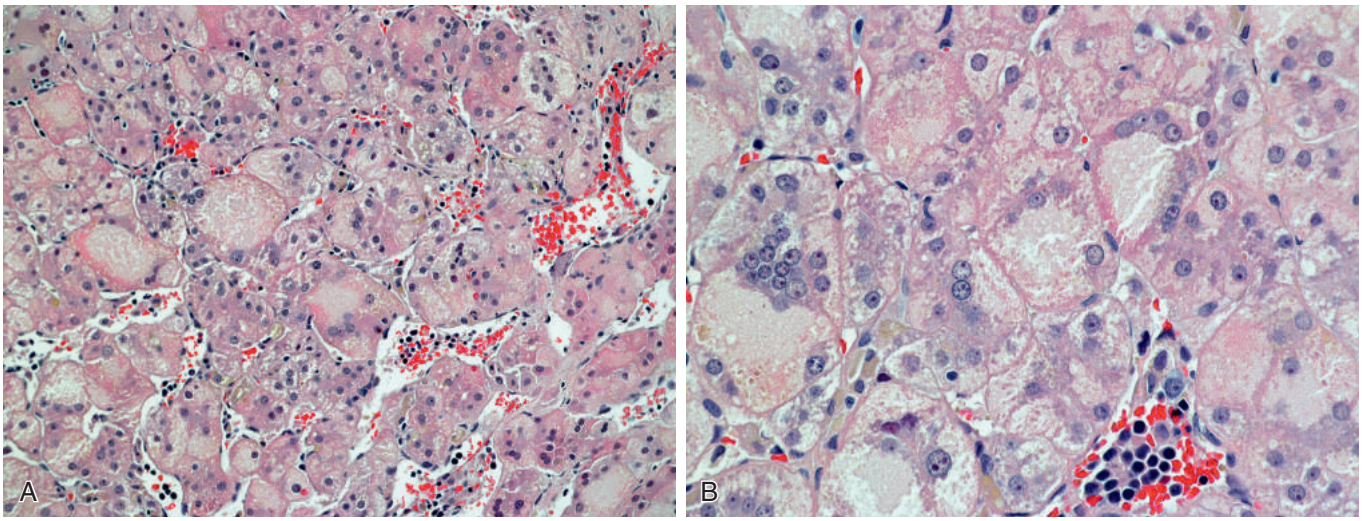
Biliary Atresia

Biliary atresia (BA) is an idiopathic hepatobiliary disorder of infancy characterized by inflammation and progressive fibrosis resulting in the obliteration of the extrahepatic biliary ducts within weeks of birth.[5,6] As the disease progresses, the end result is variable destruction and obliteration of the intrahepatic bile ducts with subsequent biliary cirrhosis. BA is the most common cause of infantile chronic liver disease and the most frequent indication for liver transplantation in the pediatric population. The reported incidence of BA is 0.5 to 3.2 per 10,000 live births but varies based on ethnicity and geography with higher incidence in the Far East and Oceania. Babies with BA generally present between 2 and 5 weeks of age. Without rapid intervention the natural history of BA is uniform fatality by 2 years of age.

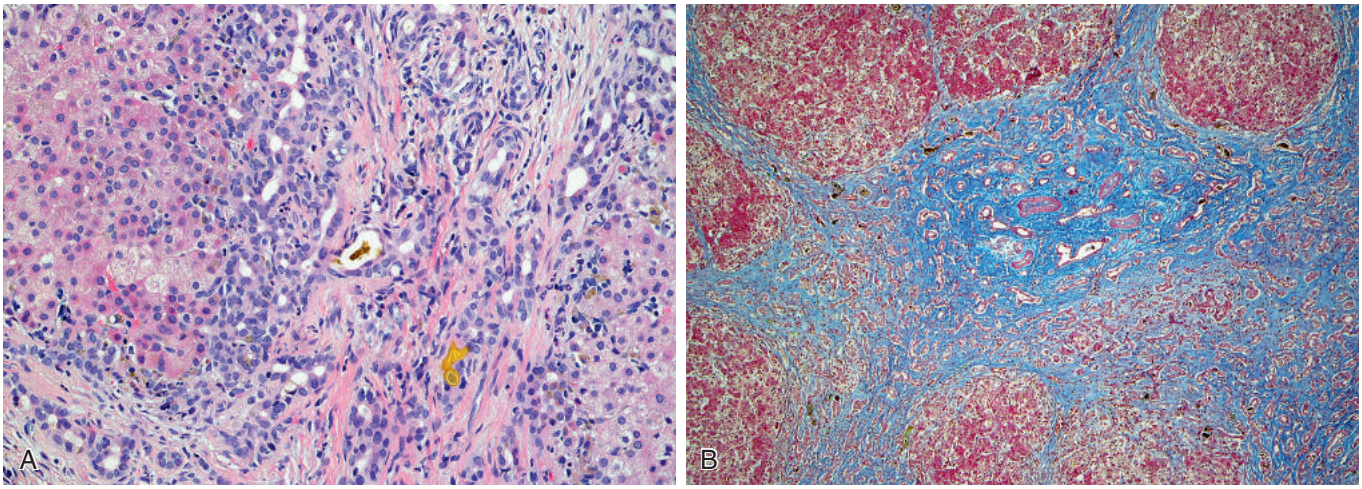
Biliary atresia generally presents in one of two broad clinical phenotypes: sporadic/nonsyndromic, which is the more common form (85% to 90%) and is not typically associated with other congenital anomalies; and embryonic/syndromic/congenital form, which accounts for 10% to 15% of the cases, and is associated with non-hepatic anomalies. The embryonic/syndromic/congenital form is commonly associated with non-hepatic congenital anomalies such as situs inversus, polysplenia or asplenia, vascular and cardiac malformations, and intestinal malrotation. In a large North American multicenter study, the latter group was further divided into two distinct subgroups based on the presence of laterality defects. The group with at least one major malformation, but without laterality defects, has a higher incidence of genitourinary defects like cystic kidney and hydronephrosis. The other group is syndromic with laterality defects.[7]

BA typically presents with cholestatic jaundice and hepatosplenomegaly between 2 and 5 weeks of age. Acholic stools suggest biliary obstruction and are frequently present, but onset is commonly well after the onset of jaundice. If the infant also had a preceding history of physiologic jaundice, the cholestatic jaundice may not be recognized as new; this highlights the importance of evaluating any prolonged or new jaundice in infants. Although splenomegaly is commonly present at diagnosis, other signs of portal hypertension such as ascites generally occur later in the course of disease. As chronic inflammation and cholestasis lead to malabsorption, many infants with BA present with inadequate weight gain.

Expedient differentiation of BA from other causes of neonatal cholestasis is critical, as surgical intervention before 2 months of age has been shown to improve surgical success and outcome.[8,9] If early laboratory evaluations are suggestive of BA, consultation with a pediatric hepatologist is mandatory. Laboratory evaluation early in the course of disease typically demonstrates conjugated hyperbilirubinemia between 2 and 7 mg/dL and total bilirubin levels between 5 and 12 mg/dL.[4] Elevations in ALT, ALP, and GGT are generally seen.[4] Abdominal US is recommended as the first-line imaging modality and may demonstrate absence of the gallbladder after adequate fasting or a fibrotic remnant of extrahepatic bile duct. However, the presence of the gallbladder does not necessarily exclude BA. In the porta hepatis, a triangular or tubular echogenic cord of fibrous tissue representing the biliary remnant may be described as "triangular cord sign." The reported sensitivity of this sonographic finding is 80%.[10] As a follow-up to abdominal US, hepatobiliary scintigraphy with technetium-labeled iminodiacetic acid derivatives (HIDA scan) may be used to assist in the differentiation between obstructive and nonobstructive causes of neonatal cholestasis.



• **Fig. 65.2** Neonatal Hepatitis Histology. (A) Low power of a hematoxylin and eosin stain from a liver biopsy demonstrating hepatocytes with giant cell transformation and ballooning. Some cholestasis is evident. (B) Higher power of same biopsy shows the hepatocytes with giant cell transformation as well as a focus of extramedullary hematopoiesis.



• **Fig. 65.3** Biliary Atresia Histology. (A) Hematoxylin and eosin stain of a liver biopsy from a 3-month-old girl demonstrating a proliferation of bile ductules. Bile plugs are present. (B) Masson trichrome stain from a liver transplant specimen from the same girl at 8 months of age. This specimen has diffuse cirrhosis with fibrous expansion of portal tracts. The portal triads lack bile ducts, but there is a marked bile ductule reaction, many containing bile plugs.

In BA, radionucleotide scans demonstrate rapid update of tracer but absence of excretion into the bowel at 24 hours. The sensitivity of the HIDA scans may be increased by pretreatment with oral phenobarbital (5 mg/kg/day) for 5 days. Care must be taken, however, to not delay definitive diagnosis by awaiting scan results and recognition that functional causes of cholestasis (such as hypothyroidism) can also result in a nonexcreting HIDA scan. Percutaneous liver biopsy is helpful in excluding alternate causes of cholestasis. Histopathologic findings supportive of a diagnosis of BA demonstrate bile ductular proliferation and bile duct plugging (Fig. 65.3). Given the progressive nature of BA, the histologic findings will vary with the point in progression. Matrix metalloproteinase-7 (MMP-7), a protease involved in tissue remodeling, was found to have good accuracy in BA diagnosis. However, more studies are needed to further validate its use in clinical practice.[11]

Failure to exclude BA after the above evaluation is complete necessitates surgical exploration with intraoperative cholangiogram. The diagnosis of BA may be made or confirmed at the time of laparotomy with the observation of an atretic biliary tree and intraoperative cholangiogram demonstrating lack of patency in the biliary ductal system. If BA is confirmed, surgical intervention with a Kasai hepatic portoenterostomy is recommended. The Kasai is a surgical procedure that works to restore the normal flow of bile by excising the obstructed bile ducts and creating an anastomosis of a jejunal limb of a Roux-en-Y with the liver at the porta hepatis, the area of the liver from which the bile ducts become extrahepatic. Restoration of bile flow may prevent or delay progression of disease, worsening of fibrosis, and development of end-stage liver disease. However, despite Kasai portoenterostomy, most children progress to cirrhosis and portal hypertension and ultimately require liver transplantation.

Choledochal Cysts

Choledochal cysts are congenital dilations of the biliary ducts which can impede bile flow, leading to liver injury. The incidence of choledochal cysts is high in the Asian population with a female predominance. Choledochal cysts can present at any age, including infancy. However, 80% of choledochal cysts are diagnosed in the first decade of life, with cholestasis being the most common sign in infants, and cholangitis or pancreatitis being less common. Choledochal cysts are associated with increased risk of choledocholithiasis, ascending cholangitis, and liver cirrhosis. There are five types of choledochal cysts based on the location of biliary dilation. The most common forms are type I (spherical or fusiform dilation of the extra hepatic biliary tree) followed by type IV (includes multiple cysts that can involve intrahepatic and extrahepatic biliary tree). The pathogenesis of choledochal cyst formation is not entirely clear. However, anomalous pancreaticobiliary union may play a role by causing reflux of pancreatic secretions into the bile duct. Diagnosis is usually made with abdominal ultrasound. Choledochal cysts need to be excised surgically, and in their entirety whenever possible. Continued surveillance for malignancy (cholangiocarcinoma and squamous cell carcinoma) is warranted after excision due to increased risk over the general population. Although not well defined for pediatric ages, screening should include regular biochemical evaluation and imaging (ultrasound or cross-sectional CT).[12,13]

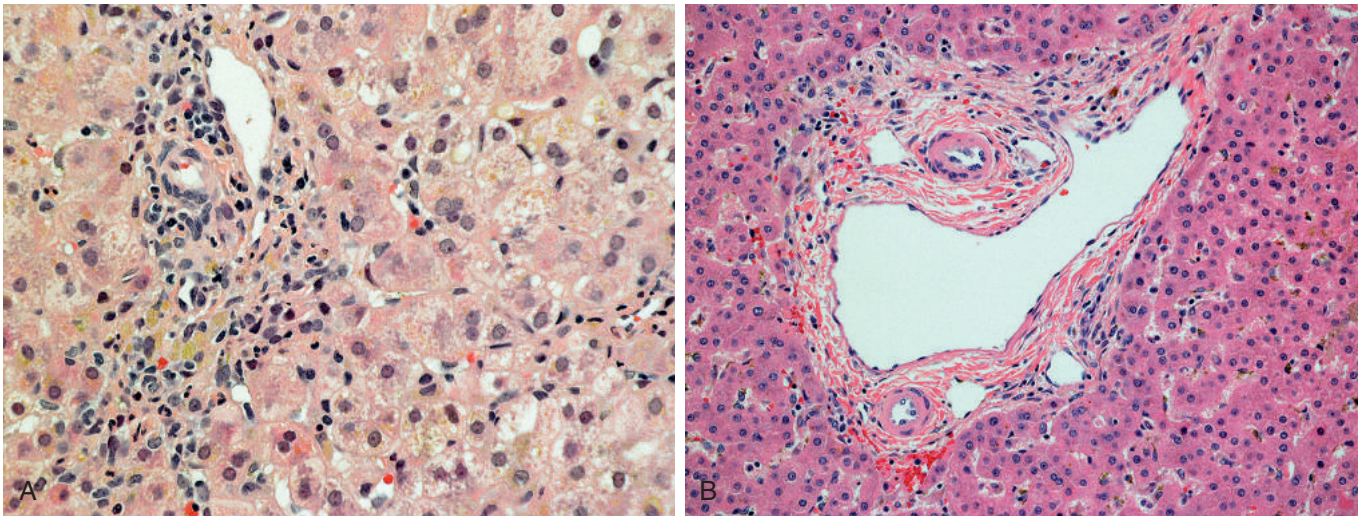
Alagille Syndrome (Arteriohepatic Dysplasia)

Alagille syndrome (ALGS) is an autosomal dominant or sporadic de novo genetic disorder with variable penetrance, characterized by chronic, progressive cholestasis secondary to a paucity of intralobular bile ducts. The estimated prevalence is 1 in 30,000 live births.[2] The majority (more than 90%) of children with ALGS carry a mutation in the gene *JAG1*, located on chromosome 20.[14–16] The product of *JAG1* is a ligand in the Notch signaling pathway that plays a key role in embryogenesis and the pathogenesis of the disorder. A small number of infants with ALGS have mutations in *NOTCH2*.[17,18]

ALGS is a multisystem syndrome characterized by cholestatic liver disease, stereotypical facial features, congenital heart disease, ocular abnormalities (most commonly posterior embryotoxon which represents prominence of Schwalbe line), skeletal abnormalities (butterfly vertebrae and rib abnormalities), and renal disease. Other features include short stature, as well as vascular and dental abnormalities. Most infants with ALGS present within the first 3 months of life with cholestasis; however, those with severe extrahepatic manifestations (usually caused by associated congenital heart disease) may present at birth or even be identified by prenatal US. Although many forms of congenital heart disease have been associated with ALGS, the most common is peripheral pulmonary stenosis. The characteristic facial features are frequently difficult to appreciate in the neonatal period but include a prominent forehead and pointed chin, giving the face a triangular appearance, deep-set eyes with hypertelorism, and a saddle nose.

When cholestatic jaundice occurs in the first 6 weeks of life, care must be taken to discriminate ALGS from alternate etiologies of neonatal cholestasis, particularly BA and other treatable etiologies for which timely initiation of treatment may change the outcome. Initial evaluation for ALGS should include serum biochemistries, abdominal US, and echocardiogram when a heart murmur is identified. In ALGS, the conjugated hyperbilirubinemia is associated with elevated serum aminotransferases and GGT, reflective of the biliary involvement. If there is clinical concern for ALGS, a spinal radiograph should be obtained to evaluate for hemivertebra or butterfly vertebra, and an ophthalmologic evaluation for posterior embryotoxon is recommended.

Although a liver biopsy is not required for the diagnosis of ALGS when other stereotypical syndromic features are present, biopsy should be performed when the diagnosis is in question. The histopathology in ALGS is characterized by bile ductular paucity; however, this is not pathognomonic. In the preterm infant the number of bile ducts is normally diminished, and hence care must be taken to not incorrectly make the diagnosis of pathologic paucity (Fig. 65.4).[19] In term infants and older children, the normal bile duct to portal tract ratio ranges from 0.9 to 1.8; ratios less than 0.9 are suggestive of paucity. Given the normal



• **Fig. 65.4** Alagille Syndrome Histology. (A) Hematoxylin and eosin stain of a liver biopsy from a 3-month-old boy demonstrating loss of bile ducts in a portal triad. This bile duct paucity is accompanied by hepatocanalicular cholestasis. The hepatocytes also demonstrate swelling. (B) At 10 months of age, another boy was transplanted for Alagille syndrome. On hematoxylin and eosin stain, his liver demonstrated paucity of bile ducts in the portal triads. Mild hepatocanalicular cholestasis is also present in this biopsy.

developmental progression of the biliary system in infancy and the importance of excluding BA, infants with cholestatic jaundice and elevated GGT usually require liver biopsy and hepatobiliary scintigraphy. Additionally, an intraoperative cholangiogram to verify patency of the extrahepatic biliary system may be required.

Metabolic bone disease is especially striking in ALGS. Elevated ALP commonly reflects abnormal bone metabolism in addition to the biliary disease. Serum bile salt levels can be extremely elevated, even in the absence of jaundice, leading to intractable and refractory pruritus. Hypercholesterolemia and hypertriglyceridemia can lead to the development of xanthomas, most prominent on extensor surfaces and areas of minor trauma, including the diaper area and plantar surfaces of feet, abdomen, and neck.

Treatment of ALGS is directed at improving or maintaining adequate nutrition, treating the complications if there is cholestasis, and supporting the cardiovascular health. The introduction of this section has details regarding nutritional management of cholestatic infants. About 21% to 31% of patients who fail medical therapy will require liver transplantation.[20]

α-1 Antitrypsin Deficiency

α-1 Antitrypsin (A1AT) deficiency is the most common genetic cause of liver disease and affects approximately 1 in 2000 live births.[21–23] It is an autosomal codominant disorder that results in a reduction in α-1 antitrypsin, a serine protease produced in the liver whose role is to inhibit other proteases and elastases that can lead to cellular destruction. The genetic defect results in a single amino acid substitution within the A1AT protein, resulting in abnormal molecular folding, and inability of the protein to be processed beyond the Golgi apparatus. Excessive hepatic accumulation of the abnormal A1AT protein results in hepatocellular injury.

The clinical phenotype of A1AT deficiency includes both liver and pulmonary manifestations. Pulmonary disease generally manifests in adulthood, whereas liver involvement commonly presents in neonates. Liver disease in A1AT is most often characterized by cholestatic jaundice, which can develop in 10% of infants with PI ZZ genotype. Hepatosplenomegaly and ascites can also be seen in the most advanced cases.[24]

A1AT deficiency is diagnosed by serologic testing and liver histology. The most specific serum test is A1AT phenotyping (Pi type). While commonly used as a less costly and widely available screening test, the serum levels of A1AT can be falsely elevated into the normal range in times of systemic inflammation or infection and so should not be used in the diagnosis of A1AT. A1AT variants are named according to their electrophoretic migration pattern.[25] The normal protein is designated M, and the S and Z variants are the most common, leading to a reduction in serum A1AT. The homozygous PiZZ is named because it has the slowest gel migration and causes the most severe disease phenotype. Generally liver disease manifests only in PiZZ, PiSZ, or, rarely, PiMZ variants.[26]

The classic, but not pathognomonic, histologic finding in A1AT deficiency is periodic acid-Schiff (PAS)-positive diastase-resistant eosinophilic globules within the hepatocytes. These globules represent the accumulated abnormal protein trapped within the endoplasmic reticulum. Liver histology may also demonstrate bile duct destruction, proliferation, and, potentially, bile duct paucity, making it important to exclude BA and ALGS.

Although recombinant A1AT has been used for the treatment of the pulmonary manifestations, management of the associated liver disease is primarily supportive, as there are no specific or targeted therapies currently available. As in all disorders resulting in cholestasis, fat malabsorption is common with A1AT deficiency. Cholestatic infants usually benefit from MCT-rich formula to aid the fat absorption and supplementation with fat-soluble vitamins as needed. Additionally, ursodeoxycholic acid may be utilized to improve bile flow; however, no study has demonstrated clear benefit. Historically, breastfeeding was thought to be of some benefit. However, in a study comparing formula-fed with breastfed infants, no benefit was found.[27,28] To prevent acceleration of pulmonary manifestations, including early emphysema, avoidance of smoking and environmental pollution is critical.

For infants and children with end-stage liver disease, liver transplantation is indicated. As most A1AT is manufactured in the liver, the recipient assumes the donor's Pi phenotype and, posttransplant, experiences normal serum levels of the functional protein, decreased risk of pulmonary disease, and no chance of recurrent disease in the transplanted organ.

Cystic Fibrosis Liver Disease

While cystic fibrosis (CF) is common, affecting approximately 1 in 2500 births in North America, CF-related liver disease is uncommon in the neonatal period. It is estimated that less than 2% of infants with CF present with cholestasis.[29] Given the low incidence of CF-related liver disease in neonates, testing for CF beyond state-mandated newborn screens should be reserved for those infants in whom alternate causes of cholestasis have been excluded or in infants with other typical features of CF, including meconium ileus or inadequate weight gain despite theoretically adequate caloric intake.

Disorders of Bile Acid Synthesis

There are several steps in the synthesis of bile acids that may be disrupted, leading to an accumulation of hepatotoxic bile acid intermediates. Bile acid synthesis disorders are rare autosomal recessive disorders, with a prevalence of 1 in 50,000 in the general population. In the neonatal period, phenotypes of bile acid synthetic disorders include acute hepatitis, acute liver failure, persistent cholestasis, and progressive chronic hepatitis.

Cholic acid and chenodeoxycholic acid are the primary bile acids in humans, and disruption at any step in their synthesis results in the accumulation of toxic intermediate metabolites. Liver injury associated with disorders of bile acid synthesis occurs secondary to direct hepatocellular injury from accumulation of toxic intermediates or secondary to the accumulation of cholesterol, drugs, and other toxins within the liver from abnormal bile excretion.

The age at presentation with a disorder of bile acid synthesis is variable, ranging from early infancy to adulthood. The most common clinical presentation includes neonatal jaundice, failure to thrive, hepatosplenomegaly, metabolic bone disease, and bleeding early in childhood. Pruritus is usually absent. Some disorders are associated with progressive neurologic disease, manifesting with seizures, developmental delay, deafness, blindness, and neuromuscular weakness.

Laboratory testing in infants with bile acid synthetic disorders demonstrates normal or low serum bile acid levels, elevated serum aminotransferases, normal GGT, and complications of fat malabsorption, including fat-soluble vitamin deficiency. If serum bile acids are found to be low, urinary bile acids should be measured

for identification of the particular synthetic defect. Liver biopsies are generally nonspecific and can demonstrate canalicular bile plugging, inflammation without bile duct proliferation, and sometime a picture similar to those observed in idiopathic neonatal hepatitis such as giant cell transformation.[30,31]

Treatment of inborn errors of bile acid synthesis focuses on supporting normal growth and supplementation of fat-soluble vitamins. Treatment with cholic acid is a preferred therapy for the most common disorders of primary bile acid synthesis because cholic acid suppresses the production of the toxic bile acid intermediates. Ursodiol is not indicated as it does not suppress production of abnormal bile acid intermediates.

Progressive Familial Intrahepatic Cholestasis

Progressive familial intrahepatic cholestasis (PFIC) is a group of autosomal recessive disorders characterized by defective bile export leading to cholestasis. This group of disorders is classified based on the genetic mutation, and they are named PFIC 1, PFIC 2, and PFIC 3. Liver disease in PFIC results from accumulation of bile salts within the hepatocytes leading to profound cholestasis, fat-soluble vitamin deficiency, and intractable pruritus.

PFIC 1, also known as Byler disease, is caused by a mutation in the gene *ATP8B1* on chromosome 18q21-22, which encodes for a protein flippase (FIC 1) that facilitates the flipping of aminophospholipids from the outer to inner canalicular membrane. As the gene is also expressed in many extrahepatic tissues, affected individuals may also have short stature, deafness, pancreatitis, and persistent diarrhea.

PFIC 2 results from a defect in the bile canalicular bile salt export pump (BSEP) caused by a mutation in the gene *ABCB11* on chromosome 2q24. BSEP is responsible for transporting bile acids from inside the hepatocyte to the canaliculus. Disruption of BSEP results in accumulation of bile acids within the hepatocyte resulting in severe cholestasis and rapid progression to end-stage liver disease. PFIC 2 presents earlier and is a more rapidly progressive liver disease than PFIC 1. Children with PFIC 2 have an increased risk of developing hepatocellular carcinoma, a risk that persists even after liver transplantation.

PFIC 3 is caused by a mutation in the gene *ABCB4* on chromosome 7q21, which encodes for multidrug resistance–associated protein 3 (MDR3) and mediates flopping of aminophospholipids from the inner to outer canalicular lipid bilayer. Rather than a deficiency in bile acid export, patients with PFIC 3 have a deficiency in phospholipid export. The resultant bile lacks phospholipids, making the micelles unstable and toxic to bile ducts, leading to a progressive intrahepatic cholangiopathy. In contrast to PFIC 1 and 2, only a third of children with PFIC 3 present with cholestasis during infancy, with most presenting in later childhood and adolescence. When infants with PFIC 3 do present with liver disease, they commonly have cholesterol gallstones complicating their intrahepatic cholestasis.

Infants with PFIC generally have markedly elevated serum bile acid levels with only mildly elevated serum bilirubin. The characteristic biochemical markers of PFIC 1 and 2 are a normal or low GGT, normal serum cholesterol, and only mild transaminitis; however, specific diagnosis requires genetic testing. PFIC 3 presents with an elevated GGT in the absence of extrahepatic biliary obstruction. The intrahepatic cholestasis that is commonly seen in PFIC often progresses to end-stage liver disease. Liver biopsy shows canalicular cholestasis and biliary plugs. More pronounced hepatocyte injury and giant cell hepatitis is noted in PFIC 2.[32]

Treatment for PFIC initially focuses on the nutritional management of cholestasis because of insufficient absorption of fat and fat-soluble vitamins. Most dietary fat should be provided from MCT oil, which does not depend on bile salts for absorption. Additionally, aggressive treatment of debilitating pruritus with ursodiol, antihistamines, cholestyramine, rifampin, and opioid antagonists is often required. In refractory cases, treatment may include partial biliary diversion, interruption of the enterohepatic circulation by surgical ileal exclusion, and liver transplantation.[33]

Congenital Hepatic Fibrosis

Congenital hepatic fibrosis (CHF) is a hereditary malformation of the bile ducts resulting from failure of remodeling during embryogenesis. It is an autosomal recessive disorder that may present in isolation but is more often seen as a feature of several syndromes, including Ivemark, Berdet-Biedl, Caroli, and Joubert syndromes; autosomal recessive polycystic kidney disease (ARPKD); and disorder of glycosylation type 1b.

Caroli syndrome is characterized by CHF and ductal ectasia with cystic dilation of intrahepatic ducts. Infants with CHF most commonly present with portal hypertension and splenomegaly, although some present with cholestasis caused by cholangitis. Characteristically, CHF is associated with normal transaminases and intact synthetic function despite advancing portal hypertension. When complicated with cholangitis, transient elevation of GGT and conjugated bilirubin are typically seen. Diagnosis of Caroli syndrome may be made by imaging with abdominal US with Doppler or magnetic resonance imaging (MRI) of the liver with magnetic resonance cholangiopancreatography. When associated with ARPKD, CHF can be diagnosed clinically, but if in isolation, histologic diagnosis is necessary.

Treatment of CHF is aimed at treating complications of portal hypertension and preventing and treating cholangitis. Portosystemic shunting is commonly required to decompress advanced portal hypertension. In Caroli syndrome, focal cystic dilation may be amenable to liver lobectomy, but, if diffuse, liver transplantation is required.

Infections

Congenital or perinatal infections and sepsis are common causes of neonatal liver cholestasis, hepatitis, and sometimes liver failure. A careful history and physical examination may suggest infection as an etiology of neonatal liver disease. For ill-appearing infants with cholestasis, a rapid evaluation for bacterial infection (such as sepsis or urinary tract infection) is recommended. Judicial selection of antimicrobials must be considered, as several are known to exacerbate cholestasis by displacing bilirubin from albumin (e.g., ceftriaxone) or cause direct hepatotoxicity (e.g., sulfamethoxazole/trimethoprim and fluconazole).[2] In addition to common bacterial infections, TORCH infections (toxoplasmosis, rubella, cytomegalovirus, herpes, and syphilis), as well as infections with hepatitis B, parvovirus B19, adenovirus, or echoviruses, can result in neonatal cholestasis and hepatitis. For more detail regarding diagnosis and specific treatments for congenital or neonatal infections, please refer to Part IX of this text, Immunology and Infections.

Parenteral Nutrition–Associated Liver Disease

Parenteral nutrition–associated liver disease (PNALD) is an important and common cause of hepatitis, cholestasis, and

liver-related morbidity in the neonatal period. It is estimated that between 33% and 85% of premature infants who receive parenteral nutrition for more than 7 days develop PNALD.[34,35] The primary phenotypic manifestation of PNALD in infants is cholestasis. When total parenteral nutrition (TPN) is utilized for a short duration, generally less than 2 weeks, the transient liver inflammation generally completely resolves. However, prolonged use increases the risk for irreversible and severe liver disease, which may ultimately result in liver failure.[36,37] PNALD often presents as hepatic steatosis with elevated serum aminotransferases, cholelithiasis, and cholestasis. Several risk factors have been identified that contribute to the development of PNALD, including prematurity, low birth weight, lack of enteral feeding, sepsis, short gut syndrome, and necrotizing enterocolitis.[38,39] The diagnosis of PNALD in an infant receiving TPN is suggested by the presence of a serum conjugated bilirubin level less than 2 mg/dL and ALT greater than two times the upper limit of normal.

Management of PNALD includes early initiation and continuation of enteral feeding, lipid minimization to less than 1 g/kg/day, and prevention of infection. Ursodiol at a dose of 20 to 30 mg/kg/day in divided doses may be utilized to improve or stimulate bile flow.[40–42] Additionally, the use of omega-6 fatty acid or fish oil–based, rather than soy-based, lipid formulations have been shown to be effective at resolving cholestasis.[2] Recognition and early intervention are required to prevent irreversible liver damage, which can progress to end-stage liver disease.

Metabolic Liver Disease

Inborn errors of metabolism often present with elevated serum aminotransferases, hepatomegaly, or metabolic derangements. There are several clinical phenotypes of metabolic liver disease, including acute liver failure, encephalopathy, cholestasis, and isolated hepatomegaly. Tables 65.2 and 65.3 summarize the clinical phenotypes of neonatal metabolic liver disease and etiologies of acute neonatal liver failure.

Disorders of Carbohydrate Metabolism

Galactosemia

Galactosemia results from an inability to metabolize galactose secondary to a deficiency in one of the following: galactokinase, galactose-1-phosphate uridyl transferase (Gal-1-PUT), or uridine diphosphate galactose-4-epimease. Classic galactosemia is caused by gal-1-PUT deficiency. This is the most common cause of galactosemia and results in the inability to metabolize galactose into glucose-1-phosphate. It is an autosomal recessive disorder with an incidence of 1 per 60,000 live births.[43] Abnormal galactose metabolism results in accumulation of toxic metabolites in the liver, brain, kidney, and eye lens.

Classically, galactosemia presents within the first few weeks of life after infants ingest breast milk or milk-based formulas that contain lactose. Presenting symptoms may include failure to thrive, jaundice, vomiting, and diarrhea. Occasionally, infants may present acutely, with *Escherichia coli* sepsis with severe acidosis, jaundice, and coagulopathy. Additional clinical findings may include hepatomegaly, ascites, bleeding, hemolysis, renal tubular acidosis, hypotonia, edema, and bulging fontanelle. Although affected infants will spill reducing sugar in their urine (positive urine reducing substances) while still ingesting galactose, the gold standard of diagnosis is demonstration of a complete absence of Gal-1-PUT activity via a quantitative red blood cell (RBC) assay.

TABLE 65.2 Common Clinical Presentations of Metabolic Liver Diseases in the Neonatal Period

Clinical Presentation	Metabolic Disorder	Laboratory Investigation
Acute liver failure	Galactosemia	Erythrocyte galactose-1-phosphate uridyl transferase activity, DNA mutational analysis
	Tyrosinemia	Urine—succinylacetone
	Hereditary fructose intolerance	Screen—urine reducing substances Genetic testing Enzyme activity analysis
	Mitochondrial defects	Serum lactate, pyruvate
	Fatty acid oxidation defects	Fibroblast enzymatic assay, acyl carnitine profile, genetic testing
	Gestational alloimmune liver disease	Ferritin, liver biopsy with C5b-9 staining
Encephalopathy	Fatty acid oxidation defects	As above (ferritin, liver biopsy with C5b-9 staining)
	Organic acidemias	Urine organic acids
	Urea cycle defects	Serum ammonia level, serum amino acid profile
Cholestasis	Peroxisomal disorders	Specialized screening for urine metabolites such as very long chain fatty acids, pipecolic acid, phytanic acid, pristanic acid
	Lysosomal storage disorders	Liver biopsy, leukocyte glucocerebrosidase activity, genetic mutational analysis
Hepatomegaly	Glycogen storage diseases	Liver or fibroblast enzymatic assay, genetic testing

Many newborn screens may identify variants of the disease, resulting in varying activity of Gal-1-PUT. Patients with clinical variants typically have 1% to 10% enzyme activity, and biochemical variant patients (also known as Duarte variant) show 15% to 35% enzyme activity. It should be noted that, because the assay is of RBC enzyme activity, analysis post-RBC transfusion will give unreliable results. Diagnosis can be established by genetic analysis as well.[44]

Treatment of galactosemia centers on the immediate stabilization of the critically ill infant as well as urgent restriction of galactose from the diet. Treatment is generally supportive and involves intravenous fluids with glucose, vitamin K, antibiotics, and initiation of a soy-based (nongalactose-containing) formula. As infants graduate to solid food, continued avoidance of lactose-containing foods is recommended. Despite treatment, many children with galactosemia will have some degree of developmental delay necessitating close neurocognitive follow up. Referral to ophthalmology is recommended at diagnosis. Infants with "Duarte variant" have initially diminished gal-1-PUT enzyme activity which improves

TABLE 65.3 Causes of Neonatal Acute Liver Failure

Infections

Herpes simplex virus
Human herpesvirus 6
Cytomegalovirus
Adenovirus
Influenza
Hepatitis B
Bacterial sepsis
Enterovirus
Parvovirus B19
Malaria

Metabolic/Genetic

Galactosemia
Tyrosinemia type 1
Hereditary fructose intolerance
Fructose 1,6-bisphosphatase deficiency
Fatty acid oxidation defects
Mitochondrial defects
Urea cycle defects

Immune-Mediated

Gestational alloimmune liver disease
Autoimmune hemolytic anemia with giant cell hepatitis
Hemophagocytic lymphohistiocytosis

Vascular

Ischemia
Heart failure

Toxic

Drugs
Toxins

Neoplastic

Leukemia

after few months. Those patients generally have normal development and can tolerate unrestricted diet.

Galactokinase deficiency results in cataract without other features of classic galactosemia. Cataract results from accumulation of galactitol within the lens. Diagnosis is suspected based on newborn screen or the presence of infantile cataract. Dietary galactose restriction is highly effective if diagnosis is done early. UDP-Galactose-4-Epimerase Deficiency (GALE Deficiency) presentation is a spectrum that varies between asymptomatic to severe symptoms similar to classic galactosemia depending on organs affected.

Hereditary Fructose Intolerance

Hereditary fructose intolerance (HFI) is an autosomal recessive disorder characterized by a deficiency in fructose-1-phosphate aldolase (aldolase B), which is important in both glycolysis and gluconeogenesis, and plays a critical role in the metabolism of fructose. The incidence is approximately 1 per 20,000 live births.[43] Approximately 75% of dietary fructose is metabolized by the liver, with the remainder metabolized by the kidneys and small bowel. The deficiency in aldolase B leads to a toxic accumulation of fructose-1-phosphate and traps phosphate in an unusable form, thus depleting adenosine triphosphate (ATP) stores and, in turn, inhibiting normal gluconeogenesis and glycogenolysis.

Most infants with HFI are healthy until ingestion of fructose or sucrose (disaccharide of glucose and fructose). Upon ingestion of fructose they begin accumulating the toxic metabolites, developing metabolic derangements that lead to vomiting, hepatomegaly, and failure to thrive. Diagnosis may be suspected after a careful dietary history is obtained, and laboratory evidence of acute liver failure, hypoglycemia, and proximal renal tubular acidosis is found. Urine can be tested for the presence of reducing substances, as fructose in the urine will give a positive test, but this is not specific. Definitive diagnosis requires confirmation with genetic testing for mutations in the *ALDOB* gene, located at 9q22.3, or enzyme analysis from liver tissue.

Treatment of HFI requires prompt removal of dietary fructose and sucrose. Complete elimination is seldom achievable, and there are no established thresholds of required restriction. Additionally, as the severity of the enzyme deficiency is heterogeneous, some patients may develop chronic symptoms despite treatment.[45] Fortunately, with nearly complete dietary restriction of fructose and sucrose, most children with HFI display normal growth and development.

Glycogen Storage Diseases

Glycogen is a glucose polymer that is primarily stored in the liver and muscle and is required for glucose homeostasis during fasting. There are 12 recognized glycogen storage diseases (GSD), but only types I, III, and IV primarily manifest as neonatal liver disease.

GSD type I, also known as von Gierke disease (named after its discoverer, Edgar von Gierke), is a rare autosomal recessive disease with an incidence of approximately 1 per 100,000 live births.[43] There are several defects that lead to the clinical manifestation of GSD type I; however, the end result is absence or decreased activity of glucose-6-phosphatase. Infants with GSD type I generally present with hepatomegaly and fasting hypoglycemia, often first noted when children begin to sleep more than 4 hours. Lactic acidosis, hyperuricemia, hyperlipidemia, hyperphosphatemia, neutropenia (specific to GSD type Ib), and bleeding secondary to platelet dysfunction are other clinical manifestations experienced by those affected with GSD type I.

Diagnosis of GSD is confirmed by DNA mutational analysis of the potentially affected genes. GSD Ia accounts for 80% of cases and results from mutation of *G6PC*, the gene for glucose-6-phosphatase, located on chromosome 17q21. GSD Ib represents less than 20% of cases and results from mutations of the gene *G6PT1*, the G6P transporter. Prenatal diagnosis is possible via chorionic villus sampling.

Treatment in infancy consists of maintaining euglycemia with frequent feedings, and continuous enteral feeds overnight with a high-glucose, low-fat formula. In older infants and children, a carbohydrate-balanced diet and frequent feedings that include the addition of cornstarch are recommended. Cornstarch provides a long-lasting source of glucose because it is slowly degraded by α-amylase and hence negates the need for hourly glucose intake. With adequate metabolic and glycemic control, children generally grow and develop normally.

GSD type III (Cori or Forbes disease) is an autosomal recessive disease that results from deficiency of the glycogen debranching enzyme, amylo-1,6-glucosidase, and occurs in 1 in 100,000 live births. The disease has two subtypes: Type IIIa, the most common form, affects both the liver and muscle. Type IIIb only affects the liver. The presentation in infancy is similar to that of GSD type I,

although the hypoglycemia is not usually as rapid and severe, and failure to thrive may be more prominent. In type IIIa, the skeletal muscle (hypotonia) and cardiac manifestations (cardiomyopathy) occur later in childhood. Diagnosis is made by directly measuring the activity of amylo-1,6-glucosidase in the liver or, more recently, doing mutational analysis on the associated gene, *AGL*. Treatment mirrors that of GSD type I, with efforts to maintain euglycemia. The difference in GSD type III is the ability to use protein as a source of glucose since gluconeogenesis in intact.[46]

GSD type IV (Andersen disease) is an autosomal recessive disorder caused by a mutation in the *GBE1* gene, resulting in deficiency of glycogen branching enzyme; it affects roughly 800,000 individuals worldwide and accounts for 3% of the cases of GSD. GSD type IV may present prenatally with hydrops or postnatally with hypotonia, heart failure (secondary to cardiomyopathy), contractures, and muscle atrophy. Diagnosis is based upon the absence of branching enzyme activity in skin fibroblasts or detection of mutations in the *GBE1* gene. Treatment consists of liver transplantation when progression to end-stage liver disease has occurred (usually in the third decade of life); however, care must be taken in patient selection as extrahepatic manifestations may be life-limiting.

Disorders of Amino Acid Metabolism

Tyrosinemia Type 1

Tyrosinemia is an autosomal recessive disorder with an incidence of 1 in 100,000 live births. It results from deficiency of fumarylacetoacetate hydrolase (FAH), the enzyme responsible for the final step of tyrosine degradation.[43] Tyrosinemia generally presents acutely in the neonatal period and should be included in the differential of fulminant neonatal liver failure. Failure to thrive, vomiting, ascites, coagulopathy, hypoglycemia, and hyperbilirubinemia may be the initial presenting signs and symptoms. In older infants and children, a more chronic presentation typified by growth failure, Fanconi syndrome, and neurologic manifestations may develop. Children with tyrosinemia type 1 carry a long-term risk of developing hepatocellular carcinoma. Serum tyrosine level is not a sensitive or specific screening tool. Blood succinylacetone, a more sensitive marker, is now being used in many newborn screening protocols. Diagnosis is confirmed by measuring elevated succinylacetone in urine or blood.[47] Molecular testing for FAH can be done to confirm the diagnosis but should not delay the treatment.

Treatment in the neonatal period consists of correcting metabolic derangements, treating sepsis, and correcting coagulopathy, followed by the restriction of dietary tyrosine and phenylalanine using specific formulas. Treatment with diet alone, however, results in less than 40% survival at 1 year of age.[48–50] Treatment with NTBC (2-(2-nitro-4-trifluromethylbenzoyl)-1,3-cyclohexanedione, nitisinone) has been demonstrated to improve survival to greater than 85% at 1 year of age, and is now the standard of care in the treatment of tyrosinemia.[51] NTBC works by reversibly inhibiting 4-hydroxyphenylpyruvate dioxygenase, hence preventing the formation of maleylacetoacetic acid and fumarylacetoacetic acid, the precursors to the hepatotoxic compound succinylacetone. Dietary changes and NTBC should be initiated as soon as the diagnosis is made.[47]

Maple Syrup Urine Disease

Maple syrup urine disease (MSUD), like other rare disorders of amino acid metabolism, is autosomal recessive and screened for on routine newborn screening tests throughout the United States. MSUD is caused by the deficiency in the branched chain α-ketoacid dehydrogenase complex. The disease results in accumulation of the branch chain amino acids and their toxic ketoacids. Most infants with MSUD present with vomiting, acidosis, and evolving neurocognitive dysfunction or intermittent altered mental status. The disorder is named from the particularly sweet odor, reminiscent of maple syrup, which emanates from the urine of affected infants. Laboratory analysis may reveal mildly elevated transaminases, hypoglycemia, and hyperammonemia. The diagnosis of MSUD is made by the identification of excessive branched chain amino acids (valine, leucine, and isoleucine) in the serum. Treatment is dietary, with restriction of branched chain amino acids by limiting protein intake, supplementation with specialized formula, and care not to induce catabolism. The clinical course and natural history of MSUD and other disorders of amino acid metabolism are characterized by intermittent episodes of metabolic crises, including ketosis, acidosis, and hyperammonemia, which may be treated with intravenous glucose administration, arginine, and, rarely, dialysis. Liver transplantation may be considered for those patients in whom severe metabolic crises are threatening to cause developmental delays.

Disorders of Organic Acid Metabolism

The autosomal recessive methylmalonic acid, isovaleric acid, and 3-hydroxy-3-methylglutaryl (HMG)-coenzyme A (CoA) lyase deficiencies are rare and make up disorders of organic acid metabolism. Most are screened for during routine newborn screening in the United States but are confirmed with the identification of a particular pattern of organic acids in the urine. Clinically, they all present with metabolic crises, similar to MSUD. Treatment is largely by avoidance of catabolism and by tailored diets.

Fatty Acid Oxidation Defects

Fatty acid oxidation (FAO) is responsible for the production of the cellular energy that is required for normal cardiac and skeletal muscle function and glucose homeostasis. FAO largely occurs within mitochondria. The end result of FAO is the production of ketones, which serve as a critical energy source when glucose stores are depleted. Fatty acid oxidation defects are autosomal recessive diseases that include medium-chain acyl-CoA dehydrogenase deficiency (MCADD), long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency (LCHADD), HMG-CoA synthase deficiency, and carnitine-acylcarnitine translocase (CACT) deficiency.

MCADD is an autosomal recessive disorder that affects the metabolism of medium-chain fatty acids and results in fasting hypoglycemia and accumulation of toxic acyl-CoA. The clinical presentation is generally recurrent lethargy, emesis, coma, and even death secondary to life-threatening hypoglycemia. Laboratory evaluation reveals hypoglycemia, hepatitis, hyperammonemia, hypoketosis, and hyperuricemia. Confirmation of diagnosis is made by fibroblast enzymatic assays or genetic testing. Treatment is avoidance of hypoglycemia, but infants are at risk of sudden death due to metabolic derangements, and many develop significant developmental delays.

LCHADD is an error of fatty acid metabolism that affects the mitochondrial trifunctional complex, the primary function of which is to metabolize long-chain fatty acids (found in oils and milk). The classic presentation of a neonate with LCHADD is acute onset of poor feeding in the newborn period, accompanied by lethargy, hypotonia, and hypoketotic hypoglycemia. Older

children may go on to develop neuropathy and retinopathy. LCHADD in a fetus predisposes the mother to develop *he*molysis, *l*iver dysfunction, *l*ow *p*latelets (HELLP) syndrome and acute fatty liver of pregnancy. Diagnosis is suggested by newborn screening and confirmed by measuring LCHADD activity in fibroblasts or muscle or mutational analysis. Treatment is largely dietary, with a low-fat, high-carbohydrate diet and supplementation with MCTs. Morbidity is high, despite dietary intervention.

HMG-CoA synthase deficiency results in the failure to form ketones in times of stress, resulting in hypoketotic hypoglycemia. Infants may present with hepatomegaly, encephalopathy, or coma in the setting of acute illness or fasting. Generally, serum aminotransferases are normal, as is serum lactate. Diagnosis is suggested by the absence of urinary ketones with normal acyl carnitine profile and confirmed by molecular testing. Treatment is largely avoidance of fasting.

CACT is necessary to transport long-chain fatty acids from the cytosol into the mitochondrion, which supports the production of ATP. Most patients with deficiency in CACT present in the neonatal period with hypoketotic hypoglycemia, hepatic dysfunction, cardiomyopathy, and seizures. Laboratory investigation often reveals hypoketosis, hypoglycemia, hyperammonemia, elevated creatinine kinase, elevated serum aminotransferases, low carnitine levels, and abnormal acyl carnitine profile. Diagnosis is confirmed by genetic testing. Treatment is dietary and involves a low-fat diet with supplementation of MCTs and carnitine. Morbidity and mortality are high in the presence of cardiomyopathy.

Urea Cycle Defects

One of the primary functions of the liver is ureagenesis, to detoxify ammonia, which is the end product of amino acid metabolism. The accumulation of ammonia and glutamine lead to hepatocellular and central nervous system dysfunction. The most common disorders of ureagenesis include ornithine transcarboxylase deficiency, carbamoyl phosphate synthase, and citrullinemia. These disorders generally present in the first few days of life with hyperammonemia and, potentially, cerebral edema. The clinical presentation may be confused with sepsis. Diagnosis is suggested by the presence of hyperammonemia with low serum blood urea nitrogen and typical patterns of abnormal elevations in serum amino acids. Diagnosis is confirmed by molecular genetic testing. Treatment should be directed by a metabolic specialist and largely focuses on maintaining serum ammonia within normal range, preventing catabolism, and utilizing a protein-restricted formula with one-half of the protein content as essential amino acids. Liver transplantation may be considered in patients with severe recurrent episodes of metabolic crises with hyperammonemia.

Mitochondrial Hepatopathies

Mitochondrial hepatopathies secondary to respiratory chain complex deficiencies may present in the neonatal period as acute liver failure. Additionally, mitochondrial disorders should be suspected in neonates who present in the first week of life with hypoglycemia, hypotonia, seizures, and evolving hepatic synthetic dysfunction. Diagnosis is suggested by elevated lactate and specifically with lactate-pyruvate ratios greater than 30. With evolving mitochondrial DNA mutational analysis some disorders may be identified; however, few targeted therapies are available. Liver transplantation may be considered if additional comorbidities do not suggest poor posttransplant survival.

Lysosomal Storage Disorders

Lysosomal storage disorders generally present with hepatosplenomegaly as well as an array of systemic symptoms. The most common lysosomal storage disorders are Gaucher disease, Niemann-Pick disease type C (NPD-C), and lysosomal acid lipase deficiency.

Gaucher disease is an autosomal recessive genetic disease that results from the deficiency of a lysosomal enzyme, glucocerebrosidase, leading to an accumulation of glycolipids within lysosomes. The incidence is approximately 1 per 75,000 live births but is more prevalent in infants of Ashkenazi Jewish descent.[43] Clinical suspicion should be raised in infants who present with hepatosplenomegaly and normal to mildly elevated liver enzymes. Diagnosis is supported by liver biopsy, which demonstrates macrophages filled with lipid, also known as Gaucher cells. However, the diagnosis is confirmed by reduced glucocerebrosidase activity in leukocytes or by mutational analysis. Prenatal diagnosis is available by chorionic villus sampling or amniocentesis. Targeted treatment with enzyme replacement therapy with recombinant glucocerebrosidase is recommended for symptomatic children.

NPD-C is autosomal recessive and caused by mutations in *NPC1* and *NPC2*. These mutations result in impaired processing and transport of low-density lipoprotein (LDL) cholesterol, which clinically manifests as hepatosplenomegaly and progressive neurocognitive degeneration with dystonia, seizures, dysphagia, and ataxia. Laboratory findings characteristic of NPD-C include decreased high-density lipoprotein, hypertriglyceridemia, and increased LDL. Diagnosis is confirmed by fibroblast cell culture and genetic testing. Treatment is largely supportive, as bone marrow transplantation, liver transplantation, and lipid-lowering medications have not been shown to confer survival benefit.

Lysosomal acid lipase deficiency results in two distinct phenotypes: Wolman disease and cholesteryl ester storage disease (CESD). Both occur secondary to abnormal lysosomal accumulation of cholesteryl esters, triglycerides, and other lipids. Phenotype severity depends on the extent of reduction in lysosomal acid lipase activity. Wolman disease is the most severe manifestation of lysosomal acid lipase deficiency. Infants typically present in the first few weeks of life with failure to thrive, steatorrhea, hepatosplenomegaly, and jaundice. Death generally occurs within the first year of life. Diagnosis is suggested by calcifications of adrenal glands, cholestasis, elevated transaminases, liver synthetic dysfunction, and a normal plasma lipid profile. Liver biopsies have a characteristic orange-yellow appearance secondary to lipid and fat accumulation. CESD generally presents in older children or even in adulthood. Treatment is largely supportive, although trials of recombinant lysosomal acid lipase are available with promising results in early and late-onset disease.

Gestational Alloimmune Liver Disease

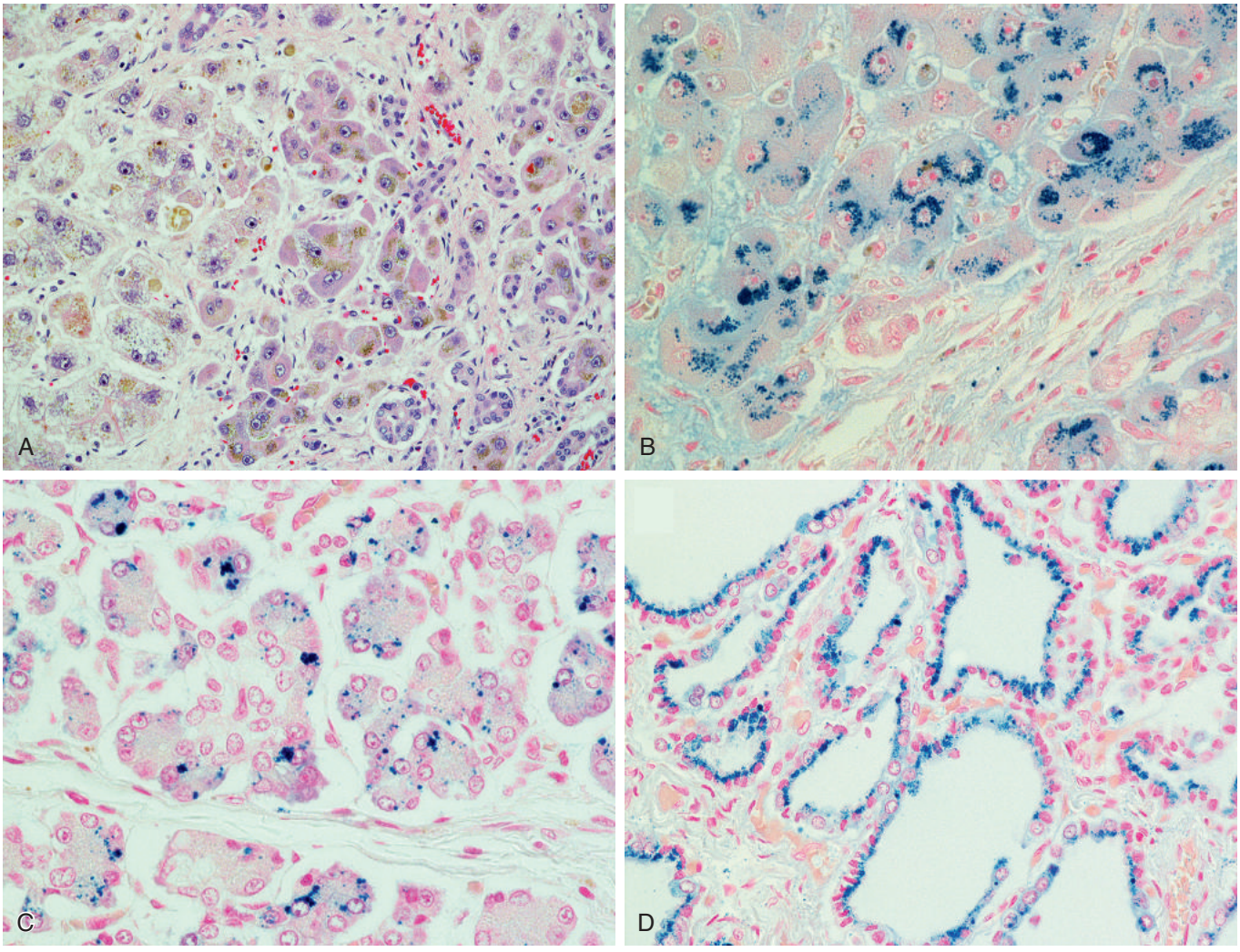
Historically, gestational alloimmune liver disease (GALD) was referred to as neonatal hemochromatosis, a term used to describe the clinical phenotype of severe neonatal liver disease resulting from an inborn error of iron metabolism, causing hepatic and extrahepatic siderosis. Recently, advances in understanding of the pathogenesis debunk earlier suppositions that GALD results from primary iron overload. Evidence now suggests that iron overload is the phenotype that results from GALD.[52–54] While nearly all nonhematopoetic iron overload is due to GALD, there are a few rare associations other than GALD which include trisomy 21, mitochondrial DNA depletion syndrome, bile acid synthetic defects, GRACILE (growth

retardation, aminoaciduria, cholestasis, iron overload, lactic acidosis, and early death syndrome), myofibromatosis, Martinez-Frias syndrome, and tricho-hepato-enteric syndrome.[55]

The pathogenesis of GALD stems from complement-mediated hepatocyte injury and alloimmunity activated by maternal immunoglobulin G (IgG) (the only immunoglobulin that freely crosses the placenta). Once sensitization has occurred, maternal anti-fetal hepatocyte IgG binds to fetal liver antigen. Subsequently, complement-mediated destruction of fetal hepatocytes results in a neonatal liver mass that is 10% to 25% less than normal. The unaffected fetal liver normally produces hepcidin to regulate placental iron delivery,[52] but in GALD the drastically reduced amount of hepcidin fails to appropriately regulate iron influx, resulting in increased iron transport through the placenta and iron overload in extrahepatic tissues including pancreas, salivary glands, heart, and thyroid gland.[56]

GALD typically presents at birth but can present anytime between 18 weeks gestation up to 3 months of age. GALD should always be suspected in infants who manifest prenatal or neonatal liver disease, especially liver failure. Infants with GALD are typically sick at birth. In the fetal period, oligohydramnios or intrauterine growth restriction are common. Affected newborns generally present in the neonatal period in acute liver failure with hypoglycemia and coagulopathy or, more rarely, with cirrhosis. Cholestasis generally evolves over time and is not present at birth. Laboratory findings may include normal to slightly elevated ALT, elevated ferritin levels (generally >800 ng/mL but commonly exceeding 15,000 ng/mL), alpha fetoprotein greater than 80,000 ng/mL but typically greater than 300,000 ng/mL, and iron saturation greater than 90%. Imaging may demonstrate extrahepatic siderosis effecting the pancreas, myocardium, thyroid, and salivary glands. Patients are typically extremely coagulopathic. Generally the reticuloendothelial system, including the spleen, bone marrow, and lymph nodes, is spared.[55]

The diagnosis of GALD is suggested by liver biopsy demonstrating reduced hepatocyte volume and the presence of C5b-9 complex on immunohistochemistry.[57] Biopsies may also show hepatocyte collapse, fibrosis, and siderosis (Fig. 65.5). However, demonstration of extrahepatic siderosis is necessary to confirm the diagnosis. This may be accomplished by tissue biopsy



• **Fig. 65.5** Gestational Alloimmune Liver Disease Histology. (A) Hematoxylin and eosin stain of a liver demonstrating pericellular fibrosis. Many hepatocytes contain pigment, some of which is hemosiderin, and some of which is bile. (B) The corresponding Gomori iron stain shows iron deposition within hepatocytes. (C) Iron stain demonstrating iron deposition in the pancreas acinar epithelial cells. (D) Iron stain demonstrating iron deposition in thyroid follicular cells.

or T2-weighted MRI. Generally, it may be preferable to obtain mucosal tissue (submucosal glandular biopsy). This may be followed by MRI if the biopsy is negative, and diagnosis is still suspected. Unfortunately, GALD is often diagnosed at autopsy where extrahepatic siderosis is observed. Diagnosis also should be considered in cases of fetal demise and stillbirth.

Treatment attempts to block existing antibody action by administration of high-dose intravenous immunoglobulin (IVIG) (1 g/kg) as well as to remove existing antibody with double-volume exchange transfusion. This regimen has been shown to improve survival to between 75% and 80% without liver transplantation.[58] Before this treatment regimen, outcomes were very poor with older medical therapies such as iron chelation and antioxidants, which have no role in current treatment recommendations. GALD remains a common indication for liver transplantation in the neonatal period. However, transplantation in these patients is complicated by small infant size and multiorgan failure. Transplant adverse outcomes (graft loss and death) are high; however, they are comparable to those of transplantation for other indications of neonatal acute liver failure.[59]

Because of the rapid progression and high mortality associated with GALD, if it is suspected, an infant should receive a dose of IVIG immediately, even before the diagnostic work-up is complete. Side effects of IVIG are minimal, and IVIG has minimal impact on the natural history of alternate causes of neonatal liver failure. If diagnosis is confirmed, then the double-volume exchange transfusion should be employed followed by a second dose of IVIG. It may take 4 to 6 weeks for INR to normalize depending on existing liver damage.

The probability of GALD diagnosis in each subsequent infant born to a mother whose previous child was born with GALD is greater than 90%.[60,61] However, GALD is thought to be congenital and familial but not hereditary.[62] Evidence for prevention of GALD is evolving. Given the high risk of subsequent infants developing GALD following an index birth of an affected infant, all mothers should be treated during pregnancy with recurring infusions of IVIG at 14 weeks gestational age, 16 weeks, and weekly from 18 weeks until delivery.[63]

Vascular Malformations

Vascular malformations of the liver generally present in the neonatal period if they are associated with cutaneous malformations or result in significant systemic shunting of blood. Types of shunts may include hepatic artery to hepatic vein, hepatic artery to portal vein, or from portal vein to systemic circulation (portosystemic shunts).

Arteriovenous Malformations

Arteriovenous malformations that shunt large volumes of blood may present in the neonatal period with anemia, heart failure, portal hypertension, or hepatomegaly, depending on the vascular involvement. Treatment includes embolization, surgical ligation, or resection. Liver transplantation is reserved for those lesions that are not amenable to more limited surgical intervention and that result in life-threatening complications.

Congenital Portosystemic Shunts

Congenital portosystemic shunts allow for intestinal venous blood to bypass the liver and shunt directly into the systemic venous system. They may be either intrahepatic or extrahepatic in origin (Figs. 65.6 and 65.7). Extrahepatic portosystemic shunts are known as "Abernathy malformations" (Fig. 65.8). Persistence of fetal circulation with a patent ductus venosus may allow for intrahepatic shunting from the left portal vein to a hepatic vein.

Portosystemic shunts are most commonly identified incidentally on abdominal imaging in individuals with portal hypertension or other vascular malformations. If diverting significant portal blood flow past the liver, they may lead to elevated serum ammonia. Portal hypertension is not usually seen with portosystemic shunts.

Treatment of congenital portosystemic shunts varies and depends on the severity of clinical sequelae. Several may close spontaneously; however, those that are contributing to encephalopathy and subsequent developmental delay or cardiopulmonary compromise should be considered for closure. Techniques may include embolization, surgical ligation, or, rarely, liver transplantation.[64,65]

Hereditary Hemorrhagic Telangiectasia

Hereditary hemorrhagic telangiectasia (HHT) is an autosomal dominant disease characterized by mucosal and cutaneous telangiectasias, and visceral vascular malformations. The visceral organs most commonly affected include the lungs, liver, intestine, and brain. The prevalence is estimated to be approximately 1 per 10,000 live births. Diagnosis of HHT is based on fulfilling greater than three of four clinical criteria and genetic testing. The clinical criteria include spontaneous, recurrent epistaxis, multiple cutaneous and oral cavity telangiectasias, the presence of visceral vascular lesions, and a first-degree relative with HHT.

Most infants with HHT will present with high-output heart failure and potentially portal hypertension secondary to arterioportal shunting. An ischemic cholangiopathy may result if hepatic arterial blood shunts away from bile ducts.

As with all liver-associated vascular lesions, diagnosis is accomplished by Doppler-enhanced US of the abdomen. In the case of HHT, US most commonly demonstrates a dilated hepatic artery with elevated hepatic artery flow and intrahepatic vascularity. On computed tomography (CT), the presence of multiple telangiectasia leads to heterogeneous enhancement of the liver. Liver biopsy carries high risk of bleeding.

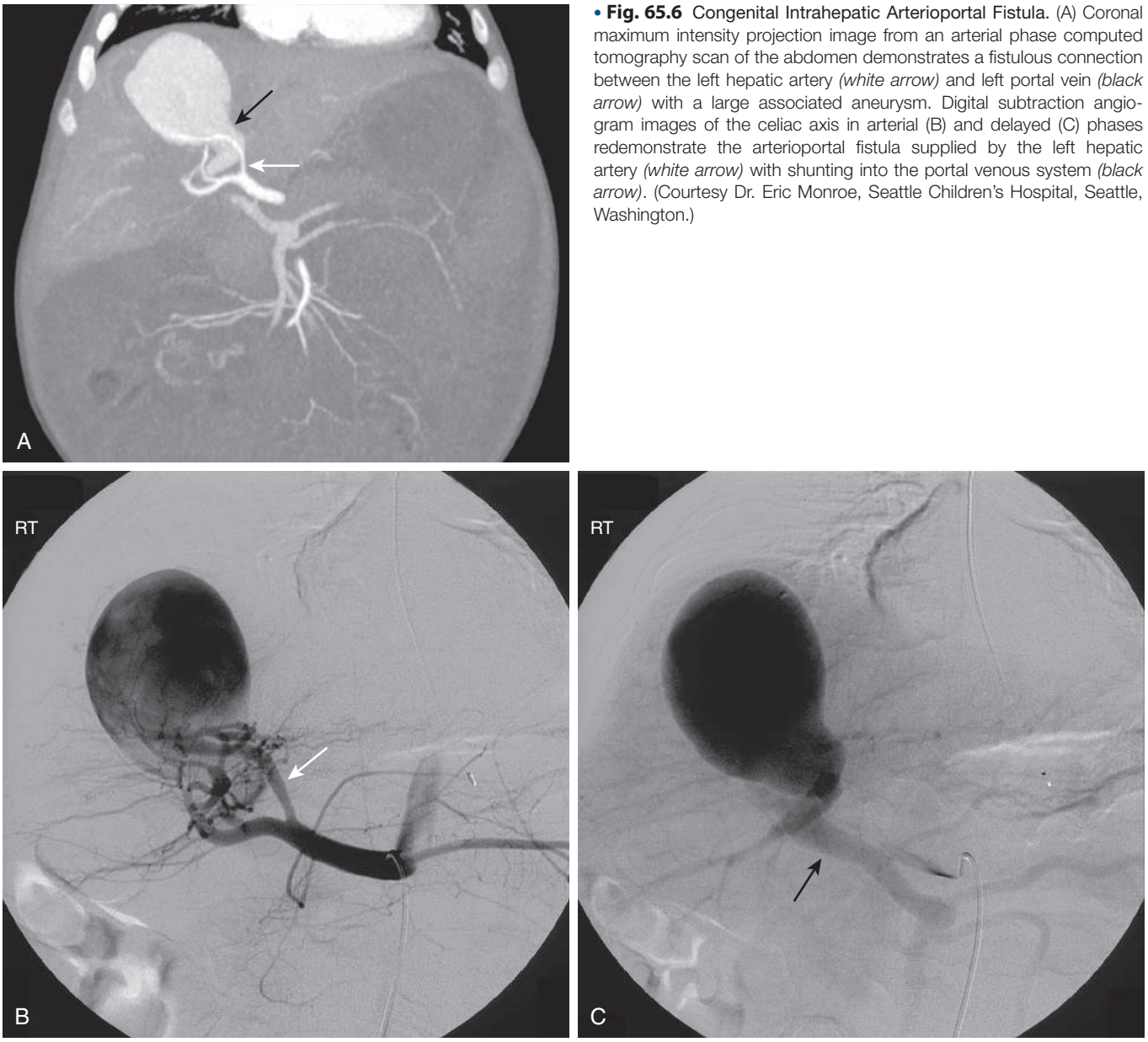
Liver transplantation may be considered in the setting of heart failure, biliary ischemia, or severe portal hypertension.

Infantile Hepatic Hemangiomas

Infantile hepatic hemangiomas (IHHs) are the most common benign tumor of infancy and the most common pediatric tumor of the liver, affecting as many as 1% to 2% of newborns and upwards of 10% of infants by 1 year of age.[66] Most infants present with associated cutaneous or subcutaneous lesions; however, many present with isolated vascular lesions of the liver, brain, and lungs. IHH should be suspected in infants with five or more cutaneous hemangiomas, and this physical finding should prompt investigation for visceral involvement.

IHH may be characterized as focal, multifocal, or diffuse. Most infants with IHH present before 6 months of age and experience initial progression of the size of lesions from birth to 12 months, followed by a period of regression and involution by about 2 to 10 years of age.

Focal IHH is usually incidental and may be initially seen on prenatal US. They are not associated with cutaneous findings and are typically asymptomatic; larger lesions may lead to high-output



• **Fig. 65.6** Congenital Intrahepatic Arterioportal Fistula. (A) Coronal maximum intensity projection image from an arterial phase computed tomography scan of the abdomen demonstrates a fistulous connection between the left hepatic artery *(white arrow)* and left portal vein *(black arrow)* with a large associated aneurysm. Digital subtraction angiogram images of the celiac axis in arterial (B) and delayed (C) phases redemonstrate the arterioportal fistula supplied by the left hepatic artery *(white arrow)* with shunting into the portal venous system *(black arrow)*. (Courtesy Dr. Eric Monroe, Seattle Children's Hospital, Seattle, Washington.)

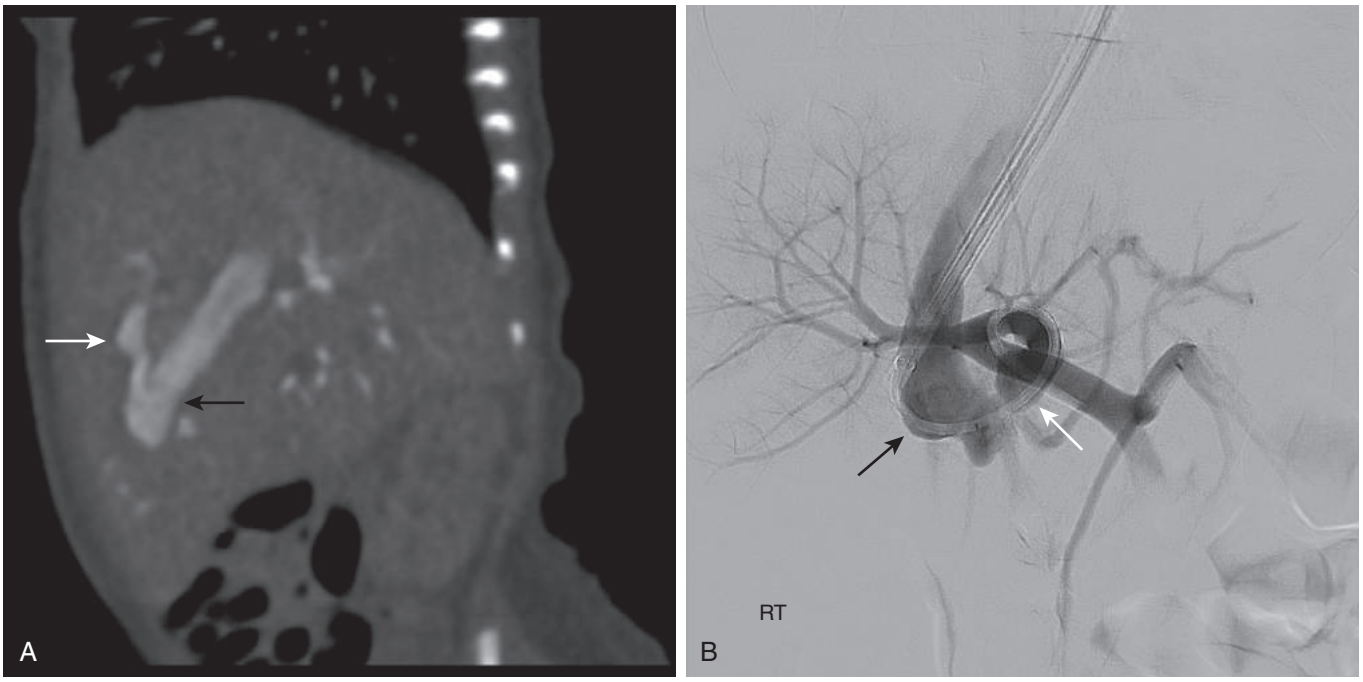
heart failure. Diagnosis is by US imaging, identifying a hyperechoic or hypoechoic lesion with heterogeneous echotexture with vascular flow on Doppler interrogation. On MRI, lesions are noted to be hypointense on T1-weighted images, and hyperintense on T2-weighted scans with centripetal enhancement with gadolinium contrast.

Multifocal and diffuse IHH are associated with multiple cutaneous hemangiomas. Multifocal IHH is typically not apparent at birth but presents within the first few weeks of life when the hemangiomas become more prominent. Symptomatic infants present with hepatomegaly, heart failure, anemia, and occasionally thrombocytopenia secondary to consumption, hemorrhage, or thrombosis within the hemangioma. Rarely, hemangiomas may stimulate inactivation of thyroxine, resulting in hypothyroidism. Occasionally, rupture of hemangiomas may result in intraperitoneal hemorrhage. US and MRI imaging are similar to that of focal IHH but with more numerous lesions. Treatment is reserved for symptomatic infants. Asymptomatic infants may be observed and followed with serial imaging. Medical therapy employs propranolol at a dose of 1 to 3 mg/kg/day, continued until the proliferation phase is completed around 9 to 12 months of age. Second-line treatments for propranolol failures include steroids, vincristine, and alfa 2a interferon.[67,68] Embolization or surgical resection may be utilized if medical therapy fails or in the setting of significant organ dysfunction. Liver transplantation has been performed in critical clinical circumstances without other options.[69,70] Rarely, multifocal IHH may be associated with hepatic angiosarcoma.

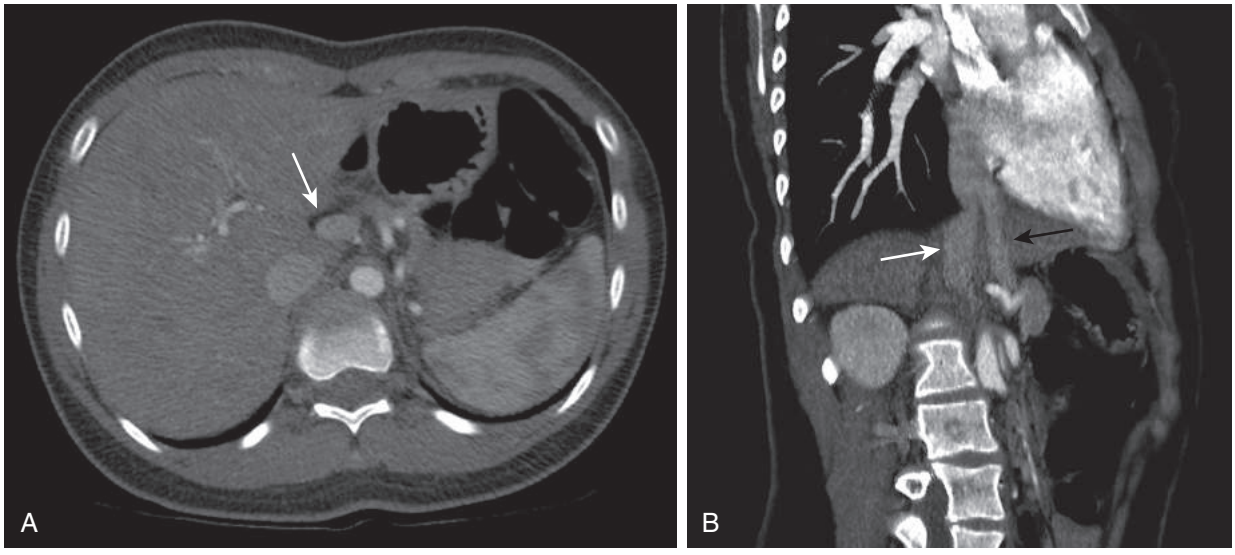
Liver Masses

Hepatoblastoma

While many solid and cystic tumors of the liver may present in the neonatal period, the most common malignant tumor is hepatoblastoma. Risk factors for the development of hepatoblastoma include prematurity, low birth weight, familial adenomatous



• **Fig. 65.7** Congenital Intrahepatic Portosystemic Shunt. (A) Sagittal image from a venous phase computed tomography scan of the abdomen demonstrates an anomalous portosystemic shunt between the anterior division of the right portal vein *(white arrow)* and middle hepatic vein *(black arrow)*. (B) Right anterior oblique digital subtraction angiogram image following trans-shunt catheterization of the portal vein redemonstrates the broad portosystemic shunt between portal vein *(white arrow)* and middle hepatic vein *(black arrow)*. (Courtesy Dr. Eric Monroe, Seattle Children's Hospital, Seattle.)



• **Fig. 65.8** Abernathy Malformation. (A) Axial image from a late arterial phase CT scan demonstrates a craniocaudal course of the portal vein *(arrow)* and absent portal vein within the hepatic hilum. (B) Multiplanar reformat image from the CT confirms an abnormal course of the portal vein *(black arrow)* draining to the right atrium, parallel to the inferior vena cava *(white arrow)*. (Courtesy Dr. Eric Monroe, Seattle Children's Hospital, Seattle.)

polyposis, trisomy 18, GSD Ia, Beckwith-Wiedemann syndrome, and Li-Fraumeni syndrome. Hepatoblastoma most commonly presents as an abdominal mass associated with weight loss, abdominal pain, and decreased oral intake. Diagnosis is made by typical radiologic findings, including a well-defined but heterogeneous mass that may have areas of necrosis, hemorrhage, or calcification. MRI is the preferred mode of imaging. Biopsy to differentiate epithelial subtype (fetal, embryonal, macrotrabecular, and small cell undifferentiated) is required. AFP is usually elevated in most hepatoblastoma patients. AFP in a full-term infant can be as high as 100,000 ng/mL, making interpretation difficult; however, in unaffected infants the value rapidly decreases over time. AFP is a

valuable prognostic marker after therapy. Treatment consists primarily of resection and is augmented by chemotherapy. When not resectable, liver transplantation may be considered.

Congenital Hepatic Cysts

Congenital hepatic cysts are one of the most common benign liver masses of infancy. With modern imaging techniques, simple hepatic cysts are frequently diagnosed prenatally by US. There is a wide spectrum of pathologies resulting in simple hepatic cysts. Simple cysts are generally thought to develop from aberrant bile ducts and are not generally associated with cystic lesions of other organs.[71] They are characterized by a layer of cuboidal or columnar epithelium surrounding a collection of serous fluid. Congenital hepatic cysts are more common in females and can be solitary or multiple.[72] Most cysts are incidental, asymptomatic, do not require intervention, and may shrink or resolve over time. It is recommended that periodic, serial US be utilized to survey for interval growth or resolution. Indications for surgical intervention include interval growth, large size, which may cause compression of nearby structures or cause risk for rupture, or those that become symptomatic.[71]

Suggested Readings

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66 Developmental Hematology

SANDRA E. JUUL AND ROBERT D. CHRISTENSEN

KEY POINTS

- Pluripotential stem cells sustain hematopoietic function throughout a person's lifetime; the fate of developing cells is influenced by the microenvironment.
- The site of erythropoiesis changes over development, progressing from the yolk sac to the aortogonadomesonephron, to the liver, and then to the bone marrow. Erythropoietin (Epo) is the principal factor regulating secondary erythropoiesis.
- Hemoglobin tetramers change over development. Oxygen affinity decreases as hemoglobin switches from embryonic to adult forms.
- While the optimal hematocrit or hemoglobin trigger for transfusion of preterm infants is not known, it has now been established that hemoglobin thresholds of 11 to 13 g/dL for critically ill or ventilated infants and 7 to 10 g/dL for stable infants can be safely used.
- The need for transfusions in the neonatal intensive care unit can be reduced by delayed cord clamping or cord milking, reducing phlebotomy-related losses, and using erythropoiesis-stimulating agents (Epo or darbepoetin).
- Most platelet transfusions given in the neonatal intensive care unit are prophylactic, meaning they are given to nonbleeding neonates with low platelet counts. For nonsurgical preterm infants less than 32 weeks of gestation, allowing the platelet count to reach 25,000/μL prior to transfusion is safe. Including metrics in addition to the platelet count, such as the "platelet mass" (platelet count × mean platelet volume), and the immature platelet fraction, might provide a better transfusion trigger than the platelet count alone. In general, 10 to 20 mL of single donor platelets per kilogram should raise the platelet count by more than 100,000/μL. The use of volume-reduced or pooled platelets should be avoided because processing results in platelet activation and decreased function.

Introduction to Embryonic Hematopoiesis

Hematopoiesis is the process by which self-renewing multipotential stem cells give rise to all the differentiated blood cells (Fig. 66.1). This process involves the coordinated expression of growth factors, some of which act on primitive progenitors that can give rise to multiple cell lineages and others that support clonal maturation of lineage-committed multipotential hematopoietic stem cells (HSCs). Hematopoiesis begins in the embryo, with the first lymphoid progenitors emerging within the embryo and yolk sac before stem cell detection at embryonic day 7.5.[1] By day 10, HSCs are present in the aortogonadomesonephron, and activity then shifts to the liver and finally to the bone marrow.[2] Each cell lineage undergoes developmental changes that are unique and specific.[3]

Stem Cell Biology

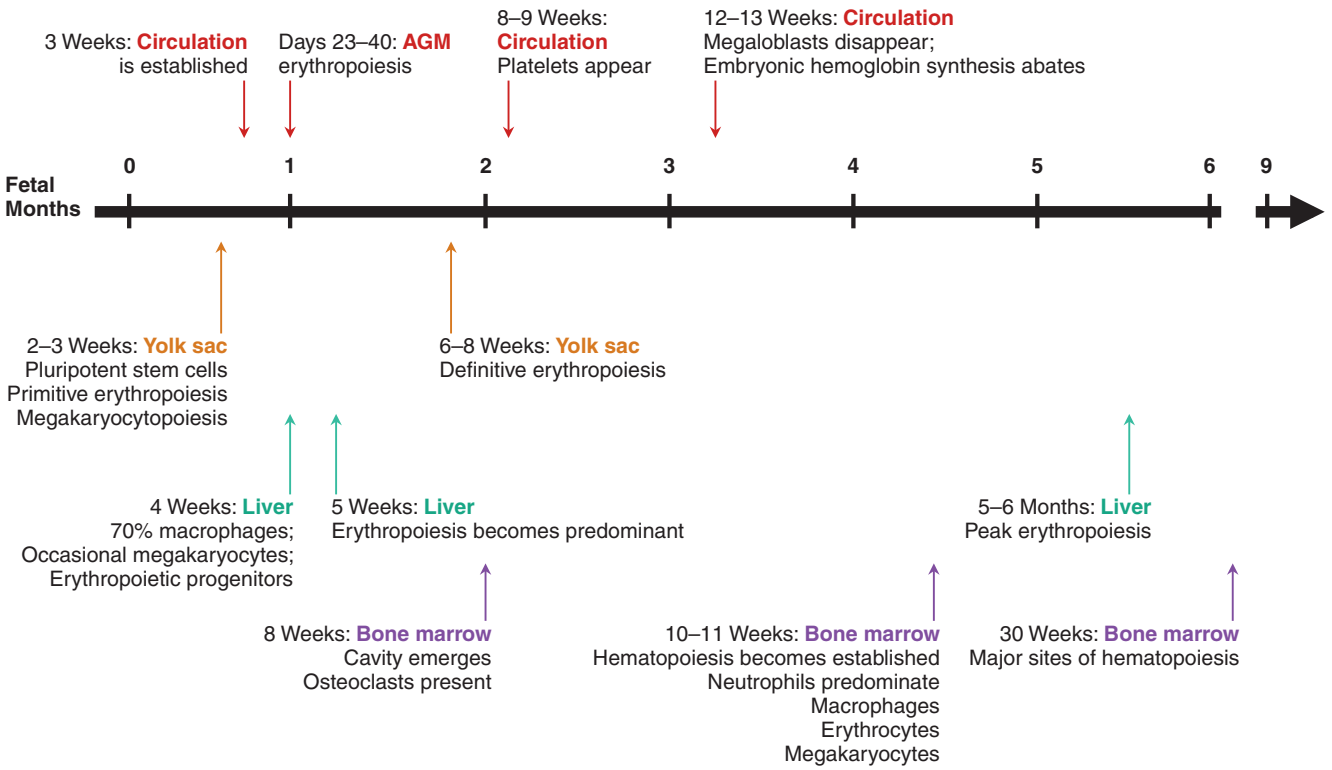
Pluripotential stem cells sustain marrow function throughout a person's lifetime. A unique characteristic of these cells is that their direct offspring include at least one identical daughter cell, thus perpetuating the population. In contrast, progenitor cells are more differentiated and give rise only to cells more differentiated than themselves. The fate of any particular developing cell is determined in large part by its microenvironment.

The developmental changes in the number, function, and location of HSCs are of interest to transplantation biologists and gene therapists. The proliferative capacity of HSCs differs with the anatomic source of the cells and with the age at which the cells are harvested. The sensitivity of these cells to recombinant cytokines also changes with age. Improving the understanding of the ontogeny of these cells may be helpful in optimizing their clinical use.

Embryonic and fetal HSCs are capable of repopulating adult organisms.[4] In contrast, transplanted adult stem cells have a lower capacity for self-renewal, sometimes resulting in late graft failure. This might be because stem cells harvested from adults continue to express the adult differentiation program, even if transplanted into a neonatal environment, indicating an irreversible change in gene expression.[5] Other explanations for the decrease in proliferative potential may have to do with DNA damage over time and loss of telomere repeats with each stem cell division, limiting the replicative potential.[6]

Ongoing research focuses on optimizing stem cell harvesting techniques. Cell-surface markers, which are dependent on cell maturity and gestational age, are often used to identify and separate HSCs with the use of monoclonal antibodies and fluorescence-activated cell sorting analysis. For example, CD34, a cell-surface sialomucin, is an antigen commonly used to select HSCs and early erythropoietic progenitor cells. Combining CD34 positivity with the absence of lineage-specific markers allows the selection of a population highly enriched for cells desired for transplant. Research is also focused on optimizing stem cell harvest sites. Both bone marrow and umbilical cord blood (UCB) are rich in stem cells and have long been used as sources of progenitor cells. The collection of stem cells from peripheral blood by stimulated apheresis, with ex vivo expansion of select populations, is now also an option.[7]

UCB can be harvested as a source of HSCs and used for transplant in patients with marrow failure, malignancy, or immunodeficiency.[8,9] Hematopoietic progenitor cells in UCB tend to have



• **Fig. 66.1** Changing Sites of Hematopoiesis During Human Gestation. Fetal gestation is shown in months along the *central horizontal arrow*. The timing of significant events during hematopoiesis is shown, beginning with primitive erythropoiesis and megakaryocytopoiesis in the yolk sac and ending with definitive hematopoiesis in the bone marrow during late gestation. *AGM*, Aortogonadomesonephron.

a high proliferation index compared with cells harvested from adult marrow.[10] Because of the immaturity of the UCB cells, HLA matching is less stringent for UCB, allowing more efficient donor unit identification compared with a bone marrow registry.[11] This feature also allows the improved matching ability for patients in minority ethnic populations since bone marrow registry matches are frequently in short supply. Promising strategies to increase cells available for transplant include combining multiple units of UCB[12] and ex vivo expansion of UCB units.

The first successful use of UCB for HSC transplant purposes was in 1989, between HLA-identical siblings, for severe aplastic anemia from Fanconi anemia.[13] Since then, thousands of UCB transplants have been performed to treat malignancies such as acute lymphoblastic leukemia, acute myeloid leukemia, and chronic myeloid leukemia; bone marrow disorders such as Fanconi anemia; immunodeficiencies such as severe combined immune deficiency; metabolic disorders; and hemoglobinopathies.[9]

UCB can be stored in public or private commercial banks. Donation of harvested cells to public UCB banks allows storage in a central repository available to all individuals in need of a transplant.[14] Private banking is expensive and less standardized. Banked cells are available for future use by individuals or a family, but there is a low likelihood of any one individual needing an autologous UCB transplant.[15]

Delaying umbilical cord clamping by 60 seconds increases the blood volume of the neonate and increases the endowment of iron. In theory, this practice might result in insufficient UCB remaining for banking. However, the Canadian Task Force on Preventive Health Care suggests that delayed cord clamping has significant benefits for the neonate and that sufficient blood typically remains in the umbilical cord and placenta after delayed clamping for UCB banking. The task force suggests that both techniques can generally be accomplished.[16]

Developmental Aspects of Erythropoiesis

Erythropoiesis is the process of perpetual production of red blood cells (RBCs). Serial adaptations occur throughout development to meet the changing oxygen demands of the embryo, fetus, and neonate. The type of cells produced, the locations in which they are produced, and the microenvironments within these locations change as development proceeds (see Fig. 66.1). The molecular mechanisms involved in instituting, regulating, and maintaining these adaptations are complex.

Primitive and Definitive Erythropoiesis

During development, two types of RBCs are formed: primitive and definitive erythrocytes. The liver is the primary organ of hematopoiesis during fetal life, but primitive RBCs are first formed in the yolk sac.[17] Large primitive RBCs are produced in blood islands of the yolk sac days after implantation of the embryo. These cells enter the newly formed vasculature of the embryo, where they continue to divide and differentiate for several days. This process is only minimally responsive to Epo.[18] Primitive erythroblasts are large (>20 μm), nucleated, CD34 negative, and contain predominantly embryonic hemoglobin. Hemoglobin synthesis continues until cell replication ceases.[19] In mice, a transition to definitive erythropoiesis occurs at embryonic day 13.5 (full gestation is 21 days). Definitive erythropoiesis is characterized by smaller (<20 μm) CD34-positive erythroblasts, which produce fetal and adult hemoglobins, extruding their nuclei when mature.

Unlike primitive erythropoiesis, this process is dependent on Janus kinase signal transduction and Epo stimulation.[20] Primitive erythroblasts normally undergo apoptosis, becoming extinct during fetal life, whereas definitive erythroblasts are able to self-renew.[21]

Switch of the Primary Site of Erythropoiesis

Humans have four main sites of embryonic and fetal erythropoiesis—yolk sac, aorta (ventral aspect), liver, and marrow. In rodents, the spleen is also an important site of hematopoiesis, but there is no evidence for this in healthy humans.[22] Studies using an in vitro embryonic stem cell differentiation system showed that endothelial cells, primitive hematopoietic cells, and definitive blood-cell colonies arise from a common fetal, liver, kinase-1 (Flk-1)-expressing progenitor.[23] Between embryonic day 8 and embryonic day 11.5, runt-related transcription factor 1 is required for the formation of HSCs and their progenitors. Primitive progenitor cells first develop in the yolk sac, followed by the rise of definitive progenitors, also in the yolk sac.[24] Another source of the early definitive progenitors is the ventral aspect of the embryonic aorta.[24,25] Once circulation is established, progenitors from all lines are detected in the blood, then in the liver, and finally in the marrow.[26]

Yolk Sac

The yolk sac is an extraembryonic structure that can be subdivided into the primary and secondary yolk sac. The primary yolk sac is transient and has no known hematopoietic function. In humans, it forms by proliferation and differentiation of primitive endodermal cells 7 to 8 days after conception. These endodermal cells give rise to mesodermal precursors (intermediate cells). The primary yolk sac then collapses into small vesicles, and the secondary yolk sac is formed from its remnants 12 to 15 days after conception. By 16 to 19 days, primitive erythropoiesis is found in the human yolk sac.[27,28] The secondary yolk sac is an active site of protein synthesis, nutrient transport, and hematopoiesis.[29] Primitive hematopoietic cells, adherent to surrounding endothelial cells, are first observed on day 16 in the mesodermal layer. These hematopoietic–endothelial cell masses have been described as *blood islands*. As maturation proceeds, these blood islands migrate toward each other, merging to form a network of capillaries. Small clusters of undifferentiated cells, the hemangioblasts, and clusters of primitive erythroblasts are observed in the small vessels present at this developmental stage.[29] As differentiation proceeds, endothelial and hematopoietic cell lineages emerge. These cell types share common molecular markers and responsiveness to a cohort of growth factors and, depending on the microenvironment, can be derived from a common stem cell in culture.[23,30,31]

After the sixth week after conception, definitive erythroblasts are found in the yolk sac. A decline in yolk sac hematopoiesis is observed after the eighth week.[29] Yolk sac–derived hematopoietic cells have more restricted potential in vivo, as only RBCs and macrophages are present in the yolk sac,[32] while progenitor cells in the liver develop into the full spectrum of hematopoietic cells. However, when yolk sac–derived stem cells are cultured in vitro or are transplanted, they are multipotent, illustrating the importance of the microenvironment in the development of committed cell lineages.

Aortogonadomesonephron

Another site of early erythropoietic activity in the developing human embryo is the ventral aspect of the aorta in the periumbilical region.[25] At around the 23rd postconceptional day in humans, the multipotent hematopoietic progenitor cells in this region are more numerous than in the yolk sac or the liver. By day 40 of gestation, hematopoiesis in this site is concluded.

Liver

A short time after the onset of blood circulation (week 4 to week 5 of gestation), erythropoiesis begins in the liver.[27] As in the yolk sac, primitive erythroblasts initially predominate. However, over the next 4 weeks, definitive erythrocytes become the predominant RBC form. During this time, the liver mass increases 40-fold, with hematopoietic cells constituting 60% of the liver from week 11 to week 12.[33.] Meanwhile, other hematopoietic cell types are also produced in the liver. Early in this process (5 weeks), macrophages predominate, with approximately one granulocyte to every nine macrophages.[34] In contrast to the yolk sac, during the period of peak hepatic hematopoiesis (week 6 to week 18), production of all hematopoietic cell lines (erythrocytes, macrophages, megakaryocytes, granulocytes, and lymphocytes) occurs. Between 18 and 21 weeks of gestation, hematopoiesis in the liver diminishes, but the liver continues as an erythropoietic organ until term.

Bone Marrow

As hepatic hematopoiesis diminishes, the bone marrow becomes the primary site of erythropoiesis and remains so throughout postnatal life. The process of erythropoiesis in marrow begins at about 8 weeks with primitive erythrocytosis.[27] Over the next few gestational weeks, a switch to definitive erythropoiesis occurs, and by 14 weeks, only definitive erythroblasts are present. As with the liver, the production of all hematopoietic cells occurs in the bone marrow. Erythropoietic cells constitute a maximum of 35% of total bone marrow cells at week 12 of gestation, falling to between 20% and 30% thereafter.[35]

Factors Influencing the Sites of Erythropoiesis

The microenvironment at each site of hematopoiesis influences the type and timing of hematopoietic development. The microenvironment includes hematopoietic growth factors and cytokines, as well as the extracellular matrix in which the cells proliferate.

Growth factors are thought to act mainly as permissive and/or selective signals, allowing already committed cell types to proliferate and differentiate. Growth factors important for definitive erythropoiesis include Epo, stem cell factor, interleukin (IL)-3, thrombopoietin (TPO), and possibly insulin and insulin-like growth factor I, both of which act as nonessential survival factors for $CD34^+$ cells.[36] These growth factors work in concert to promote definitive erythropoiesis. However, Epo is the primary growth factor: in the absence of Epo signaling, as in the case for both Epo-null and Epo receptor–null mutations, definitive erythropoiesis does not occur. Null mutations of either Epo or its receptor are lethal at 13.5 days of gestation in the mouse because of severe anemia.

Extramedullary Hematopoiesis

Extramedullary hematopoiesis can occur after the bone marrow has been established as the primary site of erythropoiesis. Diseases inducing this occurrence include hemolytic conditions, congenital rubella, cytomegalovirus infection, and parvovirus B19 infection.

Extramedullary hematopoiesis can occur in the liver, spleen, adrenal glands, pancreas, thyroid, endocardium, testes, uterus, skin, or brain.[37] When the skin is involved in a neonate, the classic "blueberry muffin" rash is seen, typical of congenital rubella or cytomegalovirus infection.

Ontogeny of Erythrocytes

The earliest precursor cells specific to the erythroid lineage are the burst-forming unit–erythroid (BFU-E) cells. BFU-E cells have low numbers of Epo receptors[38] and respond to Epo, as well as a granulocyte-macrophage colony-stimulating factor (GM-CSF) and IL-3. As these cells mature into erythroid colony-forming units–erythroid and proerythroblasts, they become highly dependent on Epo, which is reflected by the high density of Epo receptors on the cell membrane (up to 1000 per cell).[39] Mature erythroblasts have fewer Epo receptors and are less sensitive to Epo stimulation, and reticulocytes and erythrocytes have no Epo receptors and are unresponsive to Epo. The principal functions of mature erythrocyte metabolism are to maintain adequate adenosine triphosphate (ATP) stores, to produce reducing substances to act as antioxidants, and to produce 2,3-diphosphoglycerate (2,3-DPG), which modifies the oxygen affinity of hemoglobin. Recent studies have identified a previously unappreciated role for RBCs in immune regulation. The role of RBCs in the modulation of response to bacterial and viral infection, immune tolerance, tumor surveillance, and RBC effects on the microbiome is reviewed by Yang and Lewis.[40]

Important developmental changes occur in hematocrit, reticulocyte count, RBC morphology, membrane content, deformability, life span, and metabolism. Over the course of gestation, there is an expected rise in hematocrit, from (36 ± 3)% at 18 to 20 weeks of gestation (fetal samples) to (61 ± 7)% expected at term birth. To maintain this increase in hematocrit and blood volume (up to 7 mL/day during the last trimester), the production of approximately 50×10^9 erythrocytes per day is required. During this same period of fetal development, erythrocyte size (the mean cell volume) decreases from 134 ± 9 femtoliters (fL) to 119 ± 9 fL.[41] At term, the mean cell volume is larger than that of normal healthy adults and drops postnatally, reaching a nadir at 4 to 6 months. It then increases to reach adult values (88 ± 8 fL) by approximately 1 year.

Reticulocytes are near mature erythrocytes released from the bone marrow into circulation. Although the nucleus has been extruded, they retain cytoplasmic organelles such as ribosomes, mitochondria, and Golgi bodies for approximately 24 hours. These newly released cells can be differentiated from mature RBCs by staining with new methylene blue or brilliant cresyl blue, which stains the nucleic acid within the cells. They can also be enumerated using flow-cytometric gaiting of cells that are larger and contain more nucleic acid than mature RBCs. The reticulocyte count can be used to assess the level of erythrocyte production because high values indicate active erythropoiesis, while low numbers indicate low levels of erythropoiesis. At birth, reticulocyte counts in preterm infants tend to be higher than in term infants (400,000/μL to 550,000/μL vs. 200,000/μL to 400,000/μL).[42] Absolute reticulocyte counts, reticulocyte percentage of total RBCs, and corrected reticulocyte counts can be obtained. In general, when neonates are being evaluated, the absolute reticulocyte count is the most helpful.[43]

RBC morphology is quite heterogeneous in preterm and term infants as compared with adults. Irregularly shaped cells such as poikilocytes, acanthocytes, schistocytes, and burr cells are common in the blood smears of neonates. This reflects developmental changes in cell membrane deformability and flexibility. The neonatal RBC has a life span of approximately 70 to 80 days as compared with 120 days for the adult RBC.

Developmental Changes in the Regulation of Erythropoiesis

The principal growth factor that regulates erythropoiesis is Epo. This 30.4-kDa glycoprotein contains 165 amino acids and is extensively glycosylated, with 40% carbohydrate content. Epo maintains RBC production during fetal, neonatal, and adult life by inhibiting apoptosis of erythroid progenitors and by stimulating their proliferation and differentiation into normoblasts.[44] Since very little Epo crosses the placenta, the Epo concentrations measured in the fetus reflect fetal synthesis.[45,46] Epo production begins early in fetal life, and Epo has been identified in extraembryonic coelomic fluid and amniotic fluid.[47] The primary site of Epo production during fetal life is the liver, with the transition to the kidney postnatally.[48] This transition is mediated in part by the expression of *GATA4*.[49] Production of Epo is stimulated by hypoxia via hypoxia-inducible factor (HIF) 1 and 2 pathways.[50–54] HIF is a DNA-binding complex composed of two subunits: HIF-1β, which is not oxygen responsive and is constitutively expressed, and either HIF-1α or HIF-2α, which are highly oxygen sensitive. The HIF complex is, in turn, regulated by prolyl hydroxylase domain enzymes 1–3.[55] Elevated Epo concentrations (up to 8000 mU/mL) have been reported in pathologic states such as fetal hypoxia, anemia, placental insufficiency, and in infants of diabetic mothers.[56]

During fetal development, circulating Epo concentrations range from 4 mU/mL at 16 weeks of gestation to 40 mU/mL at term.[57,58] An unhealthy intrauterine environment can result in increased Epo production, reflecting fetal hypoxemia.[59,60] In healthy term infants, serum Epo concentrations decrease after birth to reach a nadir between the fourth and sixth week of age.[60] By 10 to 12 weeks, they reach adult concentrations (approximately 15 mU/mL).[61] These changes in Epo concentrations are consistent with the changes in hemoglobin and hematocrit are seen following term birth (physiologic anemia). In premature infants, the anemia is more severe and persists longer, leading to anemia of prematurity. Epo concentrations in these infants are inappropriately low, forming the rationale for recombinant human Epo therapy.

Ontogeny, Organization, and Structure of Hemoglobins

Hemoglobin is a tetrameric molecule comprising two pairs of polypeptide subunits. As development proceeds, various hemoglobins are constructed by a combination of two α-like globins (ζ or α) with two β-like globins (ε, γ, δ, or β) to form hemoglobin tetramers. These tetramers include the embryonic hemoglobins, hemoglobin Gower 1 ($\zeta_2\varepsilon_2$), hemoglobin Gower 2 ($\alpha_2\varepsilon_2$), and hemoglobin Portland 1 ($\zeta_2\gamma_2$), fetal hemoglobin (hemoglobin F) ($\alpha_2\gamma_2$), and the adult hemoglobins A ($\alpha_2\beta_2$) and A_2 ($\alpha_2\delta_2$). Their expression and proportion depend on gestational age but can be modified by external mechanisms. The basic function of the various hemoglobins is similar, but their oxygen affinity differs. As the hemoglobins switch from embryonic to fetal to adult forms, oxygen affinity decreases. Thus the switch from embryonic to fetal to adult hemoglobin synthesis is a major mechanism by which

the developing fetus adapts from the relatively hypoxic intrauterine environment to the relatively oxygen-rich extrauterine environment.[62]

Changes in Hemoglobin Synthesis With Development

The genes within the α-globin and β-globin families are expressed according to a strict ontogenetic schedule, and the quantitative expression of the genes from each of these families is strictly balanced and coordinated.[63] Hemoglobin synthesis begins around 14 days after conception, with the synthesis of ζ-globin and ε-globin chains. These are replaced by the synthesis of α-globin and γ-globin chains by the fifth to the seventh week of gestation (hemoglobin Gower 2, hemoglobin Portland 1, and hemoglobin F become predominant).[64] By 12 weeks of gestation, hemoglobin F ($\alpha_2\gamma_2$) accounts for almost all hemoglobin produced.[65] After the 20th week of gestation, no ε-globin chains are produced, but the production of the ζ-globin chains can persist through the last trimester in pathologic conditions such as homozygous α-thalassemia. Expression of the γ-globin gene peaks during midgestation and declines rapidly during the last month of fetal gestation. β-Globin synthesis, required for hemoglobin A, starts at the sixth week of gestation, increasing as γ-globin synthesis declines, a transition that continues to the sixth month of life.[66] Thus, hemoglobin A synthesis quantitatively increases first after the 30th week of gestation. At the end of the last trimester, a rapid switch from the synthesis of fetal hemoglobin to adult hemoglobin occurs, falling from 85% at 34 weeks of gestation to 60% to 80% at birth.[67] The synthesis of δ-globin chains, required for hemoglobin A_2 ($\alpha_2\delta_2$), begins at the 34th to 35th week of gestation. After birth, a rapid increase in hemoglobin A and hemoglobin A_2 synthesis occurs.

Red Blood Cell Transfusion

RBCs can be transfused to anemic patients to simultaneously increase the recipient's blood volume and RBC content, thereby increasing oxygen-carrying capacity. The technical ability to store blood for future transfusion was developed in the early 1900s.[68] In the early years, RBCs were kept viable in a citrate and glucose solution, but current solutions make it possible to store cells for up to 42 days in solutions such as citrate–phosphate–dextrose, citrate–phosphate–dextrose–adenine, and various additive solutions, which may contain additional dextrose, mannitol, and adenine.[69] The hematocrit of "packed RBCs" typically ranges from 55% to 80%. During storage, RBCs undergo metabolic and structural changes. 2,3-DGP, antioxidant, and ATP contents decrease, glycolysis decreases, osmotic fragility increases, and deformability decreases.[70] ATP-dependent membrane pumps become dysfunctional, and extracellular potassium content increases at a rate of 1 milliequivalent per day, which can be dangerous when large volumes are transfused quickly. Oxidative damage occurs in lipids and proteins during storage and irradiation.[71] Proinflammatory compounds accumulate during the storage of blood, particularly if it is not leukoreduced. After an RBC transfusion, the mean life span of the RBC is about 70 days with a mean half-life of 30 days.[72,73]

The risks of RBC transfusion may be due to the storage process, the transfusion itself, and the association with oxidative damage. Because of the storage process and increasing age of the stored blood, RBC transfusion exposes the recipient to high levels of potassium, glucose, hydrogen, inflammatory mediators, and lactic acid; the clinical significance depends on the age of the blood and the volume and speed of transfusion.[71] However, it is not clear that transfusion of recently donated RBCs decreases transfusion risks.[74] Although rare, transfusion-transmitted bacterial infections can occur because of bacterial contamination of stored blood.[75] Other risks of RBC transfusion include viral infections, transfusion-related acute lung injury, graft-versus-host reaction, and, potentially, an increased risk of neurodevelopmental impairment.[76,77] Multiple RBC transfusions in the absence of significant phlebotomy-related loss may also put the patient at risk of iron overload and oxidative injury.[78] There is 200 mg of iron in a 420-mL unit of whole blood, which is then processed into 250-mL units of packed RBCs,[79] so a unit of blood with a hematocrit of 60% has an iron content of 0.7 mg/mL, which becomes available as the cells turn over. There is increased unbound iron in stored blood, which may increase the amount of reactive oxygen species.[80] Studies comparing restrictive with liberal transfusion practices in premature infants have not demonstrated a substantial and lasting benefit to maintaining higher hemoglobin levels.[81–84]

Preterm infants are at high risk of receiving red blood cell (pRBC) transfusions during their initial hospitalization (range from 0 to >10 transfusions).[83–87] In retrospective and observational studies, increased numbers of RBC transfusions have been associated with the development of bronchopulmonary dysplasia (BPD),[88] necrotizing enterocolitis (NEC),[89–92] retinopathy of prematurity (ROP),[93,94] diuretic use,[85] and poorer neurodevelopmental assessments[77,95] and therefore are to be avoided if possible. Transfusions can be prevented or decreased by combining several approaches: use of and adherence to restrictive transfusion guidelines, delayed cord clamping,[96] use of a cord or placental blood for initial laboratory studies,[97] minimized phlebotomy draws, ensured availability of adequate iron for erythropoiesis, and use of erythropoiesis-stimulating agents (ESA) such as erythropoietin (Epo)[98] or darbepoetin (Darbe) when reticulocytosis is inadequate.[99–101]

Bilirubin Metabolism

The primary source of bilirubin in the fetus and neonate is the metabolism of heme derived from hemoglobin in circulating erythrocytes. The rate-limiting step in heme breakdown is the formation of biliverdin, a process controlled by heme oxygenase.[102] After heme breakdown, the iron is recycled, carbon monoxide is liberated and exhaled, and biliverdin is reduced to bilirubin IXα by biliverdin reductase. In utero, unconjugated bilirubin is processed by the mother after placental transfer. Exhaled carbon monoxide can be quantified to assess the heme breakdown rate.[103] Unconjugated bilirubin is lipophilic and tightly bound to circulating albumin. The conjugation of bilirubin results in a relatively polar, water-soluble molecule, bilirubin diglucuronide, which can be excreted. This process occurs in the liver and is dependent on ligandin, a transfer protein, and the enzyme uridine diphosphoglucuronyl transferase. The conjugating ability of the fetus and newborn is impaired relative to that of older individuals because of reduced transferase activity and low levels of uridine diphosphoglucuronic acid.[104]

Developmental Aspects of Megakaryocytopoiesis

Megakaryocytopoiesis is the process by which megakaryocytes, and ultimately platelets, develop. Platelets are small (7.5 fL) anucleated fragments of megakaryocytes that circulate as relatively

smooth disks when unactivated. The normal circulating life span of a platelet is 10 days. Platelets provide hemostasis when a breach of the vascular endothelial lining occurs, and they activate and adhere to the exposed subendothelium. Activated platelets generate mediators, including the potent vasoconstrictor thromboxane A_2 and adenosine diphosphate, both of which further contribute to hemostatic plug formation.[105]

Sites of Megakaryocyte Production

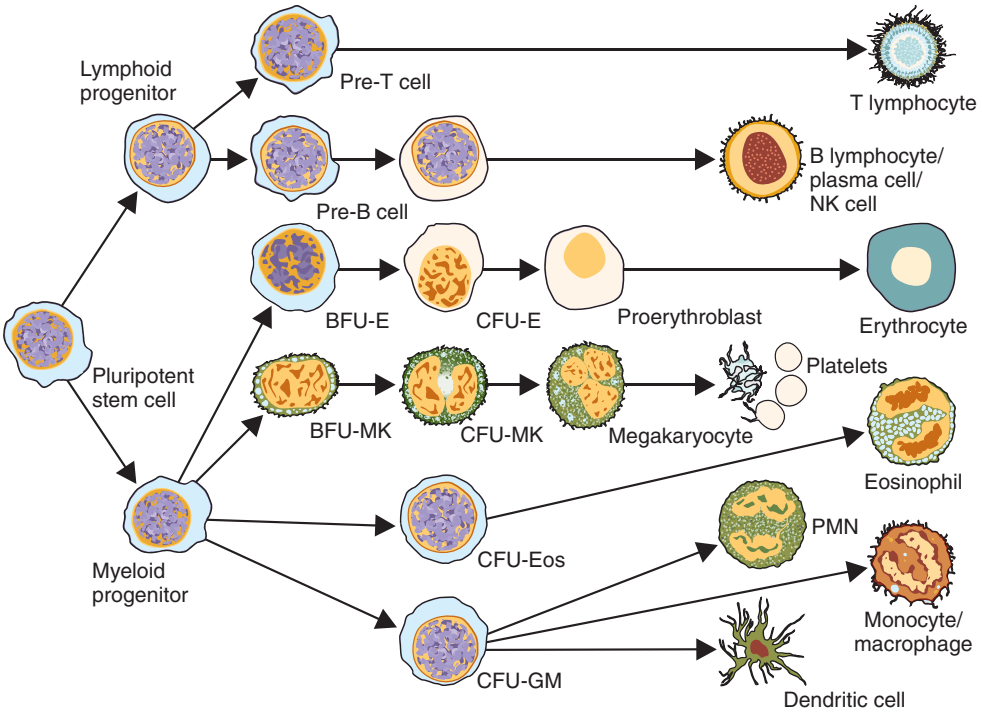
As with erythropoiesis, the sites of megakaryocytopoiesis change during embryonic and fetal development; in mouse development, megakaryocytes are found in the early yolk sac.[106] These cells, when cultured in the presence of stem cell factor, IL-3, IL-6, Epo, TPO, and granulocyte colony-stimulating factor (G-CSF), produce not only BFU-E cells but also megakaryocyte colonies. Megakaryocyte progenitors share a common progenitor with primitive hematopoietic cells.[106] In humans, electron micrograph studies have shown megakaryocytes present in the liver and circulatory system as early as 8 weeks after conception.[107]

Megakaryocyte Precursors

Megakaryocytopoiesis begins with the pluripotent HSCs, which give rise to myeloid progenitor cells (colony-forming unit–spleen cells), then burst-forming unit–megakaryocyte cells, followed by colony-forming unit–megakaryocyte cells (Fig. 66.2). Further maturation brings these small mononuclear cells that are largely indistinguishable from monocytes to large polyploid cells, which are easily recognized on the basis of their phenotype. The process of megakaryocyte differentiation has been separated into four stages. Stage I cells, or megakaryoblasts, are the smallest and most immature. As cells mature through stage II (promegakaryocytes), stage III (granular megakaryocytes), and stage IV (mature megakaryocytes), the nucleus becomes multilobed; the cytoplasm becomes increasingly eosinophilic by Wright–Giemsa staining, and cellular size increases from 6 to 24 μm up to 50 μm. The presence of granules increases steadily until, in the mature cells, they become organized into "platelet fields." Unlike in other cell lines, as the nucleus of megakaryocytes matures, it undergoes endomitosis or endoreduplication, a process by which cell ploidy is increased in the absence of cell division. Megakaryocytes from adults typically have a modal ploidy of 16 *N*, while comparable samples from preterm or term infants have a significantly lower ploidy of less than 8 *N*.[108] Megakaryocytes from newborns are also typically smaller than those in adults, although they manifest features of mature megakaryocytes.[109] Adult-size megakaryocytes appear by 2 years of age. Typically, smaller cells with lower ploidy produce fewer platelets than larger cells with higher ploidy. Despite this, the platelet counts of fetuses are only slightly lower than those of adults.[110]

Control of Megakaryocytopoiesis

Platelets serve the primary function of hemostasis but also participate in the antimicrobial defense and tissue repair.[111] Multiple cytokines participate in the process of megakaryocytopoiesis; however, TPO is the principal one. Stem cell factors, IL-3 and IL-6, increase ploidy and the size of megakaryocytes. IL-11 also stimulates the proliferation of megakaryocyte progenitors and induces megakaryocyte maturation, while still other growth factors such as Epo, stem cell factor, GM-CSF, IL-1, basic fibroblast growth factor, platelet-derived growth factor, and interferon-γ have a less clearly defined role. Some cytokines inhibit thrombopoiesis,



• **Fig. 66.2** Overview of Hematopoiesis. Hematopoietic lineages are outlined in this simplified overview of hematopoiesis. *BFU-E*, Burst-forming unit–erythroid; *BFU-MK*, burst-forming unit–megakaryocyte; *CFU-E*, colony-forming unit–erythroid; *CFU-Eos*, colony-forming unit–eosinophil; *CFU-MK*, colony-forming unit–megakaryocyte; *CFU-GM*, colony-forming unit–granulocyte-macrophage; *NK*, natural killer; *PMN*, polymorphonuclear leukocyte. (Courtesy of Alexander R. Vermillion.)

including transforming growth factor β and platelet factor 4.[112] The magnitude of the influence of these growth factors changes with development.

Thrombopoietin

The presence of a growth factor regulating platelet formation was hypothesized in the 1950s but was not realized until 1994 when the protein was isolated.[113] TPO is composed of 332 amino acids and contains two domains. The amino terminal is the active domain (153 amino acids) and bears marked homology to Epo. TPO is produced primarily by the liver, although other tissues express small amounts. It acts as a potent stimulator of all stages of megakaryocyte development by binding to its specific cell-surface receptor, c-myeloproliferative leukemia (MPL). In TPO- and c-MPL-knockout models, platelet production is 10% to 15% of that of controls, confirming that TPO is the primary regulator of platelet production but also indicating that alternative pathways exist for megakaryocytopoiesis. TPO is bound to the surface of platelets by its receptor, thereby reducing megakaryocyte exposure to the hormone.[113] Serum TPO concentrations tend to be lower in preterm infants than in older infants and children, and the TPO response to thrombocytopenia is less robust as gestational age decreases. This is counterbalanced by an increased sensitivity of megakaryocyte precursors to TPO.[114]

Early clinical trials of recombinant TPO administration to patients with immune thrombocytopenia showed significantly increased platelet counts, but the development of antibodies in a few patients, which cross-reacted with endogenous TPO, resulted in a halt to further clinical trials of recombinant TPO. In 2008 the US Food and Drug Administration (USFDA) approved the first TPO receptor agonist, molecularly and structurally unrelated to TPO but nevertheless capable of activating the TPO receptor and resulting in increased platelet production. This TPO agonist was originally called AMG531 but is now known as Romiplostim.[115]

In 2018 Romiplostim was approved by the USFDA to manage ITP in children 1 year of age or older, where safety and efficacy are reported to be good.[116] Only two case reports have been published describing the use of Romiplostim to decrease platelet transfusions in neonates with severe and prolonged thrombocytopenia. Both neonates were platelet-transfusion dependent[117,118] until Romiplostim treatment was begun, after which further platelet transfusions were not needed. No adverse effects of the medication were observed in either case.

Developmental Changes in Platelet Count

Fetal platelet counts increase with gestation. At 15 weeks, the average platelet count is 187,000/μL, increasing to 274,000/μL at term. Overall, preterm infants have slightly lower platelet counts than do adults with a broader range of normal (100,000/μL to 450,000/μL).[42,110]

Platelet Transfusions

Clinical practice regarding platelet transfusion of neonates has been inconsistent until very recently because no evidence-based guidelines were available.[119] In a 2005 Web-based survey of neonatologists in the United States and Canada, wide variations in practice were noted, with platelet transfusions frequently administered to nonbleeding neonates with platelet counts greater than 50,000/μL. This practice was particularly common during indomethacin treatment, before or after procedures or operations, or after diagnosis of intraventricular hemorrhages.[120] This is concerning because several studies show a correlation between the number of platelet transfusions received by hospitalized neonates and the mortality rate.[121,122] In a study of 1600 thrombocytopenic NICU patients, those who received platelet transfusions had higher mortality rates: 2% for those with no transfusions, 11% for those with 1 or 2 transfusions, 35% for those with more than 10 transfusions, and 50% with 20 or more transfusions.[122] This was partially due to the underlying illness that required the infant to be transfused. However, since transfusion practices differ so widely, some of the increased mortality could statistically be ascribed to the harmful effects of multiple platelet transfusions. In an attempt to create a reasonable yet safe approach to platelet transfusions, two guidelines were prospectively compared: one based on platelet count and the other based on platelet mass (platelet count times mean platelet volume). Fewer patients were transfused when platelet mass was used as a transfusion trigger.[123,124] The authors recommended that prophylactic transfusions of platelets be avoided after the first week of life unless the platelet count falls below 30,000/μL. During the first week of life, they recommend a transfusion trigger of 50,000/μL for preterm infants. For patients with platelet counts greater than 50,000/μL, transfusions should be reserved for those with active serious bleeding. Another controversy concerns the preparation of platelets: single donor, multiple donors, single unit, or volume-reduced multiple unit preparations. In general, 10- to 20-mL single donor platelets per kilogram should raise the platelet count by more than 100,000/μL. In the absence of consumption, some of the donor platelets should remain in the recipient's circulation for 1 week. The use of volume-reduced or pooled platelets should be avoided because processing results in platelet activation and decreased function.

The recently published multicentered platelet transfusion trial by Curley and collaborators[119] involved 660 premature neonates with a birth weight averaging 740 g and gestational age averaging 26.6 weeks. If the platelet count fell below 50,000 per cubic millimeter, they would either receive a platelet transfusion (randomized to the high threshold group) or do not receive a platelet transfusion unless their platelet count subsequently fell below 25,000 per cubic millimeter (the low threshold group). Outcomes were worse in those randomized to the high threshold group, who obviously received more platelet transfusions but somewhat paradoxically had fewer subsequent bleeding episodes. Thus, it is clear that platelet transfusions can have deleterious effects on preterm neonates, and the evidence from this trial strongly suggests a lower threshold for platelet transfusion is better in this population.

Subsequent to the study of Curley et al., a further analysis was performed of a subgroup of those study neonates who had a high baseline risk of death. Those also were found to have better outcomes if they had been randomized to the lower threshold platelet transfusion group. This suggests that a platelet transfusion threshold of 25,000 is better than one of 50,000, even for the smallest and most ill preterm infants.[125]

Developmental Aspects of Granulocytopoiesis

Early hematopoiesis is characterized almost exclusively by erythropoiesis, although a small number of macrophages are produced in the yolk sac. After circulation begins in the fourth to the fifth week of gestation, macrophages appear in the liver, brain, and

lungs. During the fifth week, hematopoiesis begins in the liver, and the first hematopoietic cells to appear are macrophages.[27] Whether Kupffer cells originate in the yolk sac and migrate to the liver or arise de novo in the liver is unknown. The marrow space begins to develop around the eighth week after conception, and as occurs in the liver, the first hematopoietic cells to appear in the bones are phagocytes.[27,34] These phagocytic osteoclasts seem to core out the marrow space. When hematopoiesis is established in the marrow at 10 to 11 weeks after conception, primarily neutrophils are produced, in contrast to the liver, where primarily macrophages are present.[126]

The thymus appears around 8 weeks after conception. T-cell progenitors are thought to migrate from the fetal liver to the thymus at 8 to 9 weeks after conception,[127] and by the 10th week, lymphoid cells constitute 95% of this organ, with granulocyte precursors and macrophages making up the remainder. B-cell precursors first appear in the omentum and the fetal liver 8 weeks after conception. B-cell production in the omentum occurs transiently from 8 to 12 weeks,[128] while production continues in the fetal liver. Regulatory T cells are found in the thymus and secondary lymphatic organs in the early second trimester and are proposed to be involved in self-reactivity and immune tolerance.[129,130]

The spleen is an important secondary lymphatic organ in humans. The human spleen does not have intrinsic granulocytopoiesis and erythropoiesis activities or produce hematopoietic growth factors,[22] and lymphocytes appear to migrate there through fetal blood. Lymphocytes begin to appear in the spleen around 11 weeks after conception. By the 22nd week, 70% of the cells are lymphocytes.

Hematopoietic Cytokines

Hematopoietic growth factors can be classified into two groups: those responsible for the regulation of myeloid and erythroid growth and differentiation, called *colony-stimulating factors*, and those concerned with immunity, called *lymphokines*. Once sequenced, lymphokines are assigned IL numbers. There is a great deal of functional overlap between hematopoietic growth factors (redundancy), and each growth factor has a multiplicity of biological actions (pleiotropy). Thus more than one cytokine controls cells in any cell lineage, and most factors affect cells in more than one lineage.[131]

Epo, GM-CSF, and G-CSF belong to a family of hematopoietic cytokines that share a tertiary structure and function by binding to specific cell-surface receptors. Specific ligand binding results in allosteric changes in the receptor molecules, which, depending on the type of receptor, results either in protein kinase activation, as with macrophage colony-stimulating factor,[132] or in a cascade of intracellular signaling via the Janus kinase two mechanisms, as is characterized by Epo.[133]

Many of the hematopoietic cytokines were discovered on the basis of their growth-promoting effects on hematopoietic cells or their specific immune functions. It was initially assumed that their effects were specific to the hematopoietic system. This view was incomplete. Functional receptors are expressed by other nonhematopoietic cells, with clear nonhematopoietic functions as reviewed by Li et al. and Juul and Pet.[134,135] For example, both glia and neurons produce many of the cytokines once thought to be restricted to the hematopoietic system. Furthermore, they express receptors for these peptides, indicating the capability of both paracrine and autocrine interaction. Epo and G-CSF are both available in recombinant form and are approved by the USFDA for clinical use.

Acknowledgment

This work was supported in part by a Eunice Kennedy Shriver National Institute of Child Health and Human Development Grant R01HD052820 and in part by National Institute of Neurologic Disorders and Stroke Grants U01NS077953 and U01NS092764.

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67

Neonatal Bleeding and Thrombotic Disorders

MATTHEW A. SAXONHOUSE AND ASHLEY P. HINSON

KEY POINTS

- Acquired or inherited coagulation disorders should be considered in any neonate that suffers significant hemorrhage.
- Treatment for specific coagulation disorders should be provided in consultation with pediatric hematology and based on the most current guidelines.
- Thromboembolism (TE) is a significant problem affecting both term and preterm neonates.
- Most neonates that experience a significant TE event have acquired risk factors and/or a prothrombotic disorder.
- Proper imaging is essential for accurately identifying neonatal TE events.
- The use of central venous/arterial catheters significantly increases a neonate's risk for thrombosis.
- Recommendations for treatment for neonatal TE events are based on expert opinion and data from case studies/series.
- Care for neonates with coagulation disorders or significant TE events should occur in a tertiary referral center that has appropriate subspecialty support.

Neonatal bleeding and thrombotic disorders may present a diagnostic challenge to the caregiver. Excess and/or deficiencies of certain coagulation/anticoagulation proteins coupled with multiple acquired and/or prothrombotic risk factors and/or thrombocytopenia can result in a hemorrhagic or thromboembolic (TE) emergency in the neonatal period. The timely diagnosis of a congenital hemorrhagic disorder can potentially avoid significant long-term sequelae, while the lack of randomized clinical trials addressing the management of neonatal thromboses can leave a neonatologist guessing on what the optimum treatment strategy should be. In this chapter, we briefly review the neonatal hemostatic system. Neonatal hemorrhagic disorders are presented with a discussion of current treatment options. Congenital and acquired risk factors and common sites for neonatal thromboses are reviewed. Finally, suggested evaluations for neonates with TE emergencies as well as the latest treatment options are presented.

The Neonatal Hemostatic System

Blood vessel injury causes clinical bleeding, which can be severe. Hemostasis refers to the process in which bleeding is controlled at the site of damaged endothelium.[1] The process of hemostasis involves the interaction between endothelium, subendothelium, platelets, circulating cells, and plasma proteins. Blood vessel injury leads to an immediate, local response of both plasma and cellular components. Thrombosis is confined to the area of injury, leading to eventual tissue repair. A model of hemostasis combining the vascular, platelet, and plasma phases is presented in Video 67.1. The result is a localized, firm thrombosis, leading to cessation of bleeding. The thrombosis then serves as a scaffold for tissue repair, leading to complete restoration of the endothelial lining.[2] Fibrinolysis must then occur for blood vessel repair and return of blood flow (Video 67.2).

Hemostatic processes are regulated by natural anticoagulants (Video 67.3), whose job is to contain these processes to the site of injury and to prevent these reactions from becoming systemic and pathologic.[2] Deficiencies in natural anticoagulant proteins, as well as decreased fibrinolysis in neonates, may lead to the formation of pathologic thromboses during the neonatal period, and these disorders are described later. The complex interaction of the hemostatic, fibrinolytic, and anticoagulant components of the hemostatic system results in a well-balanced machine that allows for hemostasis to occur at the site of injury and for fibrinolysis to follow, facilitating a localized tissue repair process.

Developmental Hemostasis

The neonatal hemostatic system differs significantly from that of older children and adults.[3] The concentrations of the procoagulant, anticoagulant, and fibrinolytic proteins, compared with adults', are shown in Table 67.1, and age-related normal values should be referenced when assessing for bleeding or clotting abnormalities. Essentially, all the procoagulant proteins, except for fibrinogen, factors V and VIII, and von Willebrand factor (vWF), are lower. These lower levels are balanced by lower levels of anticoagulant proteins, except for alpha2-macroglobulin, which is the only anticoagulant protein that is elevated during the neonatal period. All the fibrinolytic proteins are reduced. These differences place a neonate in a "relative" prothrombotic state, but other factors balance the system and prevent a term or "well" premature neonate from experiencing spontaneous thrombosis. However, many acquired (Table 67.2) and/or prothrombotic risk factors (Box 67.1) may disrupt this balance, shifting the neonate into a prothrombotic state.

The exact reasons for the differences in the fetal and neonatal hemostatic systems, compared with adults, are unclear but

TABLE 67.1 Neonatal Coagulation/Anticoagulation/Fibrinolytic Protein Levels Compared With Adult Levels

	Protein Levels Elevated Compared With Adult Values	Protein Levels Decreased Compared With Adult Values
Procoagulant	Fibrinogen Factor V* Factor VIII vWF	Factors II, VII, IX, X, XI, XII, XIII† Prekallikrein High-molecular-weight kininogen
Anticoagulant	Alpha2-macroglobulin	Antithrombin III Heparin cofactor II Protein C Protein S
Fibrinolytic	Tissue plasminogen activator Plasminogen activator inhibitor	Plasminogen α_2-antiplasmin

*Factor V levels are low on day of life 1 but reach adult values within days after birth.[3]
†Levels are 50% of adult values.[3]
vWF, von Willebrand factor.
Adapted from Manco-Johnson M. Controversies in neonatal thrombotic disorders. In: Ohls RY, ed. *Hematology, Immunology and Infectious Disease: Neonatology Questions and Controversies.* Philadelphia: Elsevier; 2008.

TABLE 67.2 Acquired Risk Factors for the Development of Neonatal Thromboses

Maternal Risk Factors	Delivery Risk Factors	Neonatal Risk Factors
Infertility	Emergent cesarean section	Central venous/arterial catheters*
Oligohydramnios	Fetal heart rate abnormalities	Congenital heart disease
Prothrombotic disorder	Instrumentation	Sepsis
Preeclampsia	Meconium-stained fluid	Meningitis
Diabetes		Birth asphyxia
Intrauterine growth restriction		Eosinophilia
Chorioamnionitis		Respiratory distress syndrome
Prolonged rupture of membranes		Dehydration
Autoimmune disorders		Congenital nephritic/nephrotic syndrome
Fetal thrombotic vasculopathy		Necrotizing enterocolitis
		Polycythemia
		Pulmonary hypertension
		Surgery
		Extracorporeal membrane oxygenation
		Medications (steroids)

*Greatest risk factor for thrombosis with a significant risk if present ≥14-days.
From Saxonhouse MA, Manco-Johnson MJ. The evaluation and management of neonatal coagulation disorders. *Semin Perinatol.* 2009;33:56; with permission. Data from the following references 58,60,62,73,75,78,87,90,123,135,162–169.

supported by recent theories surrounding overall fetal development.[4] In the fetus, the lower levels of antithrombin III (ATIII) are balanced by elevated concentrations of alpha2-macroglobulin, allowing for unrestricted angiogenesis during intense growth while maintaining an effective anticoagulant pathway.[5,6] Lower levels of vitamin K during fetal growth may reduce the synthesis of osteocalcin, preventing premature fetal cartilage maturation.[7]

Despite having a balanced hemostatic system at birth, neonatal prothrombin time (PT) and activated partial thromboplastin times (APTT) are elevated when compared with adults. Elevated tissue factor (TF) in neonates compensates for the lower levels of tissue factor pathway inhibitor (TFPI) and ATIII. Some aspects of decreased platelet aggregation in neonates are compensated with higher levels of vWF and factor VIII (FVIII). Therefore, despite the lower levels of coagulation proteins, neonates can form effective thromboses.

The neonatal hemostatic system undergoes numerous changes over the first 6 months of life, and many of the lower protein levels reach adult values during this time.

Bleeding Disorders in the Neonate

When faced with either an actively bleeding infant or one that has suffered a significant hemorrhage, acquired or inherited, coagulation defects must be considered, especially if the neonate has a normal platelet count. Laboratory diagnostic criteria for the bleeding newborn are shown in Table 67.3. Inherited and acquired coagulation defects and the most common symptoms associated with their presentations in the neonate are shown in Table 67.4. It is important to obtain a detailed family and delivery history for any neonate experiencing significant bleeding, as information gained may allow the clinician to perform a quicker, more focused approach.

Laboratory Investigation

The first approach (initial screen) to any neonate with a suspected bleeding disorder should be a complete blood count (CBC) and coagulation screen (PT, APTT, and fibrinogen). More specific testing can then be performed to make the correct diagnosis. It is important to remember that the method of sample collection may affect sample results. For example, heel-stick samples should

never be used for coagulation screening, and they may result in platelet clumping, which will produce a falsely low platelet count. Elevated hematocrit levels greater than 55% may result in prolonged diluted coagulation times. In addition, the collection of blood samples through heparinized arterial or venous lines will prolong the APTT, unless the specimen has the heparin absorbed and/or adequate blood is removed from the line before sample collection to clear heparin from the line. When interpreting values from coagulation screening and more detailed testing, values should be interpreted using age-adjusted normal ranges based on gestational age and days of life; these are published elsewhere.[8–10]

• BOX 67.1 Prothrombotic Disorders Implicated in the Development of Neonatal Thromboembolism

Factor V Leiden mutation (most common)
Factor II *G20210A* gene mutation (1%–2% of Caucasians)
Increased apolipoprotein(a)
Methylenetetrahydrofolate reductase gene mutation (*MTHFR C677T*) genotype
Hyperhomocysteinemia
Protein C deficiency
Protein S deficiency
Antithrombin III deficiency
Heparin cofactor II deficiency
Dysfibrinogenemia
PAI-1 4g/5G gene mutation
Increased levels of factors VIIIC, IX, XI, or fibrinogen
Antiphospholipid antibodies (including anticardiolipin antibodies, lupus anticoagulant)
Chromosome 2q
Chromosome 2q13 deletion

References 55,65,73,78,90,166,180,181.
Adapted from Saxonhouse MA. Thrombosis in the neonatal intensive care unit. *Clin Perinatol.* 2015;42:651–673.

Hemophilia

The most common congenital bleeding disorders are hemophilia A (FVIII deficiency) and hemophilia B (factor IX deficiency), both being inherited as X-linked recessive. The incidence of hemophilia A is 1 per 5000 males and for hemophilia B is 1 per 20,000 males.[11] Approximately one-third of cases will occur in the absence of a positive family history.[12] Heterozygous females may have mild hemophilia as a result of nonrandom X-chromosome inactivation.[2] The severity of hemophilia is determined by the type of mutation and the part of the protein that is affected, with severity of bleeding phenotype inversely proportional to the infant's factor level.[2] The lower the factor level, the greater the potential for more severe early onset bleeding. With the absence of either FVIII or factor IX, there is reduced thrombin formation on the surface of activated platelets, resulting in a thrombosis with poor structural integrity that is more susceptible to fibrinolysis, and as a result, bleeding occurs. Approximately 70% of patients with hemophilia are diagnosed during the first month of life, with the mean age of patients with hemophilia having their first bleed by 28.5 days.[12,13] The most common presentation of hemophilia in the neonate is excessive bleeding, either after circumcision or surgery; but these infants can also present with severe intracranial hemorrhage. Further classifications of hemophilia A and B, based on factor activity level, and the types of bleeding that neonates may present with are shown in Table 67.4.

TABLE 67.3 Laboratory Diagnostic Criterion for the Bleeding Newborn*

Disorder	PT	APTT	Platelets	Fibrinogen
Hemophilia A	Normal	↑↑↑	Normal	Normal
Hemophilia B	Normal	↑↑↑	Normal	Normal
Hemophilia C	Normal	↑↑	Normal	Normal
Factor XIII deficiency	Normal	Normal	Normal	Normal
Factor II, V, and X deficiency	↑↑	↑↑	Normal	Normal
Hemorrhagic disease of the newborn	↑↑↑	Normal/↑†	Normal	Normal
DIC‡	↑↑↑	↑↑↑	Low	Low
Liver disease‡	↑↑↑	↑↑↑	Low	Low
vWD	Normal	Normal/↑	Normal	Normal
Hypofibrinogenemia	↑↑↑	↑↑↑	Normal	Low
Dysfibrinogenemia	↑↑↑	↑↑↑	Normal	Normal/Low

*A complete blood count and coagulation screening test should be performed for any bleeding infant.
†APTT values may be prolonged but not as severe as the elevated PT value.
‡To differentiate between DIC and liver disease, a factor VIII value should be obtained. Factor VIII values will be normal in infants with liver disease but low in infants with DIC.
APTT, Activated partial thromboplastin time(s); *DIC*, disseminated intravascular coagulation; *PT*, prothrombin time(s); *vWD*, von Willebrand disease.
References 2,22,170.

TABLE 67.4 Acquired and Inherited Bleeding Disorders in Neonates

Inherited	Classification	Symptoms
Hemophilia A (FVIII) Hemophilia B (FIX)	Severe (<1%) Moderate (1%–5%) Mild (>5%)	Bleeding after circumcision and/or blood draws, ICH, extracranial hemorrhage, excessive bruising, muscle hematomas, bleeding after surgery
Fibrinogen deficiency	Decreased levels (heterozygote): hypofibrinogenemia Absent levels (homozygote): afibrinogenemia Dysfunctional Dysfibrinogenemia	Prolonged bleeding from umbilical stump, bleeding after circumcision, ICH, or mucocutaneous bleeding
Factor II (prothrombin) Factor V	Commonly associated with congenital anomalies, particularly cardiac defects	Mucocutaneous bleeding, ICH, prolonged bleeding from umbilical stump, bleeding after procedures
Factor VII Factor X	Levels do not correlate well with bleeding phenotype	
Factor XI	Levels do not correlate well with bleeding phenotype	
Factor XIII		Umbilical cord stump bleeding, ICH, bleeding after procedures

Acquired	Classification	Symptoms
DIC Liver disease		Prolonged bleeding after venipuncture/heel sticks, jaundice, pulmonary hemorrhage
Vitamin K deficiency	Early <24 hours	Cephalohematoma, umbilical stump bleeding, ICH
	Classical 1–7 days	GI bleeding, umbilical stump bleeding, mucocutaneous, circumcision, ICH
	Late ≥2 weeks	ICH, mucocutaneous, GI bleeding

DIC, Disseminated intravascular coagulation; *FVIII*, factor VIII; *FIX*, factor IX; *GI*, gastrointestinal; *ICH*, intracranial hemorrhage.
From Saxonhouse MA, Manco-Johnson MJ. The evaluation and management of neonatal coagulation disorders, *Semin Perinatol*. 2009;33:56; with permission.
References 2,22,170.

Most symptomatic infants with hemophilia A and B will have significant prolongation of their APTT; however, if the diagnosis is suspected, specific factor levels should always be obtained. If there is a strong family history for hemophilia, factor levels may be screened from cord blood samples at birth, but severity of disease should always be confirmed from samples obtained after the infant is born. Infants suspected to have hemophilia, or with known family histories, should have strategies employed to reduce the risk of bleeding, such as subcutaneous vitamin K at birth, applied pressure to an intramuscular injection site for 5 minutes, use of smallest possible needles and lancets for venipunctures, and avoidance of arterial punctures.[14]

Because FVIII levels are increased during the neonatal period, the diagnosis of mild hemophilia A may be difficult, and confirmatory testing should be done at 6 to 12 months of age. In addition, lower levels of factor IX at birth may also make the diagnosis of mild hemophilia B difficult, and testing should also be repeated at 6 to 12 months of life.

Treatment for bleeding episodes is replacement of the specific factor and should be done as quickly as possible and in consultation with pediatric hematology, as there are a number of factor concentrates that are commercially available for both FVIII and factor IX deficiency (Table 67.5).[2] Many neonates do not require treatment during the neonatal period, as they may not manifest any bleeding symptoms. Adjuvant therapies for hemophilia, such as desmopressin, may increase factors to hemostatic levels, and can be useful for management of minor bleeding or procedures in patients with mild factor VIII deficiency. Desmopressin should be used with caution in infants, and only in conjunction with pediatric hematology, as use carries a risk of symptomatic hyponatremia.[2] Fresh frozen plasma (FFP) should only be used in the instance of acute hemorrhage when confirmatory testing is not yet available.[15,16] Any infant with concern for hemophilia and intracranial bleeding should receive immediate factor replacement as this can be life-threatening if not treated rapidly and appropriately.

von Willebrand Disease

The primary plasma protein required for platelet adhesion, vWF, also has a role in platelet aggregation and serves as the carrier for FVIII.[2] Absence, reduction, or abnormal function of vWF results in defects in platelet adhesion and aggregation, increasing one's risk for bleeding. The effectiveness of vWF as an adhesive protein relies on multimerization of the protein, resulting in very

TABLE 67.5 Treatment for Congenital/Acquired Bleeding Disorders

Factor	Replacement Therapy
Hemophilia A	Factor VIII concentrate
Hemophilia B	Factor IX concentrate
Fibrinogen	Fibrinogen concentrate FFP or cryoprecipitate if concentrate unavailable
Factor II	Prothrombin complex concentrate (if available) FFP if concentrate unavailable
Factor V	FFP
Factor VII	Recombinant factor VIIa or prothrombin complex concentrate (if available)
Factor X	Factor X concentrate (if available) FFP if concentrate unavailable
Factor XI	Factor XI concentrate (if available; use with caution) FFP if concentrate unavailable
Factor XIII	Plasma derived and recombinant (only for A subunit deletions) FXIII concentrate Cryoprecipitate if concentrate unavailable
Early vitamin K deficiency Classical vitamin K deficiency Late vitamin K deficiency	Parenteral vitamin K Prothrombin concentrates for active bleeding Recombinant factor VIIa for severe active bleeding
DIC*	FFP and cryoprecipitate, platelets
Liver disease*	Cryoprecipitate, FFP, prothrombin complex concentrates, platelets, vitamin K, recombinant factor VIIa

*Therapies for bleeding patients, not abnormal laboratory testing.
DIC, Disseminated intravascular coagulation; *FFP*, fresh frozen plasma; *FXIII*, factor XIII.
From Saxonhouse MA, Manco-Johnson MJ. The evaluation and management of neonatal coagulation disorders. *Semin Perinatol*. 2009;33:56; with permission.
References 2,22,170.

large molecules comprising what are known as high-molecular-weight multimers.[2] Neonates have higher plasma concentrations of vWF and an increased proportion of high-molecular-weight vWF multimers than older children or adults. As a result, presentation of von Willebrand disease (vWD) during the neonatal period is rare.[17]

The spectrum of vWD is presented in Table 67.6. Suspicion for vWD in a neonate requires specialized testing. Coagulation testing may demonstrate an isolated prolonged APTT and prolongation of epinephrine and ADP closure times as measured by the PFA-100. Further testing would evaluate the levels of vWF (vWF antigen assay), platelet-binding function (ristocetin cofactor assay), and FVIII-binding function (FVIII activity).[18,19] Assistance by a pediatric hematologist should occur if a diagnosis is suspected.[2]

The management of type 3 vWD should consist of factor replacement using an intermediate purity FVIII concentrate containing the high-molecular-weight multimers of vWF. Desmopressin should be reserved for those patients with type 1 vWD but, as noted above, should only be used after consultation with a pediatric hematologist, as it carries a risk of symptomatic hyponatremia in infants.[2]

TABLE 67.6 Spectrum of Neonatal von Willebrand Disease

Type	Description	Clinical Findings
1	Quantitative	Reduced amounts of vWF due to decreased production/secretion of vWF or increased clearance
3		Complete absence of vWF due to severe gene mutations*
2A	Qualitative	Defects in multimerization resulting in absence of large and medium-sized multimers
2B		Mutation that leads to increased binding of the high-molecular weight multimers to platelets
2M		Mutation leading to inability of vWF to bind to platelets
2N		Affects vWFs ability to bind to FVIII

*Highest potential for presentation during the neonatal period
vWD, von Willebrand Disease; *vWF*, von Willebrand factor; *FVIII*, factor VIII.
References 8,19,171,172.

Other Rare Inherited Coagulation Disorders

There are other rare factor deficiencies, inherited as autosomal recessive, in 1 in 500,000 to 1 in 2,000,000 live births, representing 3% to 5% of all coagulation disorders.[20,21] A complete listing of the disorders and their potential symptoms are displayed in Table 67.4. Severe deficiencies of fibrinogen, factor VII, factor X, and factor XIII are the most likely (of the rare coagulation disorders) to present during the neonatal period. The majority of these deficiencies will present with an abnormality in the coagulation screen (see Table 67.3). Further testing for the specific abnormality will then confirm the diagnosis.[22] One must remember that newborn levels of many of these factors are lower at birth, and therefore diagnosis may be difficult and must be confirmed by 6 to 12 months of life. Treatment for the various deficiencies is shown in Table 67.5.

Factor XIII is composed of two subunits and cross-links with fibrin to stabilize clots. Factor XIII deficiency is extremely rare, with an incidence of one per 1 to 3 million people but can cause a range of bleeding symptoms in the neonatal period from skin or umbilical cord bleeding, to severe intracranial hemorrhage.[23] Low levels of factor XIII do not prolong the PT or APTT. Therefore, any neonate with concerns for a coagulation disorder that has a normal platelet count, normal fibrinogen levels, and normal PT and APTT values should be screened for factor XIII deficiency via a quantitative assay. Treatment of factor XIII deficiency is shown in Table 67.5.

Acquired Coagulation Disorders

Vitamin K Deficiency

Vitamin K is found in leafy green vegetables as vitamin K_1 (phytonadione) and is synthesized as vitamin K_2 in intestinal bacteria.[24]

It is an essential cofactor for the γ-carboxylation process of procoagulant factors II, VII, IX, and X and anticoagulant proteins C and S.[22] Insufficient bacterial colonization of the colon at birth, inadequate dietary intake in solely breastfed infants, poor intestinal absorption of vitamin K, and poor transfer across the placenta place neonates at risk for vitamin K deficiency bleeding (VKDB). Further, preterm infants are at higher risk for VKDB than term infants, due to hepatic and gut flora immaturity. The different forms of VKDB and their clinical presentation are shown in Table 67.4. Treatment is shown in Table 67.5.

Early VKDB may be due to maternal malabsorption disorders or maternal ingestion of oral medications which inhibit vitamin K such as warfarin, anticonvulsants, and antituberculosis agents. These agents cross the placenta and interfere with vitamin K metabolism. Classical VKDB occurs because of a physiologic deficiency in vitamin K at birth combined with a sole breast milk diet (low vitamin K in breast milk), inadequate vitamin K prophylaxis at birth, or inadequate feeding. Late VKDB presents in an infant that is either solely breastfed who receives an inadequate dose of vitamin K (none or one oral dose) or has an associated disease process that interferes with the absorption or supply of vitamin K such as diseases of malabsorption or cholestasis like diarrhea, cystic fibrosis, α_1-antitrypsin deficiency, biliary atresia, hepatitis, and celiac disease.[25,26] In the absence of vitamin K prophylaxis, the incidence of late VKDB is 4 to 10 per 100,000 births.[27] When intramuscular (IM) vitamin K prophylaxis is provided, the incidence of late VKDB decreases to 0.24 to 3.2 per 100,000 live births.[28]

If VKDB is suspected, coagulation screening should be performed and will usually demonstrate isolated prolongation of the PT, followed by prolongation of the APTT. The prolongation of the PT is usually out of proportion to the elevation of the APTT. Fibrinogen concentration and platelet counts will be normal. In addition, decreased concentrations of factors II, VII, IX, and X will occur. An alternative is to obtain an undercarboxylated or abnormal coagulation factor II measurement.[29] This factor is released into the bloodstream in the very early stages of vitamin K deficiency, and it can be detected well before changes in the PT become manifest. The adoption of this single test in clinical practice may improve the early diagnosis of VKDB, resulting in decreased incidences of intracranial hemorrhage (ICH).

When presented with a patient with suspected VKDB, parenteral treatment (intravenous, IM, or subcutaneous injection) with vitamin K should immediately occur. Improvement of the PT and APTT 2 to 6 hours after the administration of parenteral vitamin K will confirm the diagnosis. However, if a patient suspected of having VKDB presents with severe hemorrhage, additional therapy with prothrombin complex concentrates (contain all the vitamin K-dependent factors; not available at every institution; use FFP if not available) aimed at immediate correction of factor deficiencies should occur.[2] Additionally, recombinant factor VIIa can be used in the treatment of severe intracranial hemorrhage due to vitamin K deficiency.

Common practice in the United States is to provide all infants 1.0 mg (0.3 mg/kg for infants <1000 g and 0.5 mg for infants >1000 g but <32 weeks' gestation) of IM vitamin K on the first day of life.[24] This single dose has been found to prevent both classical and late VKDB, even in infants with cholestatic jaundice.[30,31] The safety of IM vitamin K has been questioned because of the reported association between IM vitamin K administration in newborns and an increased incidence of childhood cancer.[32,33] Further studies have concluded no association between IM vitamin K and childhood leukemia or other cancers.[34] Further research has suggested a prenatal origin of childhood leukemia, further weakening the plausibility of a causal relationship between IM vitamin K and cancer.[35]

Proper oral administration of vitamin K has been shown to have an efficacy similar to that of IM vitamin K in the prevention of early and classical VKDB.[36,37] However, cases of late VKDB began to increase and surveillance data from four countries revealed oral prophylaxis failures of 1.2 to 1.8 per 100,000 live births,[38] with higher rates of 2 to 4 per 100,000 cases when incomplete oral dosing was observed. More recent data have demonstrated that weekly or daily oral dosing of vitamin K until 3 months of age protects almost all babies from VKDB, and late VKDB remains confined to breastfed infants who received no vitamin K or just one oral dose. [39,40]

A more recent study evaluated the association of VKDB in breastfed infants with unrecognized biliary atresia and oral versus IM dosing of vitamin K.[41] Of 91 breastfed infants diagnosed with biliary atresia, 25 received a 2-mg IM dose after birth compared with 55 and 11 infants receiving 25-μg and 150-μg oral daily dosing, respectively; 4% of the infants that received IM dosing experienced vitamin K deficiency bleeding compared with 82% (both groups) of the infants that received only oral dosing. More importantly, 0% of the infants who received IM dosing experienced ICH compared with 40% and 27%, respectively, in the oral dosing groups.[41] Thus, a single dose of IM vitamin K at birth remains standard of care for preventing vitamin K deficiency bleeding; but repeated oral vitamin K until 3 months of age is an option for those who decline or refuse the IM dose.

Disseminated Intravascular Coagulation

A complex process involving the activation and dysregulation of the coagulation and inflammatory systems, disseminated intravascular coagulation (DIC), results in massive thrombin generation with widespread fibrin deposition and consumption of coagulation proteins and platelets, ultimately leading to multiorgan damage and hemorrhage.[12] DIC always occurs as a secondary event, and many perinatal and neonatal problems are associated with its development. These include birth asphyxia, respiratory distress syndrome, meconium aspiration syndrome, infection, necrotizing enterocolitis, hypothermia, severe placental insufficiency, homozygous protein C/S deficiency, and thrombosis.[12]

The diagnosis of DIC is made in an ill neonate with supporting laboratory parameters (see Table 67.3). The most important aspect of treatment for DIC is to treat the underlying disorder. However, once DIC is established, it can be difficult to control.[12] The focus of acute management in the neonate is to support adequate hemostasis to reduce the risk of spontaneous hemorrhage. This is usually achieved with platelet transfusions, FFP, and/or cryoprecipitate.[42] Another option is to inhibit the activation of the coagulation system using heparin. However, trials in neonates have not been conclusive, and the risk of bleeding may be increased, so this is generally not advised.[43,44]

Liver Disease

Acute liver disease is rare in the neonatal population. Illnesses that can lead to acute liver failure include congenital heart disease with low cardiac output, birth asphyxia, extrahepatic biliary atresia, inborn errors of metabolism, hemophagocytic syndromes, and viral hepatitis. Mechanisms contributing to hemostatic abnormalities include decreased synthesis of coagulation factors, activation of the coagulation and fibrinolytic systems, poor clearance of activated hemostatic components, loss of coagulation proteins into ascitic fluid, thrombocytopenia, platelet dysfunction, and vitamin

K deficiency.[44] The diagnosis of acute liver disease in the neonate should include elevated liver enzymes, direct hyperbilirubinemia, prolonged PT and APTT, thrombocytopenia, elevated ammonia concentrations, decreased fibrinogen levels, and decreased concentrations of factors VII and V (see Table 67.3). A normal FVIII concentration, reflecting extrahepatic synthesis, can help distinguish primary liver disease from DIC.[44] Treatment, in addition to treating the underlying cause of liver disease (if able), includes prothrombin complex concentrates (if available),[2] cryoprecipitate (if fibrinogen is low), FFP, recombinant factor VIIa (for severe bleeding not responsive to platelets and plasma products), platelets, and vitamin K.

Neonatal Thrombosis

Epidemiology

Neonatal TE is becoming an increasingly recognized condition due to improved diagnostic modalities and the survival of the smallest and sickest neonates. The exact incidence of neonatal TE varies with the most recent centers reporting 6.9 to 15 per 1000 NICU admissions.[45] Thromboses occur in both term and preterm infants, with a slight predominance in male neonates.[45] The majority of venous thromboses in neonates are associated with central venous catheters (CVCs).[46] The recurrence rate for neonatal TE remains low and ranges from 3.3% to 7%.[47]

Risk Factors for Neonatal Thromboembolism

Hypercoagulability, disturbances in blood flow, and endothelial damage/disruption (Virchow triad) all pertain in some degree to neonates admitted to the NICU.[2] Therefore, it is understandable that neonates have the highest rate of TE events among pediatric patients. As demonstrated in Table 67.1, neonates, particularly premature neonates, are deficient in the anticoagulant proteins, especially protein C and protein S. Prothrombotic disorders may also be present, shifting the neonate into a hypercoagulable state. Hypovolemic, septic, or cardiogenic shock may affect an ill neonate, resulting in hypotension and thus disrupting blood flow to vital organs. Polycythemia or certain cardiac lesions may also result in sluggish blood flow and/or hyperviscosity, increasing the risk for thrombosis. However, the presence of CVC and arterial catheters is by far the greatest acquired risk for the development of TE in neonates.[46] Insertion, infusion of hyperosmolar substances, and length of time that a catheter remains in place may disrupt the endothelium, increasing the risk for thrombosis. The effects of different types of catheter materials, catheter design, and lumen number have been evaluated. These reviews have demonstrated that fewer thrombotic complications were associated with end-hole umbilical arterial catheters (UACs).[48–51] Risk factors that have been implicated in neonatal TE are shown in Table 67.2.

Prothrombotic Disorders: Pathophysiology and Their Role in Neonatal Thromboembolism

A genetic mutation resulting in the complete absence or severe deficiency of an inhibitor of hemostasis, the production of an inhibitor of hemostasis that has inadequate function despite normal levels, or an overproduction of a procoagulant protein or cofactor greatly increases a neonate's risk for TE. The more severe the mutation, the greater the risk for the development of severe thrombosis. Homozygous prothrombotic disorders, such as severe protein C, protein S, or ATIII deficiency, usually present in newborns with severe clinical manifestations, mainly purpura fulminans.[52] The inheritance of a heterozygous prothrombotic mutation and a neonate's risk for symptomatic TE is less well defined.[53] Despite having a heterozygote prothrombotic mutation, a neonate may never experience a symptomatic TE event, thus demonstrating that the inheritance of a prothrombotic trait does not guarantee a symptomatic TE event. Other acquired risk factors (see Table 67.2) are usually present. Prothrombotic disorders that have been associated with neonatal TE are shown in Box 67.1.

Neonatal registries have demonstrated that greater than 80% of neonates with symptomatic thromboses have acquired risk factors present (see Table 67.2).[53,54] Therefore, it appears that the combination of acquired factors, and the presence of a prothrombotic mutation, may represent the perfect storm for neonatal TE.[54] Registry data and other case studies have demonstrated that the majority of symptomatic neonatal TE events stem either from the association of multiple prothrombotic defects or the combination of prothrombotic defects and environmental or clinical conditions.[55–61] It is therefore currently recommended that pediatric patients, including neonates, with thrombosis (regardless of other acquired risk factors), be tested for prothrombotic traits (see Box 67.1).[62,63] A more detailed laboratory approach is presented later.

The most common prothrombotic disorder is the factor V Leiden mutation, which reduces the inactivation of activated factor V by its regulator, activated protein C.[64] Heterozygous mutations affect about 5% of the Caucasian population, and confer a 5-fold increased TE risk in adults.[65] The presence of the prothrombin 20210 mutation has the potential to increase circulating concentrations of prothrombin by 15% to 30%.[66,67] The exact role regarding mutations in the methylenetetrahydrofolate reductase (MTHFR) enzyme and risk for neonatal TE remains unclear.[68] The mutation results in a defect in the remethylation of homocysteine into methionine, with the end result being hyperhomocysteinemia. Increased levels of homocysteine have been speculated as an increased risk for premature vascular disease and arterial thrombosis.[61,69] However, most studies have associated an increased risk for thrombosis because of elevated homocysteine levels (kidney damage) and not with the genetic mutation. Thus, the *MTHFR* gene mutation and the risk of neonatal arterial thrombosis remain controversial.[70,71]

The activated protein C system, consisting of protein C plus protein S and factor V as cofactors, cleaves and inactivates factors V and VIII. Mutations that affect the quantity or quality of these proteins may contribute to an increase in neonatal venous thrombosis. ATIII, when bound to heparin sulfate, inhibits thrombin and activated factors IX, X, and XI. Mutations may result in deficiencies in ATIII production, increasing thrombosis risk.[72] Elevations in lipoprotein(a) (reduces fibrinolytic capacity) have been implicated as a significant risk factor for both venous and arterial thrombosis in the German and Dutch registries; however, generalizing these findings to all populations remains unclear.[73,74] Lupus anticoagulant, anticardiolipin antibody, anti-β_2-glycoprotein-I antibodies, and other maternal antiphospholipid antibodies may play a role in neonatal TE, especially neonatal stroke. These antibodies either lead to thromboses in the placenta or are transported across the placenta and may serve as a nidus for thrombosis formation.[75–77] A recent review of 16 infants with a history of maternal antiphospholipid antibodies and perinatal thrombosis found that 13/16 had arterial thromboses, with 8/16 presenting as strokes. Therefore, mothers of and/or infants with

significant arterial or venous TE events should be screened for the presence of antiphospholipid antibodies.[78]

Locations of Neonatal Thromboses, Imaging Modalities to Diagnose Them, and Management Guidelines for Specific Thromboses

Neonatal TE events may occur in a variety of locations and are usually identified by proper imaging modalities (Table 67.7). Difficult intravenous access, desire to limit radiation exposure, lower glomerular filtration rates, and inaccessibility to perform specific testing at the bedside limit neonatal use of many of the gold standard techniques used in adults and children.[79] Therefore, doppler ultrasonography is the most widely and safely used modality. The small diameter of certain vessels, low pulse pressure, and the presence of a CVC at the site of thrombus may limit interpretation of the results.[79]

Arterial Thromboses

Neonatal Arterial Ischemic Stroke

The majority of neonatal arterial TE events are represented by neonatal arterial ischemic stroke (NAIS). Neonatal stroke (NS) refers to a cerebrovascular incident causing interruption of blood flow that presents in the first 28 days postnatally and is confirmed by neuroimaging or pathological studies.[80] The subcategories of neonatal stroke and their presenting symptoms are displayed in Table 67.8.[81,82] Arterial stroke in neonates is referred to as NAIS for the remainder of the chapter (cerebral sinovenous thromboses are presented later). NAIS refers to a condition with acute encephalopathy, seizures, or neurologic deficits presenting in the term or preterm infant before the 29th postnatal day, with brain imaging confirming a parenchymal infarct in the appropriate arterial territory (Fig. 67.1).[82,83] NAIS is responsible for 22% to 70% of congenital hemiplegic cerebral palsy in neonates,[75,84,85] as well as other neurologic comorbidities, including seizure disorders, delayed language development, and behavioral disorders.[86]

TABLE 67.7 Locations of Neonatal Thromboses and the Best Imaging Modalities to Diagnose Them

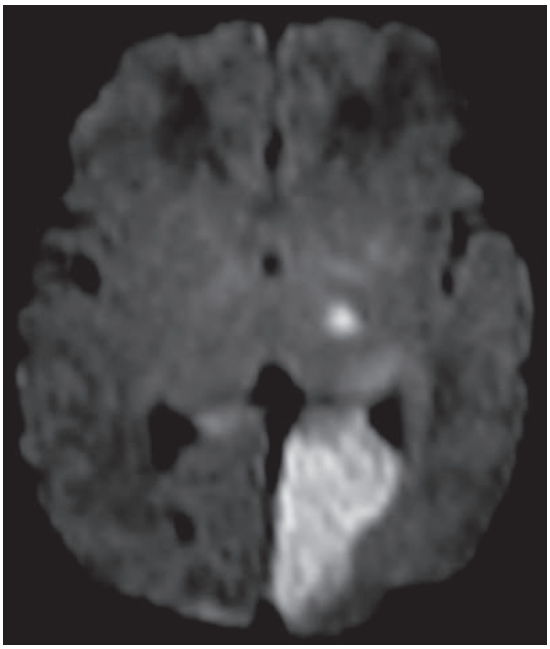
Vessel	Type of Thromboses *(Vessels Potentially Involved)*	Imaging Modality
Arterial	Perinatal arterial ischemic stroke *(Left middle cerebral artery, anterior cerebral artery, posterior cerebral artery)*	Diffusion-weighted MRI/MRA
	Iatrogenic *(Abdominal aorta, radial artery, renal artery, mesenteric artery, popliteal artery)*	Doppler ultrasound
	Spontaneous *(Iliac artery, left pulmonary artery, aortic arch, descending aorta, renal artery)*	
Venous	Iatrogenic/spontaneous vessel occlusion *(SVC, IVC, hepatic vein, subclavian vein, abdominal veins, peripheral veins)*	
	Renal vein	
	Portal vein	
	Cerebral sinovenous *(Superior sagittal sinus, transverse sinuses of the superficial venous system, straight sinus of the deep system)*	Diffusion-weighted MRI w/ venography
	Congenital heart disease related *(Right/left atria, right/left ventricle, SVC, IVC)*	Echocardiography

IVC, Inferior vena cava; *MRA*, magnetic resonance angiogram; *MRI*, magnetic resonance imaging; *SVC*, superior vena cava.
Adapted from Saxonhouse MA. Management of neonatal thrombosis. *Clin Perinatol.* 2012;39:192–193; with permission.
References 74,91,134,161,172–175.

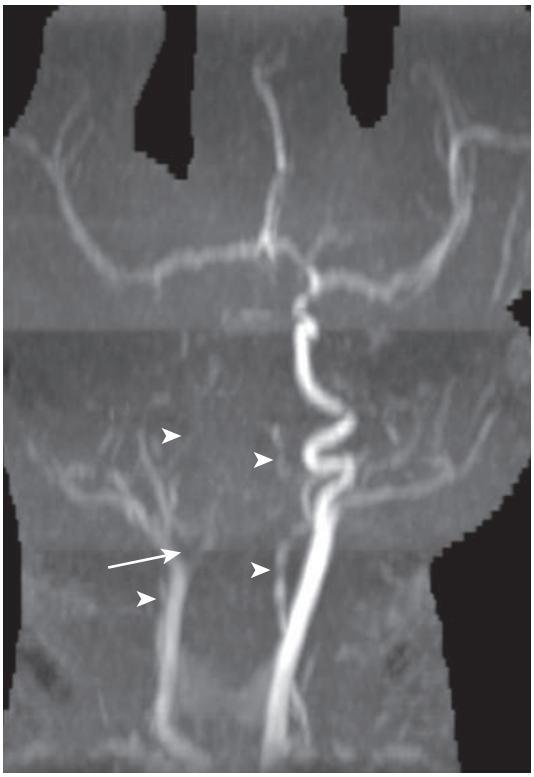
TABLE 67.8 Subcategories of Neonatal Stroke

Subcategory	Description	Symptoms
Neonatal arterial ischemic stroke	Occurs after birth but before 29th day of life	Seizures (focal), apnea, chewing or bicycling movements, persistent feeding difficulties
Perinatal arterial ischemic stroke	Difficult to establish whether stroke occurred before, during, or after childbirth but occurs from 20 weeks' gestational age until birth	
Neonatal hemorrhagic stroke	Predominant signal intensity is blood with no underlying vascular lesion or bleeding abnormality	
Neonatal cerebral sinovenous thrombosis		Seizures, apnea, lethargy, irritability, poor feeding, jitteriness, thrombocytopenia, anemia, changes in muscle tone

References 75,81,82,87,93,176,177.



• **Fig. 67.1** Axial diffusion-weighted MRI demonstrating areas of high signal in bilateral frontal and parietal lobes, left occipital lobe, left thalamus, and left pons.



• **Fig. 67.2** Three-dimensional (3D) maximum intensity projection image from 3D time-of-flight MRA demonstrates occlusion of the right internal carotid artery at its origin *(arrow)*. The vertebral arteries are diminutive *(arrowheads)*, and the basilar artery is not visualized.

The incidence of NAIS ranges from 25 to 35 per 100,000 live births.[81,87] Most abnormalities occur in the left hemisphere within the distribution of the middle cerebral artery, with anterior and posterior cerebral artery lesions being less common.[75,88] The origin of the left carotid artery from the aorta allows a more direct vascular route to the brain as a corridor for cardiac emboli. Rarely, multifocal cerebral infarctions can occur, and these tend to be embolic in origin (Fig. 67.2). Many potential risk factors have been implicated in the etiology of NAIS (see Table 67.2).

Placental pathology is a major risk factor for NAIS. Maternal/fetal conditions that may result in decreased placental reserve, thromboinflammatory processes leading to placental blood vessel thrombosis, and sudden catastrophic events may result in emboli that break off from the placental circulation and enter the fetal circulation, passing through a patent foramen ovale with a predilection to enter the left middle cerebral artery.[89]

Prothrombotic disorders (see Box 67.1) may have a role in NAIS as results have varied in population-based studies.[81,90] In a study of 91 full-term neonates with NAIS, prothrombotic risk factors, most commonly lipoprotein(a), were identified in 68% of these neonates compared with 25% in controls.[91] A more recent study of 27 neonates with NAIS did not identify any prothrombotic disorders.[81]

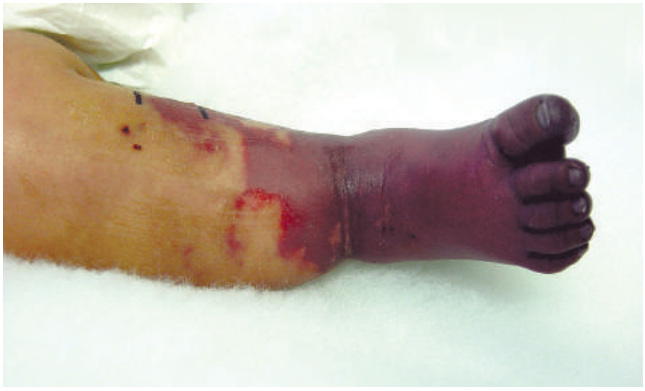
There are no randomized clinical trials addressing the management of neonates with NAIS. Current guidelines from the American College of Chest Physicians recommend anticoagulation for neonates with NAIS if there is an ongoing cardioembolic source or if there is evidence of recurrent NAIS. Otherwise, supportive care is recommended.[92,93] Treatment, if necessary, ultimately depends on the acute clinical situation and the specific hemorrhagic risks for that neonate.

Magnetic resonance imaging (MRI) with diffusion-weighted imaging (DW MRI) is the most sensitive technique for the early detection of NAIS.[94] This technique allows for the detection of cerebral edema, which is an early sign of cerebral ischemic damage.[95,96] Angiography allows for the detection of thromboses if there is a history of instrumentation/difficult delivery. Although cranial ultrasound (CUS) is the least-invasive method for diagnosing NAIS, it is also the least sensitive. One study found that 75% of cases of neonatal NAIS were missed when CUS was used.[97] Therefore NAIS should never be excluded in any neonate based on negative CUS results.

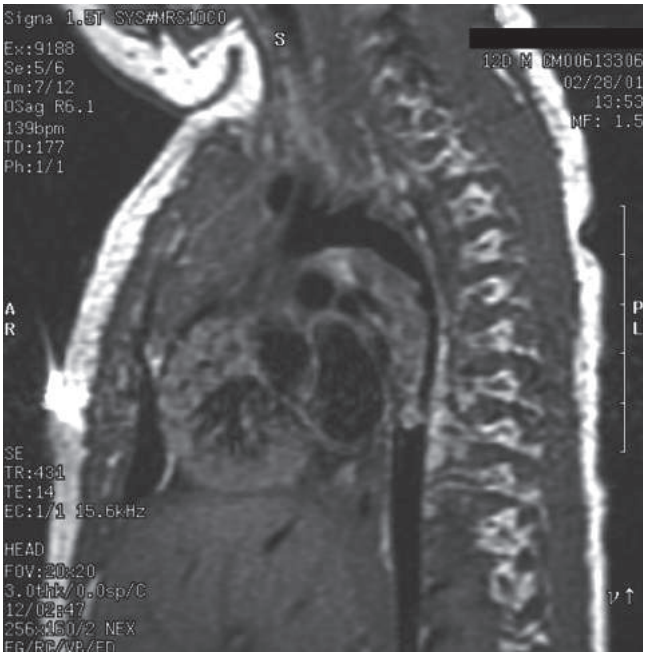
Iatrogenic/Spontaneous Arterial Thromboses

Used for continuous monitoring of arterial blood pressure and blood gases, umbilical arterial catheters (UACs) and peripheral arterial lines (PALs) are frequently used in ill neonates admitted to the NICU. Approximately 10% to 64% of newborns admitted to the NICU require placement of a UAC.[4] Femoral arterial catheters are used less frequently but are more commonly used in neonates with congenital heart disease or those requiring extensive surgery. Despite their important role, one must remember that complications from arterial catheters can occur and range from line dysfunction/infection to limb-/organ-threatening ischemia, as demonstrated in Figs. 67.3–67.5.

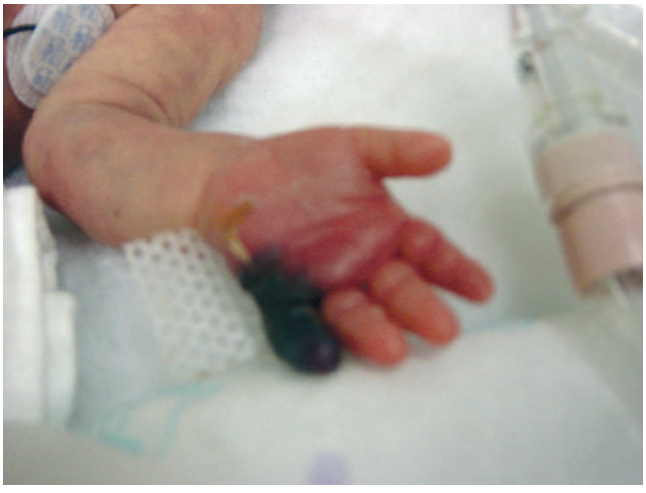
The incidence of arterial thromboses from UACs based on autopsy reports has ranged from 9% to 28%,[98] whereas the incidence of PAL-related thromboses is unknown. Potential short-term and long-term complications of UACs include mesenteric ischemia, hypertension, renal dysfunction/failure, limb loss, and congestive heart failure.[99,100] High UAC positioning (T6-9) has been found to have fewer clinical complications,[51] while low-dose continuous heparin infusion at 1 U/mL prolongs catheter patency but does not reduce the risk of thrombosis.[50] Clinical signs of PAL-related thrombosis include limb ischemia, pale or cold extremities distal to the cannulation site, weak or absent pulse, and decreased



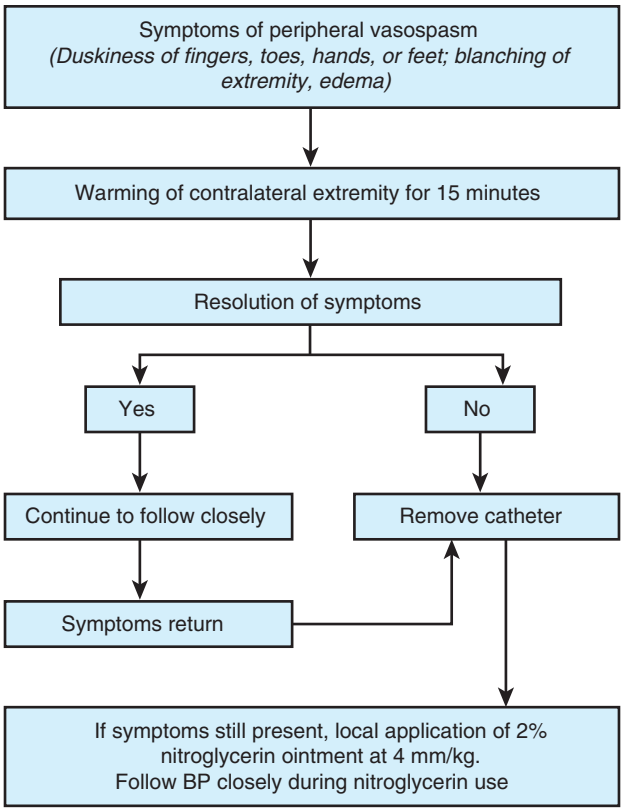
• **Fig. 67.3** Arterial thrombosis with skin necrosis.



• **Fig. 67.4** Aortic thrombus diagnosed by MRI.



• **Fig. 67.5** Arterial thrombosis of digital artery.



• **Fig. 67.6** Management of peripheral vasospasm. Current recommendations for the evaluation and management for neonates with peripheral vasospasm caused by complications from peripheral arterial lines and umbilical arterial catheters. Nitroglycerin dosing is provided. *BP*, Blood pressure.

or immeasurable blood pressure (see Fig. 67.5).[101] Catheter material, duration of placement, and solutions infused influence the risk for both UAC-related and PAL-related thrombosis.

Suspicion or confirmation of an arterial thrombosis should warrant immediate removal of the arterial catheter. Vascular spasm may occur, and removal of the catheter may simply resolve symptoms. If symptoms of spasm persist, further actions are needed. An approach to the neonate with vascular spasm is shown in Fig. 67.6. If thrombosis is suspected, the most sensitive method to diagnose an arterial catheter-related thrombosis is contrast angiography,[102] but because of its risk in neonates, it is generally reserved for older children and adults. As a result, Doppler ultrasound is mainly employed in neonates.

Management options for neonates with a UAC and peripheral arterial catheter or femoral arterial catheter-related TE event are to (1) remove the catheter and provide supportive care, (2) administer anticoagulation following catheter removal if there are non-limb-threatening symptoms, or (3) attempt thrombolysis if there are limb-threatening symptoms.[92]

Venous Thrombosis

The biggest risk for the development of venous thromboses in neonates admitted to the NICU is the presence of a CVC. Additional risk factors (see Table 67.2) combined with the presence of a CVC further increase this risk.

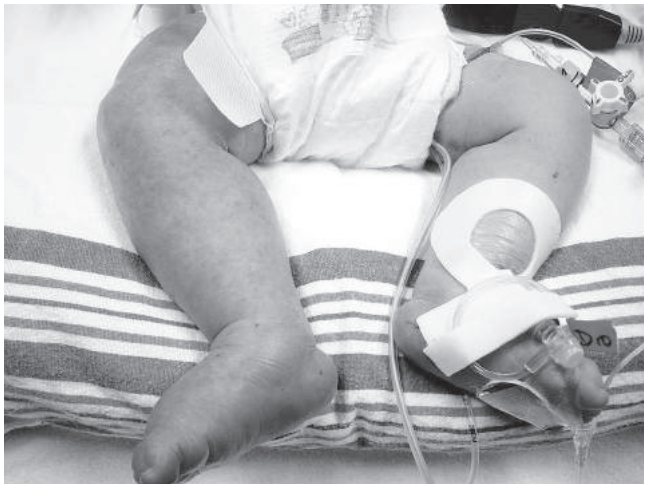
Catheter-Related Venous Thrombosis (Umbilical Venous Catheters and Peripherally Inserted Central Venous Catheters)

Premature and sick newborns greatly benefit from CVCs. Infusion of life-saving medications and enhanced nutrition lead to better outcomes, but risks for thrombosis, infection, malfunction, and death exist. Those caring for neonates admitted to the NICU must weigh the benefit versus the risk every day that a CVC remains in place, and the catheter should be removed as soon as necessary. Damage to blood vessel walls during insertion, disrupted blood flow, infusion of substances that damage endothelium, and thrombogenic catheter materials are the main reasons that thromboses develop.[103,104]

Neonates, especially very low birth weight infants (<1500 g), routinely have umbilical venous catheters (UVCs) placed during the first few days of life as peripheral access may be difficult to secure. Despite their clinical importance, UVCs represent the most common cause for thrombosis in premature infants.[79] Appropriate catheter position is vital to avoid severe complications.[105] The exact incidence of UVC-related thrombosis varies, but reports have ranged from 21% to 71% (dependent on how aggressive centers screened for asymptomatic thrombosis) and include portal vein, renal vein, and inferior vena cava (IVC) thrombosis.[103] Autopsy studies have estimated that 20% to 65% of infants who die with a UVC in situ have microscopic evidence of TE.[106,107] Intracardiac thrombosis from UVC placement ranges from 1.8% to 5.3%.[103]

Clinical signs and symptoms suggestive of a UVC-related thrombosis include persistent infection, persistent thrombocytopenia, line dysfunction, and bilateral lower limb edema (Fig. 67.7).[92] The Centers for Disease Control and Prevention recommend that use of UVCs be limited to 14 days.[108] A randomized trial comparing long-term UVC (up to 28 days) use with short-term (7 to 10 days) use followed by peripheral central venous line placement demonstrated that 4% of neonates in the short-term group, compared with 7% of neonates in the long-term group, developed significant thromboses (detected by echocardiogram). However, all of the thrombi were at the site of the UVC tip, and none of the neonates required treatment.[109] Most centers remove UVCs within 7 days of placement, but malpositioned lines should be removed as soon as possible.

To enhance nutrition, and to administer long-term parenteral medications, peripherally inserted central venous catheters (PCVCs), or surgically placed CVCs, are routinely placed in preterm and ill neonates. These lines tend to remain in place for weeks until treatment is finished or an infant reaches full enteral feedings. Clinical signs of PCVC and surgically placed CVC-related thrombosis include unilateral limb swelling/pain/discoloration, superior vena cava syndrome, chylothorax, chylopericardium, intracardiac thrombosis, unresolving sepsis, thrombocytopenia, and cardiac failure.[55,79,110]



• **Fig. 67.7** Femoral deep vein thrombus. Note the swollen leg, with venous congestion pattern on the skin.

The gold standard for diagnosing venous TE is MR (magnetic resonance) venogram; however, this modality is difficult to perform in neonates.[102] Ultrasound is therefore the most widely and safely used modality. False-negative results often occur when evaluating for thromboses in the upper central venous system because of obstruction of the distal subclavian veins by the clavicles. In addition, compression of veins in a central location by the probe is not feasible because of the neonate's rib cage.[44]

Long-term complications of venous TE include chronic venous obstruction with prominent cutaneous collateral circulation, chylothorax, portal hypertension, and embolism. More children are now presenting with postthrombotic syndrome who had a previous history of a neonatal venous TE.

Management options for CVC-related thromboses are displayed in Table 67.9 and depend on whether the CVC is vital for patient care and/or the neonate is symptomatic. If anticoagulation is used, it should continue until there is resolution of the thrombus. Treatment may last from 2 weeks to 3 months, depending on the size, location, and symptoms of the thrombus.[92]

Intracardiac Thromboses and Thromboses in Infants With Complex Congenital Heart Disease

The placement of a CVC into the right atrium may lead to damage of the endocardium, inducing either pericardial tamponade and/or the development of intracardiac thrombi. The development of intracardiac vegetations secondary to CVC infections may expose an infant to prolonged infection and dissemination of septic emboli. Thrombus formation in the right atrium and superior vena cava has been associated with a high endocarditis risk, sepsis persistence, pulmonary artery obstruction, ventricular dysfunction, acute hemodynamic compromise, and death.[56,111–113]

Thrombosis is a common complication reported in neonates undergoing repair for complex congenital heart disease.[114,115] Less is known about neonates undergoing initial palliative procedures for single ventricle physiology. A retrospective review of neonates who underwent palliative repair found that in patients who died and had autopsies available, 33% of deaths were attributable to thrombosis.[116] A more recent review of 22 neonates who underwent palliative repair between 1 and 11 days of life found evidence of thrombi in five infants (23%).[115]

A recent case-control study performed at a high-level referral NICU demonstrated an incidence of intracardiac thrombosis of 22.5 cases per 1000 admissions.[113] Many of these infants were critically ill and had complex congenital heart disease. Major risk factors from this study included prematurity, maternal history of gestational diabetes/diabetes mellitus, surgical cut-down technique to place a CVC, and *Staphylococcus epidermidis* infection. In addition, almost one-quarter of these infants died despite receiving treatment for the thrombosis.[113]

Echocardiography is the preferred modality for diagnosing either right atrial thrombus formation, intracardiac vegetations, or thrombus formation in infants with single ventricle physiology. Signs suggestive of an atrial thrombus include new-onset murmur, persistent sepsis, persistent thrombocytopenia, and cardiac failure. A surgical approach to remove the thrombus in neonates

TABLE 67.9 Recommendations for Central Venous Catheter-Related Thromboses

	Vital for Patient Care*	Symptomatic	Worsening	Recommendation
CVC	Yes	Yes	No	Initiate anticoagulation; no removal of the catheter
	Yes	Yes	Yes	May remove catheter vs continue to use catheter but anticoagulation should have already been initiated
	No	Yes	N/A	Initiate anticoagulation for 3–5 days prior to removal of the catheter
	No	No	N/A	Immediate removal of the catheter

*Assumed that catheter is still functioning; if not functioning, considered nonessential.
CVC, Central venous catheter.
Monagle P, Cuello CA, Augustine C, et al. American Society of Hematology 2018 Guidelines for management of venous thromboembolism: treatment of pediatric venous thromboembolism. *Blood Adv.* 2018;2(22):3292–3316.

TABLE 67.10 Management of Renal Vein Thrombosis (RVT), Portal Venous Thrombosis (PVT), and Cerebral Sinovenous Thrombosis (CSVT)

TE		Clinical Description	Treatment/Monitoring
RVT	Unilateral or bilateral (symptomatic)	Normal renal function	Anticoagulation
		Renal failure	Initial thrombolytic therapy with rt-TPA, followed by anticoagulation
PVT		Nonocclusive thrombus and no evidence of portal hypertension	Observation with repeat US in 10-days
		Occlusive thrombus (extension into the IVC, RA, and/or RV)	Anticoagulation *rTPA with UFH may be considered if there is evidence of end-organ compromise*
CSVT		Evidence of CSVT and infant symptomatic	Anticoagulation Repeat MRI and MRV in 6 weeks for vessel recanalization. If complete, stop therapy. If not, consider 6+ weeks of treatment

IVC, Inferior vena cava; *MRI*, magnetic resonance imaging; *MRV*, magnetic resonance venography; *RA*, right atrium; *rt-TPA*, recombinant tissue type plasminogen activator; *RV*, right ventricle; *TE*, Thromboembolism; *UFH*, Unfractionated heparin; *US*, ultrasound.

is not feasible, especially the premature infant. There have been case reports using recombinant tissue type plasminogen activator (rt-TPA) for thrombolysis of catheter-related atrial thrombus in the neonate.[111,117–120] rt-TPA has also been used successfully in the management of premature infants with infective endocarditis.[121]

Renal Vein Thrombosis

The renal vein is the most common location for spontaneous venous TE in neonates,[56] with most cases presenting during the first month of life.[122,123] The most recent population-based cohort study demonstrated an annual incidence of 2.6 per 100,000 live births.[124] Reviews of neonatal renal vein thrombosis (RVT) cases have demonstrated that 70% were unilateral, with 64% of these involving the left kidney, and males were more frequently affected.[123,125,126] The classic triad of signs and symptoms suggesting RVT are macroscopic hematuria, a palpable abdominal mass, and thrombocytopenia, although varying numbers of neonates presenting with these exact three symptoms varies.[45,125] Other symptoms include oliguria, proteinuria, acute renal failure, and hypertension. Risk factors (shown in Table 67.2) are frequently found in RVT cases, with prematurity and perinatal asphyxia being the most common.[125] Prothrombotic risk factors have been found in varying numbers of neonatal cases of RVT, with studies finding at least one prothrombotic risk factor in 17% to 67% of cases.[123,125,127,128] Because of the potential association of prothrombotic disorders and RVT, an evaluation for an inherited thrombophilia is warranted.

The diagnosis of RVT is usually made via Doppler ultrasound. Radiographic criteria for RVT include presence of echogenic clot, venous distention secondary to the presence of a thrombus, or absence of flow.[125]

The treatment for RVT is controversial, as there are no large randomized clinical trials addressing the issue. However, complications of RVT are serious and include adrenal hemorrhage, extension of the clot into the IVC, renal failure, hypertension, and death.[123] A recent cohort study demonstrated a 12.3-fold increased risk of chronic kidney disease or death and a 15.7-fold increased risk for hypertension.[124] The most recent treatment recommendations based on observational and case series and expert opinion are shown in Table 67.10.[129]

Portal Vein Thrombosis

The two main risk factors for the development of portal vein thrombosis (PVT) in neonates are sepsis/omphalitis and UVC use.[130] Most cases of PVT are isolated to the left portal vein, but some involve the right or main portal vein and extend into the inferior vena cava.[130] PVT tends to be clinically silent, making the diagnosis difficult. Incidences vary (because of aggressiveness of imaging in asymptomatic patients following UVC use) between 1% and 75%.[130–133] Ultrasound is the preferred modality for diagnosis, and findings of cavernous transformation of the portal vein with subsequent splenomegaly and reversal of portal flow are used to document its severity.[130] Spontaneous resolution of asymptomatic PVT is relatively common, but the detection of PVT, even in asymptomatic patients, warrants close observation to follow for signs of portal hypertension.[45] This complication may manifest itself up to 10 years after the neonatal period.[130,134] Management options, based on limited clinical data, are provided in Table 67.10.[129,130]

Cerebral Sinovenous Thrombosis

Cerebral sinovenous thrombosis (CSVT) represents a subcategory of neonatal stroke[81] with symptoms being like those with NAIS (see Table 67.8).[81,135] Anemia and/or thrombocytopenia may accompany these symptoms. The incidence of CSVT ranges from 3 to 12 per 100,000 live births.[136,137] A review of neonates with CSVT demonstrated that 90% of children who presented with CSVT had predisposing risk factors (see Table 67.2 and Box 67.1), with 16% of these patients having multiple risk factors.[90,135] The most common risk factors were infections (40%), perinatal complications (25%), and prothrombotic traits (13%).[135] More recent studies have reported CSVT in neonates following therapeutic hypothermia[138] and cardiac surgery.[139]

The superficial and lateral sinuses are the most frequently involved vessels,[75] and up to 30% of cases have reported venous infarction with subsequent hemorrhage.[140] Impaired or absent venous drainage in one of the cerebral sinuses leads to increased venous pressure, vasogenic edema, and secondary infarction. Intraventricular hemorrhages (especially in term neonates) and hemorrhages within the caudate nucleus and thalamus are associated with thrombosis of the deep cerebral venous sinuses. Therefore, the presence of an intraventricular hemorrhage or thalamic hemorrhage in a term or late preterm infant warrants exclusion of CSVT.[140] Diagnosis of CSVT is best made through diffusion MRI with venography.[63,79,92,141]

Neonatal CSVT mortality rates have ranged from 2% to 24%.[142] Long-term follow-up of neonates diagnosed with CSVT has demonstrated neurologic deficits, consisting of cerebral palsy, epilepsy, and cognitive impairments in 10% to 80% of infants.[142,143]

The role of anticoagulation for CSVT remains controversial, and recommendations for management are not based on clinical trials.[129,143] Current treatment recommendations are shown in Table 67.10. Surgery is reserved for those with hydrocephalus or large intracerebral hematomas with mass effect.

Evaluation of Neonatal Thromboses

The question remains whether every neonate diagnosed with a clinically significant thrombosis should have a full evaluation for a prothrombotic disorder (see Box 67.1). Does the presence of acquired risk factors (see Table 67.2) provide enough risk to avoid searching for a possible prothrombotic trait? Does a critically ill neonate with a CVC-related venous thrombosis warrant a full prothrombotic evaluation?[102,144,145] Definitive answers to these questions remain unknown, but based on the current evidence from data registries, case series, case reports, and expert opinion, a stepwise investigation for a prothrombotic disorder should be performed based on the type and severity of the thrombosis and the number of acquired risk factors. Timing of the evaluation is important, with certain aspects being performed immediately following an acute event, and other aspects being performed at 3 to 6 months of life. Key points and a comprehensive laboratory evaluation based on acquired risk factors are listed in Table 67.11 and Box 67.2. The clinician may choose to add or delete tests based on the presentation, family history, or timeframe of the event.

The large amount of blood that traditionally has been required to perform this evaluation has always been a criticism; therefore, the evaluation should take place at an experienced tertiary care center that has the ability to perform these tests.[120] This approach dramatically reduces the amount of blood required, as it is specifically designed for neonates. Although anticoagulation treatment is not usually started based on a prothrombotic evaluation, the identification of a disorder may help prevent future thromboses during childhood and adolescence. Anticoagulation before certain medical or surgical procedures may help to avoid the development of a significant thrombosis and truly be beneficial to the future health of the patient.

Management of Arterial and Venous Thromboses

The use of anticoagulant/fibrinolytic therapy in the NICU carries the significant risk for bleeding, especially ICH. Many clinicians are hesitant to start therapy, even when the benefits outweigh the risks. Specific neonatal formulations for antithrombotic agents are limited, making accurate and reproducible weight-adjusted dosing difficult.[92,129]

The majority of recommendations and dosing regimens for anticoagulant/fibrinolytic therapy in neonates are based on case series, cohort studies, and expert opinion. The goals for fibrinolysis are restoration of blood flow, preserving organ function, and preventing catastrophic long-term complications, including death. The goals for anticoagulation are prevention of recurrent thromboses and serious sequelae from embolism.[146] Before initiating any therapy, serious complications must be considered, and the treatment's benefits must outweigh its risks, especially in the premature infant. Family discussions highlighting the risks and goals for treatment must be documented before initiating any therapy. Treatment should occur at a tertiary care center that has proper laboratory, blood bank, pharmacy, pediatric radiological subspecialty, pediatric hematological subspecialty, and pediatric surgical support.[92,120] The clinician should refer to the most recent guidelines,[46,129] or may use the service 1-800-NO CLOTS to receive up-to-date management guidance. Before initiating any treatment, absolute and relative contraindications to antithrombotic therapy should be reviewed (Table 67.12).

Unfractionated Heparin

The use of unfractionated heparin (UFH) should be limited to clinically symptomatic thromboses that are not life or limb threatening. UFH achieves its main anticoagulant effect by binding to antithrombin, catalyzing its ability to inactivate thrombin and factor Xa.[147] The dosing of UFH in neonates is affected by

TABLE 67.11 Evaluation for Prothrombotic Disorder

Laboratory Testing If Other Acquired Risk Factors Present	Laboratory Testing If Other Acquired Risk Factors Not Present
Antiphospholipid antibody panel, anticardiolipin and lupus anticoagulant (IgG, IgM)*	Antiphospholipid antibody panel, anticardiolipin and lupus anticoagulant (IgG, IgM)*
Protein C activity†	Protein C activity†
Protein S activity†	Protein S activity†
Lipoprotein (a)†	Antithrombin (activity assay)†
Plasminogen level (if considering thrombolytic therapy)†	Factor V Leiden‡
Antithrombin III (AT-III) (activity assay)†	Prothrombin G‡
Factor V Leiden‡	PAI-1 4 G/5 G mutation‡
Factor II G20210A (prothrombin G)‡	Homocysteine† (If elevated, screen for methylenetetrahydrofolate reductase gene mutation)
	Lipoprotein a†
	FVIII activity†
	FXII activity†
	Plasminogen activity†
	Heparin cofactor II†

*May be performed from maternal serum during first few months of life.
†Protein-based assays are affected by the acute thrombosis and must be repeated at 3 to 6 months of life, before a definitive diagnosis may be made. Therefore, recommend that complete evaluation (excluding DNA-based assays) be performed at 3 to 6 months of life.[140,164] If anticoagulation is being administered, then these assays should be obtained 14 to 30 days after discontinuing the anticoagulant. Lipoprotein(a) levels may need to be repeated at 8 to 12 months of life.
‡DNA-based assays.
ATIII, Antithrombin III; *EDTA*, ethylenediaminetetraacetic acid; *IgG*, immunoglobulin G; *IgM*, immunoglobulin M.
Adapted from Saxonhouse MA, Manco-Johnson MJ. The evaluation and management of neonatal coagulation disorders. *Semin Perinatol*. 2009;33:59; with permission.

• BOX 67.2 Key Points Regarding the Laboratory Evaluation for a Prothrombotic Disorder

- Because of many of the pro/anticoagulation protein levels being lower than adult values, the diagnosis of a coagulation disorder may be difficult in the immediate neonatal period.
- Certain protein-based assays may aid in treatment during the neonatal period (antithrombin III and plasminogen assays) and may be performed during the neonatal period.
- DNA-based assays are accurate and may be obtained at any time.
- Lipoprotein(a) concentrations increase during the first year of life and should be repeated at 8–12 months of life if values obtained at 3–6 months are low, especially in Caucasian individuals.
- The different evaluations listed below are based on the presence of acquired risk factors, type of thrombosis, severity of thrombosis, and treatment regimen.
- Baseline complete blood count, prothrombin time, activated partial thromboplastin time, and fibrinogen levels should be obtained shortly after the acute event.
- Antiphospholipid antibody panels should be obtained from the mother and may warrant coordination with obstetrics.
- Placental pathology, especially in cases of perinatal arterial ischemic stroke, should be requested.

Adapted from Saxonhouse MA. Thrombosis in the neonatal intensive care unit. *Clin Perinatol*. 2015;42:651–673.

TABLE 67.12 Contraindications for Anticoagulation/Thrombolysis

	Absolute	Relative
Medical conditions	1. CNS surgery or ischemia (including birth asphyxia) within 10 days	1. Platelet count $<50 \times 10^4/\mu L$ ($100 \times 10^4/\mu L$ for ill neonates)
	2. Active bleeding	2. Fibrinogen concentration <100 mg/dL
	3. Invasive procedures within 3 days	3. INR >2
	4. Seizures within 48 h	4. Severe coagulation deficiency
		5. Hypertension

CNS, Central nervous system; *INR*, international normalized ratio.
Adapted from Manco-Johnson M. Controversies in neonatal thrombotic disorders. In: Ohls RY, ed. *Hematology, Immunology and Infectious Disease: Neonatology Questions and Controversies*. Philadelphia, PA: Saunders Elsevier; 2008:68; with permission.
Data from references 92,102,141,157,168.

many factors, including lower AT levels, increased binding of UFH to plasma proteins, and faster clearance in neonates compared with adults.[148–150] These differences explain why neonates tend to require a higher infusion rate to reach therapeutic levels than do adults.[151,152] Current dosing guidelines for neonates are provided in Table 67.13. Routine use of AT-replacement therapy

TABLE 67.13 Recommended Dosing for Anticoagulant and Thrombolytic Therapy in Neonates

Gestational Age	UFH*	LMWH†	rTPA‡
≤32-weeks	15 units/kg/h	1.5 mg/kg SQ q 12 h	For both age groups: 0.03 mg/kg/h Dose escalation up to 0.3 mg/kg/h may be considered, but must be done slowly with continuous monitoring of the patient (see Box 67.3) Supplementation with plasminogen (FFP at 10 mL/kg) prior to commencing therapy is recommended to ensure adequate thrombolysis Infuse UFH at 10 units/kg/h during rTPA treatment
>32-weeks	28 units/kg/h	2 mg/kg SQ q 12 h For both age groups: Prophylactic dosing 0.75 mg/kg SQ q 12 h	

Complete blood count, platelet count, and coagulation screening (including aPTT, PT, and fibrinogen) should be performed prior to starting anticoagulation/thrombolytic therapy.
Monitoring for UFH: Maintain anti-Xa UFH assay level of 0.3 to 0.7 U/mL. Levels should be checked 4 to 6 h after initiating therapy. If loading dose provided, check level 4 to 6 h after loading dose provided. If need to make changes in dosing, check levels 4 to 6 h after each change in infusion rate.
Bolus dosing for UFH should be performed only if there is a significant risk or evidence of thrombus progression. Otherwise, avoid bolus dosing in neonates. If bolus dosing is recommended: ≤32-weeks 25 U/kg IV over 10-min; >32-weeks 50 U/kg IV over 10-min.
If infant with renal dysfunction, dosing should be discussed with pharmacy.
FFP, fresh frozen plasma; *UFH*, Unfractionated heparin.
*Dosing applies also to post-conceptional age (GA + weeks of life).
†**Monitoring for LMWH**: Maintain anti-Xa LMWH assay level of 0.5–1.0 U/mL. Check level 4-h after the 2nd or 3rd dose. If therapeutic, repeat level within 24- to 48-hours. If remain therapeutic, then may check weekly.
‡Dose titrations for rTPA may be made every 12 to 24 h and are as follows: 0.06 mg/kg/h → 0.1 mg/kg/h → 0.2 mg/kg/h → 0.3 mg/kg/h. Max dose is 0.3 mg/kg/h.
Data from references 92,120,141,155,156,158,159,178.

is not recommended in neonates receiving UFH for standard anticoagulation.[129]

The most recent review of UFH dosing in neonates does not recommend a loading dose.[146] A loading dose is not recommended because of increased bleeding risk and low risk for recurrent thrombosis. Additionally, there is no evidence suggesting that recurrent thromboses are due to subtherapeutic antifactor Xa levels during the first 24 hours of anticoagulation.[146] Loading doses should be reserved for neonates with significant risk or evidence for thrombus progression.

Proper laboratory monitoring of UFH therapy is crucial to reducing bleeding complications (see Table 67.13). Guidelines for adjusting UFH therapy are presented elsewhere.[92]

Therapy is usually limited to 2 to 14 days, but data to support this recommendation are lacking.[92] Bleeding is the most significant complication of UFH therapy in neonates with concerns for IVH in preterm neonates. Therefore, CUS should be performed before and during treatment. Treatment of hemorrhage usually only requires cessation of the infusion because of UFH's short half-life. Other complications such as heparin-induced thrombocytopenia, although common in adults and children, rarely occurs in neonates.[153,154]

Low-Molecular-Weight Heparin

Low-molecular-weight heparin (LMWH), specifically enoxaparin, has become the neonatal anticoagulant of choice because of a low risk of hemorrhage, subcutaneous (SQ) dosing, and reduced monitoring requirements.[155–157] LMWH specifically acts against factor Xa, and its administration can be achieved either through SQ injection or the use of an indwelling SQ catheter (Insuflon; Unomedical, Birkerod, Denmark).[141,156] The use of the SQ catheter reduces the number of needle sticks from 14 per week to 1 per week. However, case reports and reviews of SQ catheter usage in neonates have reported that greater than 50% of neonates experience minor adverse events, including induration, leakage, and bruising.[158] Other case reports have reported major adverse events caused by SQ catheters in three infants and one case of an infected hematoma.[156–159]

Recommended dosing and monitoring for infants less than 2 months of age are provided in Table 67.13. Guidelines for adjusting LMWH therapy are presented elsewhere.[92]

Maintaining therapeutic levels in neonates may be a challenge. Lack of pediatric formulations and rapid growth rates in premature infants are the main reasons.[141] A recent review evaluating enoxaparin use in 240 neonates found that the mean maintenance dose of enoxaparin ranged from 1.48 to 2.27 mg/kg every 12 hours for all infants, but was higher for preterm neonates at 1.9 to 2.27 mg/kg every 12 hours.[158,159] These findings have influenced current recommended dosing (see Table 67.13).[159]

LMWH therapy has been effective in the NICU with centers reporting either partial or complete resolution of TE events in 59% to 100% of neonates treated.[158,159]

Recombinant Tissue Type Plasminogen Activator

Fibrinolytic agents, specifically recombinant tissue type plasminogen activator (rt-TPA), convert plasminogen to plasmin, which cleaves fibrinogen to fibrin and fibrin degradation products. rt-TPA use in neonates should *only* be considered for limb-threatening or organ-threatening thromboses and acute atrial thrombi.[76,92,118,129] Treatment in neonates is affected by low baseline plasminogen levels.

The safety and efficacy of rt-TPA treatment in neonates have been reported in case series and cohort studies demonstrating complete or partial clot lysis in 84% to 94% of cases.[118,120,160] Based on limited data, recommended dosing is provided in Table 67.13, with very careful laboratory and radiologic monitoring required, and these recommendations are provided in Table 67.14. rt-TPA treatment does not inhibit clot propagation or directly affect hypercoagulability; therefore, simultaneous infusion of UFH at 10 U/kg per hour is recommended.[118,141] Once the clot is adequately lysed, rt-TPA therapy should be discontinued, with anticoagulants, preferably LMWH, started (Box 67.3).

Surgery

The use of microsurgical techniques, and combined microsurgical and thrombolytic regimens, has the potential to rapidly restore

TABLE 67.14 Monitoring Recommendations for Thrombolytic Therapy in Neonates

Testing	When Performed	Levels Desired (If Applicable)
Imaging of thrombosis	Before initiation of treatment Every 12–24 h during treatment	
Fibrinogen level	Before initiation of treatment 4–6 h after starting treatment Every 12–24 h	Minimum of 100 mg/dL Supplement with cryoprecipitate
Platelet count	Before initiation of treatment 4–6 h after starting treatment Every 12–24 h	Minimum of 50–100 × 10^4/μL, dependent upon bleeding risk
Cranial imaging	Before initiation of treatment Daily during treatment	
Coagulation testing	Before initiation of treatment 4–6 h after starting treatment Every 12–24 h	
Plasminogen	Before initiation of treatment 4–6 h after starting treatment Every 12–24 h	Adequate to achieve thrombolysis Supplementation with plasminogen (FFP) before commencing therapy is recommended to ensure adequate thrombolysis
Line associated or mucosal oozing	At all clinical assessments	Topical thrombin as needed

FFP, Fresh frozen plasma.
Adapted from Saxonhouse MA. Management of neonatal thrombosis. *Clin Perinatol.* 2012;39:191–208; with permission.
Data from references 118,157,179.

• BOX 67.3 Key Points Regarding rTPA Therapy in Neonates

- When using for arterial thrombi, reimage at 12-hour intervals.
- When using for venous thrombi, reimage at 12- to 24-hour intervals.
- If repeat imaging reveals clot lysis <50%, *increase* infusion to next dosing level and repeat imaging in 12–24 h.
- If repeat imaging reveals clot lysis 51%–94%, continue same dose of infusion and repeat imaging in 12–24 h.
- If repeat imaging reveals clot lysis >95%, stop infusion, and initiate anticoagulation protocol.
- If no clot dissolution occurring 12–24 h after starting infusion and/or d-dimers are not increasing, may also give additional 10 mL/kg of FFP to provide plasminogen to increase efficacy of rTPA.
- Maintain fibrinogen levels >100 mg/dL (provide Cryo if <100) and platelet counts >50,000 during rTPA treatment.
- rTPA infusion should not be used for >96 h unless deemed appropriate by neonatology and hematology staff taking care of baby.
- When starting rTPA therapy, please have the staff place a sign at the bedside alerting all caregivers that the patient is you.

blood flow, avoiding tissue loss, without major bleeding complications, especially in patients with peripheral arterial occlusion.[161] One center's experience of 11 patients with arterial vascular access–associated thrombosis, secondary to PAL complications, described 5 patients that required arteriotomy, embolectomy, and subsequent microvascular reconstruction.[161] When faced with a significant arterial thrombosis, especially one from a PAL, surgery may be entertained, but should only take place at an experienced institution.

Suggested Readings

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68

Neonatal Platelet Disorders

EMÖKE DESCHMANN AND MARTHA SOLA-VISNER

KEY POINTS

- The most common cause of mild to moderate, early-onset thrombocytopenia in well-appearing neonates is placental insufficiency, frequently manifesting as small-for-gestational status at birth. This thrombocytopenia resolves spontaneously, usually within 10 days, and carries good prognosis. Thrombocytopenia in sick infants is usually associated with sepsis or necrotizing enterocolitis (NEC) and requires prompt intervention.
- Neonates with platelet counts $<50 \times 10^9$/L in the first day of life, particularly if well appearing, should be screened for neonatal alloimmune thrombocytopenia (NAIT). Random donor platelet transfusions (± IVIG) are the first line of therapy for these infants, unless HPA-1b1b and 5a5a platelets are immediately available for use (as is the case in some European countries, or if a prior child was affected). In those cases, these platelets are the preferred first line treatment.
- The risk of bleeding in thrombocytopenic neonates is multifactorial and is not related to the severity of the thrombocytopenia. Gestational age <28 weeks, postnatal age <10 days, and a diagnosis of NEC are more important predictors of bleeding than the platelet count.
- Historically, there has been significant variability in platelet transfusion thresholds used in the NICU. The recently published large multicenter PlaNeT-2 trial found a *higher* incidence of death or major bleeding among neonates randomized to receive platelet transfusions for platelet counts $<50 \times 10^3$/mcL compared to those transfused only for platelet counts $<25 \times 10^3$/mcL, supporting the hypothesis that platelet transfusions might be harmful to neonates.
- A subsequent risk stratification analysis of neonates enrolled in PlaNeT-2 demonstrated that infants with a high baseline risk of bleeding and mortality benefit from the low platelet transfusion threshold as much as (or more than) low-risk neonates.
- Most platelet function defects seen in the NICU are acquired, and are related to medications, medical conditions (i.e., uremia), or medical interventions (i.e., ECMO, therapeutic hypothermia). The most severe platelet function defects, which can present with bleeding in neonatal life, are Bernard-Soulier syndrome, caused by deficiency of glycoprotein Ib (the vWF receptor), and Glanzmann thrombasthenia, caused by a deficiency of glycoprotein IIb/IIIa (the fibrinogen receptor) on the platelet surface.

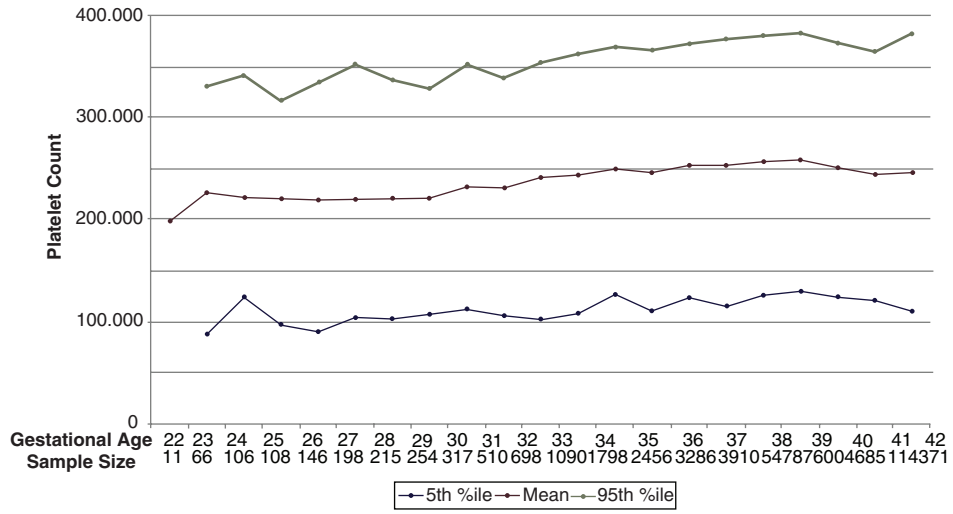
Fetal and Neonatal Platelet Production

A mounting body of evidence arising over the last decade has clearly demonstrated that there are substantial morphological and biological differences between fetal/neonatal and adult megakaryocytes and platelets. Developmental stage-specific differences are ontogenetically important because they allow the fetus to maintain stable platelet counts while the blood volume is rapidly expanding in a time characterized by exceptionally rapid growth. The complex process of platelet production can be represented as consisting of four main steps: (1) the production of thrombopoietic factors, mainly thrombopoietin (TPO), (2) the proliferation of megakaryocyte progenitors, (3) the differentiation and maturation of megakaryocytes through a unique process of endomitosis, and finally (4) the production and release of platelets into the circulation.

Studies culturing megakaryocyte progenitors derived from term and preterm umbilical cord blood, fetal blood (18 to 22 weeks' gestation), or fetal bone marrow have documented that fetal/neonatal megakaryocyte progenitors proliferate at a significantly higher rate as compared to their adult counterparts. Additionally, fetal and neonatal megakaryocytes are substantially smaller and have lower ploidy levels than adult megakaryocytes. Contrary to adult megakaryocytes, which mature as their ploidy level increases, neonatal megakaryocytes are fully mature and capable of platelet production despite their small size and low ploidy. This dissociation between proliferation, polyploidization, and cytoplasmic maturation is a hallmark feature of neonatal megakaryopoiesis. The net result of this process is the production of large numbers of low-ploidy but highly mature megakaryocytes, with which fetuses and neonates populate their rapidly expanding bone marrow space and blood volume, while maintaining normal platelet counts. As developmental processes are easily disturbed, however, sick neonates, and particularly very low birth weight infants (birth weight <1500 g), are at high risk of thrombocytopenia.

Platelet Counts During Development and Reference Ranges

In 2009, Wiedmeier et al. published the largest study on neonatal platelet counts conducted to date, which included approximately 47,000 infants delivered between 22 and 42 weeks' gestation.[1] This study showed that platelet counts at birth increased with advancing gestational age (Fig. 68.1) by approximately 2×10^9/L for each week of gestation. Importantly, while the mean platelet count was $\geq 200 \times 10^9$/L even in the most preterm infants, the 5th percentile was 104×10^9/L for those ≤32 weeks' gestation, and 123×10^9/L for late-preterm and term neonates (see Fig. 68.1).[1] These findings suggested that different definitions of thrombocytopenia should be applied to preterm infants. In that regard, however, it is important to emphasize that the reference ranges in that study were generated by eliminating the top 5% and the bottom 5% of all available values, rather than excluding values based on diagnoses. Thus, these should be considered "epidemiological reference



• **Fig. 68.1** First recorded platelet counts, obtained in the first 3 days after birth, in neonates born at 22 to 42 weeks' gestation. Mean values are indicated by the red line, and the 5th and 95th percentiles are shown in the blue and green lines, respectively. (From Wiedmeier SE, Henry E, Sola-Visner MC, et al. Platelet reference ranges for neonates, defined using data from over 47,000 patients in a multihospital healthcare system. *J Perinatol*. 2009;29:132.)

ranges" rather than "normal ranges." Nevertheless, these data suggest that platelet counts between 100 and 150 × 10^9/L might be more frequent among otherwise healthy extremely preterm infants than among full term neonates or older children/adults.

Thrombocytopenia in neonates (as in adults) has traditionally been defined as a platelet count <150 × 10^9/L and has been classified as mild (100 to 150 × 10^9/L), moderate (50 to 99 × 10^9/L), and severe (<50 × 10^9/L). However, consistent with the data by Wiedmeier et al., platelet counts in the 100 to 149 × 10^9/L range are more common among healthy neonates than adults. The incidence of thrombocytopenia in neonates varies significantly, depending on the population studied. Based on the traditional definitions, large studies in unselected populations of live-births (including healthy and sick neonates) established an overall incidence of neonatal thrombocytopenia of 0.7% to 0.9%.[2,3] However, when focusing on neonates admitted to the NICU, the incidence of thrombocytopenia is much higher, ranging from 18% to 35%.[4–6] The incidence of thrombocytopenia is also inversely correlated to the gestational age, so that the most immature neonates are the most frequently affected: platelet counts <150 × 10^9/L were found at least once during the hospital stay in 70% of infants with a birth weight <1000 g.[7]

Platelet Function and Primary Hemostasis

Multiple studies evaluating platelet adhesion, aggregation, and activation have shown that neonatal platelets are hyporesponsive in vitro to most agonists compared with adult platelets,[8,9] and this low level of reactivity is more pronounced in preterm infants.[10,11] Platelet aggregation studies demonstrated that platelets from neonatal (full term) cord blood were less responsive than adult platelets to agonists such as adenosine diphosphate (ADP), epinephrine, collagen, thrombin, and thromboxane analogues.[12] Similar results were obtained in flow cytometric platelet activation studies, which showed decreased expression of surface activation markers in neonatal platelets stimulated with thrombin, ADP, and epinephrine.[10,12] Different mechanisms account for the hyporeactivity of neonatal platelets to various agents: (1) the hyporesponsiveness to epinephrine is due to fewer α2-adrenergic receptors, the binding sites for epinephrine[13]; (2) the reduced response to collagen likely results from impaired calcium mobilization,[14] although recent studies also showed a mildly reduced expression of GPVI (the collagen receptor) coupled with an intracellular signaling defect[15]; (3) the decreased response to thromboxane results from differences in signaling downstream from the receptor in neonatal platelets[8]; and (4) the decreased responsiveness to thrombin is related to reduced expression of PAR-1 and PAR-4 in neonatal platelets.[16] Recently, developmental differences have also been described in regard to platelet *inhibitory* pathways, specifically a hypersensitivity of neonatal platelets to the inhibitory effects of prostaglandin E1 (PGE_1) during ADP- and collagen-induced platelet aggregation.[17]

Surprisingly, while the hypofunctional platelet phenotype in vitro would predict a bleeding tendency, healthy full-term neonates have normal to *enhanced* primary hemostasis, compared to older children or adults. Bleeding times (BTs) in healthy term neonates are shorter than bleeding times in adults.[18] Similarly, studies using the Platelet Function Analyzer (PFA-100, an in vitro test of primary hemostasis that measures the time it takes to occlude a small aperture, or Closure Time) found that cord blood samples from term neonates exhibited shorter closure times (CTs) than samples from older children or adults.[19,20] The results of these studies suggest that there is an enhanced platelet/vessel wall interaction in full-term neonates, likely related to their higher hematocrits, higher mean corpuscular volumes, and higher concentrations of von Willebrand factors (particularly its ultralong polymers),[18] all factors that, when combined, effectively counteract the neonatal platelet hyporeactivity. Taken together, the available evidence strongly suggests that the in vitro platelet hyporeactivity of healthy full-term infants is an integral part of a carefully balanced and well-functioning neonatal hemostatic system, rather than a developmental deficiency.

These compensatory mechanisms might be less well developed in preterm infants, whose platelets are also more hyporeactive than those of full-term infants, leading to longer BTs[21] and therefore a less balanced and probably more vulnerable hemostatic system. Specifically, BTs performed on the first day of life were longer in preterm compared with term infants, with neonates <33 weeks' gestation exhibiting the longest BTs.[21] Saxonhouse et al. found that PFA-100 CTs from non-thrombocytopenic neonates were inversely correlated to gestational age in both cord blood and

neonatal peripheral blood samples obtained on the first day of life.[22] Importantly, however, while these BTs and CTs were longer in preterm compared to term neonates, they were still near or within the normal range for adults, suggesting that healthy preterm neonates also have adequate primary hemostasis. Data regarding how disease processes perturb this delicate system, particularly in the preterm neonate, are lacking.

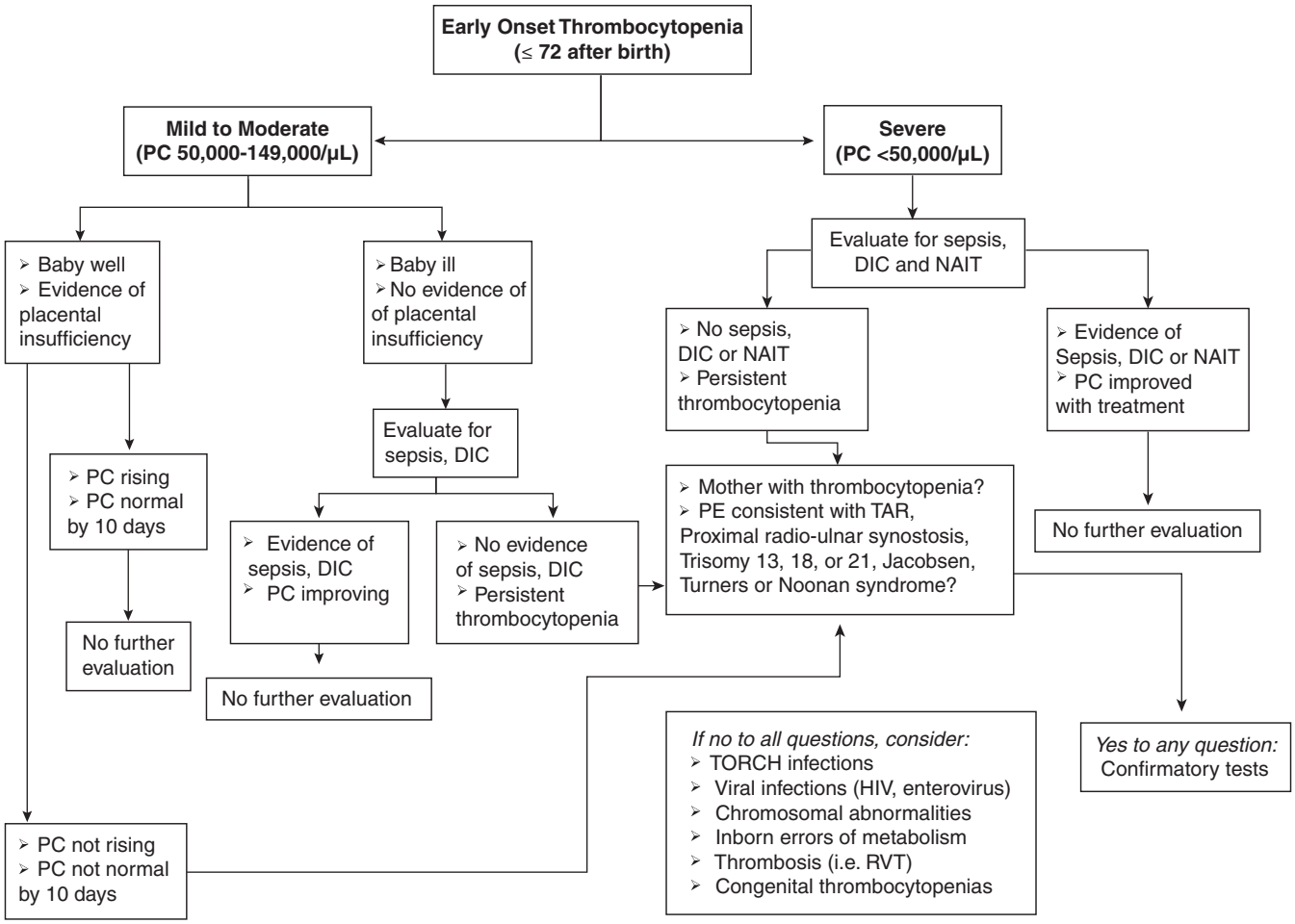
In vitro studies using flow-cytometry or the cone and platelet analyzer showed that the neonatal platelet function improves significantly and nearly normalizes by 10 to 14 days, even in preterm infants.[10,11,23] Consistent with this, Del Vecchio et al. found that, by day of life 10, all infants had shorter BTs than at birth, and early gestational age-related differences had disappeared. Moreover, little or no further shortening of BTs occurred between days 10 and 30.[21] While no causal association has been demonstrated, this period overlaps with the period of highest risk of bleeding among preterm NICU patients, namely during the first 10 days of life.[24]

Neonatal Thrombocytopenia

When evaluating a thrombocytopenic neonate, the first step to narrow the differential diagnosis is to classify the thrombocytopenia as either early onset (within the first 72 hours of life) or late onset (after 72 hours of life), and to determine whether the infant is clinically ill or well. Importantly, infection/sepsis should always be considered near the top of the differential diagnosis (regardless of the time of presentation and the infant's appearance), as any delay in diagnosis and treatment can have life-threatening consequences.

Early-Onset Thrombocytopenia (Fig. 68.2, Table 68.1)

The most frequent cause of early-onset thrombocytopenia in a well-appearing neonate is placental insufficiency, seen in infants born to mothers with pregnancy-induced hypertension/preeclampsia, and in those with intrauterine growth restriction (IUGR).[25] This thrombocytopenia is always mild to moderate, presents immediately or shortly after birth, reaches a nadir on day of life 4, and resolves within 10 days. In a recent large cohort study, approximately one third of small for gestational age (SGA) infants had thrombocytopenia ($<150 \times 10^9$/L) during the first week of life, compared to only 10% of non-SGA gestational age-matched infants. This type of thrombocytopenia was associated with low mortality (2%), as long as there was no identified cause for the SGA other than placental insufficiency (i.e., genetic syndrome or congenital infection).[26] If an SGA otherwise non-dysmorphic



• **Fig. 68.2** Guidelines for the evaluation of neonates with early onset thrombocytopenia (≤72 h of life). *DIC*, Disseminated intravascular coagulation; *NAIT*, neonatal alloimmune thrombocytopenia; *PC*, platelet count; *PE*, physical exam; *TAR*, thrombocytopenia absent radii; *RVT*, renal vein thrombosis. (From Cloherty JP, Eichenwald EC, Stark AR. *Manual of Neonatal Care*, 8th ed, Chapter 47: Neonatal Thrombocytopenia.)

TABLE 68.1 Classification of Neonatal Thrombocytopenia By Time of Presentation

Early-Onset Thrombocytopenia

Chronic fetal hypoxia (hypertension/preeclampsia, diabetes)—IUGR/SGA
Immune-mediated thrombocytopenia (NAIT, autoimmune thrombocytopenia)
Early-onset sepsis (GBS, *Escherichia coli*)
Congenital viral/parasitic infection (HIV, CMV, toxoplasma, enterovirus)
Birth asphyxia
Renal vein thrombosis
Polycythemia
Chromosomal anomalies (trisomy 21, 18, 13)
Congenital thrombocytopenia (thrombocytopenia-absent radii [TAR], amegakaryocytic thrombocytopenia with proximal radio-ulnar synostosis [ATRUS] syndrome, Wiskott-Aldrich syndrome)
Congenital leukemia

Late-Onset Thrombocytopenia

Late-onset infection/sepsis (bacterial, fungal)
NEC
Viral infection (HSV, acquired CMV, enterovirus, adenovirus)
Thrombosis (catheter related)
Drug-induced thrombocytopenia (antibiotics, heparin)
Fanconi anemia

Can Present Both Early and Late

Sepsis/DIC
Inborn errors of metabolism (Gaucher disease, methylmalonic acidemia)
Kasabach-Merritt syndrome

ATRUS, Amegakaryocytic thrombocytopenia with proximal radio-ulnar synostosis; *CMV*, cytomegalovirus; *DIC*, disseminated intravascular coagulation; *IUGR*, intrauterine growth restriction; *NAIT*, neonatal alloimmune thrombocytopenia; *NEC*, necrotizing enterocolitis; *TAR*, thrombocytopenia-absent radii syndrome; *TORCH*, toxoplasmosis, other, rubella, cytomegalovirus, herpes simplex virus infection complex.

infant with mild to moderate thrombocytopenia remains clinically stable and the platelet count normalizes within 10 days, no further evaluation is necessary. However, if the thrombocytopenia becomes severe and/or persists >10 days, further investigation is indicated.

Severe early-onset thrombocytopenia in an otherwise healthy infant should trigger suspicion for an immune-mediated thrombocytopenia, either autoimmune (if the mother is also thrombocytopenic) or alloimmune (if the mother has a normal platelet count). These varieties of thrombocytopenia are discussed in detail below. Early-onset thrombocytopenia of any severity in an *ill-appearing* term or preterm neonate should prompt evaluation for sepsis, congenital viral or parasitic infections, or disseminated intravascular coagulation (DIC). DIC is most frequently associated with sepsis but can also be secondary to birth asphyxia.[27]

In addition to these considerations, the affected neonate should be carefully examined for any radial and thumb abnormalities (suggestive of thrombocytopenia-absent radii [TAR] syndrome, amegakaryocytic thrombocytopenia with radio-ulnar synostosis, or Fanconi anemia). The inability to rotate the forearm on physical examination, in the presence of severe early-onset thrombocytopenia, suggests the rare diagnosis of congenital amegakaryocytic thrombocytopenia with proximal radio-ulnar synostosis (ATRUS). Dysmorphic features on physical exam should warrant investigation for other genetic disorders associated with early-onset thrombocytopenia, most commonly trisomy 21, trisomy 18, trisomy 13, Turner syndrome, Noonan syndrome, and Jacobsen syndrome.

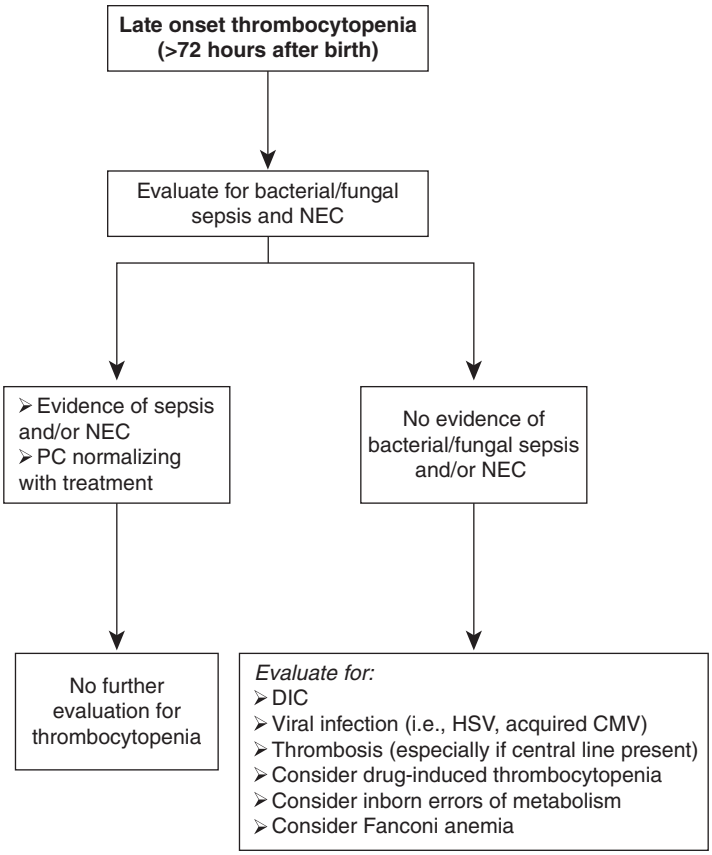
The presence of hepato- and/or splenomegaly is suggestive of viral infection, although it can also be seen in hemophagocytic syndrome and liver disease from different etiologies. Other diagnoses, such as renal vein thrombosis, Kasabach-Merritt syndrome, and inborn errors of metabolism (mainly propionic acidemia and methylmalonic acidemia) should be considered and evaluated for based on specific clinical indications (i.e., hematuria in renal vein thrombosis, presence of a vascular tumor in Kasabach-Merritt syndrome).

Late-Onset Thrombocytopenia (Fig. 68.3, see Table 68.1)

The most common causes of thrombocytopenia of any severity presenting after 72 hours of life are sepsis (bacterial or fungal) and necrotizing enterocolitis (NEC). Affected infants are usually ill-appearing and have other signs suggestive of sepsis and/or NEC. However, it is important to keep in mind that thrombocytopenia can be the first presenting sign of these processes and can precede clinical deterioration. Appropriate treatment (i.e., antibiotics, supportive respiratory and cardiovascular care, bowel rest in case of NEC, and surgery in case of surgical NEC) usually improves the platelet count in 1 to 2 weeks, although in some infants the thrombocytopenia persists for several weeks. The reasons underlying this prolonged thrombocytopenia are unclear.

If bacterial/fungal sepsis and NEC are ruled out, viral infections such as herpes simplex virus, cytomegalovirus (CMV), or enterovirus should be considered. These viral infections are frequently accompanied by abnormal liver enzymes. If the infant has or has recently had a central venous or arterial catheter, thromboses should be part of the differential diagnosis, although thromboses typically only cause thrombocytopenia if the thrombus is enlarging or is infected. Finally, drug-induced thrombocytopenia, while rare in neonates, should be considered if the infant is clinically well, other potential etiologies have been ruled out, and he/she is receiving heparin, antibiotics (penicillins, cephalosporins, metronidazole, vancomycin, or rifampin), indomethacin, famotidine, cimetidine, phenobarbital, or phenytoin, among others.[28,29] Other less common causes of late-onset thrombocytopenia include inborn errors of metabolism and Fanconi anemia (rare).

Novel tools to evaluate platelet production, which aid in the evaluation of thrombocytopenia of unclear etiology, have been recently developed, and are likely to become widely available to clinicians in the near future. Among those, the immature platelet fraction (IPF) measures the percentage of newly released platelets (<24 hours). The IPF can be measured in a standard hematological cell counter (Sysmex 2100 XE Hematology Analyzer) as part of the complete cell count, and can help differentiate thrombocytopenias associated with decreased platelet production from those with increased platelet destruction, in a manner similar to the use of reticulocyte counts to evaluate anemia.[30] Thus, an elevated IPF would suggest platelet consumption (as in neonatal alloimmune thrombocytopenia [NAIT] or DIC), while a decreased IPF would be consistent with a hyporegenerative thrombocytopenia, as in bone marrow suppression or failure.



• **Fig. 68.3** Guidelines for the evaluation of neonates with late-onset thrombocytopenia (>72 h of life). *CMV*, Cytomegalovirus; *DIC*, disseminated intravascular coagulation; *HSV*, Herpes simplex virus; *NEC*, necrotizing enterocolitis; *PC*, platelet count. (From Cloherty JP, Eichenwald EC, Stark AR. *Manual of Neonatal Care*, 8th ed, Chapter 47: Neonatal Thrombocytopenia.)

Reference intervals for the IPF% and the absolute IPF (also known as the immature platelet count, or IPC) have been evaluated in healthy adults and full term neonates, using umbilical cord blood samples (Table 68.2),[31] as well as in nonthrombocytopenic NICU patients (IPF% = 4.1 ± 1.8).[32] In the largest IPF study published to date, MacQueen et al. examined 24,372 platelet counts and IPFs from 9172 term and preterm neonates 0 to 90 days old. Data from nonthrombocytopenic infants in this cohort were used to generate age-specific reference intervals for IPF% and IPC (calculated as IPF% × platelet count) (Fig. 68.4).[33] As seen in the figure, the IPF at the time of birth was higher in preterm infants and decreased through gestation until 32 weeks, at which time it stabilized at full-term values (similar to adult values). Postnatally, the IPF increased progressively over the first 2 weeks of life and returned to baseline by 1 month in infants of all gestational ages. This study also assessed IPF percentages in neonates with thrombocytopenia, and found significantly higher values in neonates with consumptive etiologies compared to those with thrombocytopenia secondary to decreased production (Table 68.3). Thus, when available, the IPF can help differentiate rare congenital thrombocytopenias (usually low) from NAIT or other consumptive disorders (usually elevated). Other studies have shown the usefulness of the IPF to predict platelet recovery in neonates.[32,34] In patients with NEC and severe thrombocytopenia, a low absolute IPF has also been associated with a poor prognosis and high mortality.

Immune Thrombocytopenia

Immune thrombocytopenia occurs due to the passive transfer of antibodies from the maternal to the fetal circulation. There are two distinct types of immune-mediated thrombocytopenia: (1) NAIT and (2) autoimmune thrombocytopenia.

Neonatal Alloimmune Thrombocytopenia

Epidemiology

NAIT is the most common underlying cause of early-onset severe thrombocytopenia, with an incidence among liveborn neonates of 0.5 to 1.5 per 1000 births.[35] The true incidence of the disease is likely higher, however, since the milder cases might go undetected and the most severe cases lead to intrauterine death. Intrauterine death or intracranial hemorrhage (ICH) may occur as early as at 14 to 16 weeks of gestation, resulting in a relatively high incidence of intrauterine ICH (>10%).[35] The overall incidence of ICH (prenatal and postnatal) is particularly high in this population, affecting up to 20% of infants with NAIT and potentially leading to lifelong consequences. ICH may occur during the first pregnancy and has a recurrence risk close to 100% in subsequent pregnancies in the absence of prenatal treatment.[36]

TABLE 68.2 Reference Intervals for Platelet Counts and Immature Platelet Fractions (IPF) in Healthy Adults and Term Neonates (Umbilical Cord Blood)

	HEALTHY INDIVIDUALS			
	Total ($n = 2152$)	Men ($n = 1252$)	Women ($n = 900$)	Umbilical Cord Blood ($n = 133$)
Platelet counts ($\times 10^9$/L)				
Reference interval	162–347	161–338	164–360	191–392
Lower limit (95% CI)	160–164	158–164	160–169	168–208
Upper limit (95% CI)	340–353	326–344	351–372	364–447
%-IPF (%)				
Reference interval	0.5–3.3	0.5–3.1	0.5–3.4	0.7–3.8
Lower limit (95% CI)	0.5–0.5	0.5–0.6	0.5–0.5	0.7–0.9
Upper limit (95% CI)	3.2–3.4	3.0–3.3	3.3–3.5	3.0–3.8
A-IPF ($\times 10^9$/L)				
Reference interval	1.25–7.02	1.30–6.80	1.21–7.15	1.94–9.69
Lower limit (95% CI)	1.19–1.30	1.20–1.41	1.10–1.27	1.66–2.58
Upper limit (95% CI)	6.75–7.24	649–7.16	6.9–7.48	7.96–10.57

A-IPF, Absolute immature platelet fraction; *IPF*, immature platelet fraction.
From Ko YJ, Kim H, Hur M, et al. Establishment of reference interval for immature platelet fraction. *Int J Lab Hematol*. 2013;35(5):528–533.

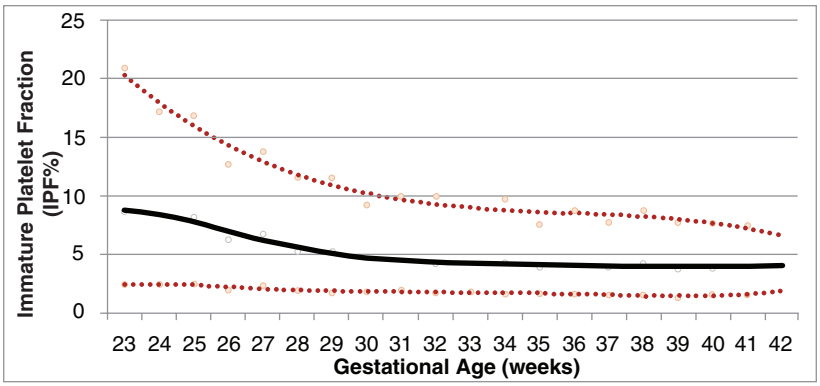
Pathophysiology

In NAIT, the antibody is produced in the mother against a specific human platelet antigen (HPA) present in the fetus but absent in the mother. The antigen is inherited from the father of the fetus. The antigens responsible for NAIT are caused by single nucleotide polymorphisms on any of the main glycoproteins located on the platelet surface, particularly GPIIb/IIIa. The first platelet antigen was identified in 1959 by von Loghem et al. and was designated Zw-a (later PLA1).[37] The initial nomenclature for these antigens came from the name of the patients, leading to confusion in the field. In 1990, a simplified system for HPA nomenclature was described, in which each antigen was given a HPA number.[38] Antigens were numbered chronologically, according to the date of their initial report. The bi-allelic antigens were given an alphabetic designation of "a" or "b" in the order of their frequency (higher frequency for "a"). Thus, the Zw-a/PLA1 antigen was named HPA-1, with its two serological forms designated as HPA-1a for the common form, and HPA-1b for the less common form (the latter corresponding to PLA2). Currently, there are at least 33 HPA antigens identified. The frequency of each antigen varies within ethnic groups: in Caucasians, antibodies to HPA-1a are the

TABLE 68.3 Immature Platelet Fraction Values in Neonates With Thrombocytopenia of Different Etiologies

Mechanism of Thrombocytopenia	*N*	IPF%
Hypoproliferative*	92	10.4 ± 2.9
Consumptive**	98	20.9 ± 7.9
Both	76	17.9 ± 5.9
Indeterminate***	14	12.8 ± 8.1

From MacQueen BC, Christensen RD, Henry E, et al. The immature platelet fraction: creating neonatal reference intervals and using these to categorize neonatal thrombocytopenias. *J Perinatol*. 2017;37(7):836.
*Small for gestational age, birth asphyxia, or a syndrome associated with hypoproliferative thrombocytopenia.
**Immune-mediated, necrotizing enterocolitis (NEC), sepsis, or disseminated intravascular coagulation (DIC).
***None of the above diagnoses.



• **Fig. 68.4** Immature platelet fraction on the day of birth according to gestational age. The top and bottom dotted red lines represent the 5th and 95th percentile reference intervals, and the solid black line represents the median. Circles are the actual medians for 5th, median and 95% each day. The dotted and solid lines are generated by smoothing values in the circles. (From MacQueen BC, Christensen RD, Henry E, et al. The immature platelet fraction: creating neonatal reference intervals and using these to categorize neonatal thrombocytopenias. *J Perinatol*. 2017;37[7]:836.)

major cause of NAIT, followed by HPA-5a and, less frequently, HPA-9b, HPA-3a and b, and HPA-15. Antibodies to HPA-4b are the predominant cause of NAIT in the Japanese population.[39]

The anti-HPA antibody produced in the maternal serum crosses the placenta and reaches the fetal circulation, leading to platelet destruction, apoptosis of early megakaryocyte progenitors (therefore decreased platelet production),[40] and thrombocytopenia.

Clinical Presentation

NAIT should be considered in any neonate who presents with severe thrombocytopenia at birth or shortly thereafter, particularly in the absence of other risk factors, clinical signs, or abnormalities in the physical exam. In a study of more than 200 neonates with thrombocytopenia, using a platelet count $<50 \times 10^9$/L in the first day of life as a screening indicator identified 90% of the patients with NAIT.[41] Based on this observation, it is currently recommended that all neonates with platelet count $<50 \times 10^9$/L in the first day of life be screened for NAIT. In addition, the combination of severe neonatal thrombocytopenia with a parenchymal (rather than intraventricular) ICH is highly suggestive of NAIT.

Evaluation

When NAIT is suspected, blood should be collected from the mother and father and submitted for testing. The initial antigen screening should include HPA-1, 3, and 5. This evaluation should identify approximately 90% of cases of NAIT. However, if the diagnosis is strongly suspected and the initial evaluation is negative, further testing should be undertaken for HPA-9 and 15 (and HPA-4 if the parents are of Asian descent).[42] If positive, these tests will reveal an antibody in the mother's plasma directed against the specific platelet antigen in the father. If blood cannot be collected from the parents in a timely fashion, neonatal serum may be screened for the presence of antiplatelet antibodies. However, a low antibody concentration in the neonate, coupled with binding of the antibodies to the infant's platelets, can lead to false-negative results. It is still unclear if there is any correlation between the affinity of the antibodies and the severity of disease.[43] Due to the complexity of testing, evaluations should be performed in an experienced reference laboratory that has a large number of typed controls available for antibody detection, and the appropriate DNA-based technology to type multiple antigens. In rare cases, antibodies may be hard to detect in samples drawn at the time of delivery; therefore, when the clinical diagnosis is most likely NAIT, follow-up serology tests should be performed.

Brain imaging studies (beginning with cranial ultrasound) should be performed as soon as NAIT is suspected, regardless of the presence or absence of neurologic manifestations, because findings from these studies will dictate the aggressiveness of the treatment regimen for the affected infant and for the mother's future pregnancies. The clinical course of NAIT is short in most cases, often resolving almost entirely within 2 weeks. However, to confirm the diagnosis, it is imperative to follow the platelet count frequently until a normal count is documented.

Management

The management of NAIT differs depending on the specific clinical scenario: suspected NAIT (unknown pregnancy); known case of NAIT; or antenatal management of pregnant woman with previous history of NAIT.

Management of the Neonate With Suspected NAIT (Unknown Pregnancy)

Based on recent data demonstrating that a large proportion of infants with NAIT respond to random donor platelet transfusions, this is now considered the first line of therapy for infants in whom NAIT is suspected.[44] If the patient is clinically stable and does not have an intracranial hemorrhage, platelets are usually given when the platelet count is $<30 \times 10^9$/L, although this is arbitrary. In addition to platelets, if the diagnosis of NAIT is confirmed or strongly suspected, intravenous immune globulin (IVIG) (1 g/kg/day for up to 2 consecutive days) may be administered to increase the patient's own platelets and potentially to protect the transfused platelets.[39] Because in NAIT the platelet count usually falls after birth, IVIG may be given when the platelet count is between 30 and 50×10^9/L in a stable neonate, to try to prevent a further drop.

If the patient has evidence of an ICH, the goal is to maintain a platelet count $>100 \times 10^9$/L. However, this may be challenging in the setting of NAIT. In all scenarios, it is important to keep in mind that some infants with NAIT fail to respond to random donor platelets and IVIG. For that reason, the blood bank should be immediately alerted about any infant with suspected NAIT, and arrangements should be made to secure a source of antigen-negative platelets (either from HPA-1b1b and 5a5a donors, which should be compatible in >90% of cases, or from the mother) as soon as possible, so they are available if there is no response to the initial therapies. If maternal platelets are used, they need to be concentrated to decrease the amount of antiplatelet antibodies, which are present in the mother's plasma, infused into the infant. Platelets can also be washed to eliminate the plasma, although this induces more damage to the platelets than concentrating them.[45] Of note, in some European countries, HPA-1b1b and 5a5a platelets are maintained in the blood bank inventory and are immediately available for use. In those cases, these are preferable to random donor platelets and/or IVIG, and should be the first line of therapy.

Management of the Neonate With Known NAIT

When a neonate is born to a mother who had a previous pregnancy affected by confirmed NAIT, genotypically matched platelets (e.g., HPA-1b1b platelets) should be available in the blood bank at the time of delivery, and should be the first line treatment if the infant is thrombocytopenic.

Management of Pregnant Women With Previous History of NAIT

Mothers who previously delivered an infant with NAIT should be followed in high-risk obstetric clinics during all future pregnancies as the reoccurrence rate is high, reaching 100% if the father is homozygous dominant for the causative HPA. However, in all cases fetal genetic testing should be performed. Non-invasive methods have recently become available through cell-free DNA testing from the mother's plasma. The intensity of prenatal treatment will be based on the severity of the thrombocytopenia and the presence or absence of ICH in the previously affected fetus. This is particularly important to assess the risk of developing an ICH in the current pregnancy, and to minimize this risk. Current recommendations involve maternal treatment with IVIG (0.5 to 2 g/kg/week) ± steroids (0.5 to 1 mg/kg/day prednisone), starting at 12 or at 20 to 26 weeks of gestation, depending on whether the previously affected fetus

suffered an ICH, and if so, at what time during pregnancy.[45] Most recent studies showed that the combination of IVIG and steroids is the most efficient treatment.[35] Regarding mode of delivery, elective cesarean section is recommended in most countries to avoid ICH, regardless of ICH status in previous and current fetus.[43]

Autoimmune Thrombocytopenia

Epidemiology

The diagnosis of neonatal autoimmune thrombocytopenia should be considered in any neonate who has early-onset thrombocytopenia and a maternal history of either immune thrombocytopenic purpura (ITP) or an autoimmune disease (with or without thrombocytopenia). A retrospective study of obstetric patients who had ITP (including a high number of mothers who had thrombocytopenia during their pregnancies) demonstrated a relatively high incidence of affected babies: 25% of neonates had thrombocytopenia at birth, the thrombocytopenia was severe in 9%, and 15% received treatment for it.[46] Other large studies confirmed an incidence of severe neonatal thrombocytopenia in this population ranging from 8.9% to 14.7%, with ICH occurring in 0% to 1.5% of affected neonates.[47–49]

Pathophysiology

In autoimmune thrombocytopenia, the antibody is directed against an antigen on the mother's own platelets (autoantibody) as well as on the infant's platelets. The maternal autoantibody crosses the placenta (both passively and actively transported), resulting in destruction of fetal platelets and thrombocytopenia.

Evaluation

We recommend that all neonates born to mothers who have autoimmune diseases undergo a screening platelet count at or shortly after birth. If the platelet count is normal, no further evaluation is necessary. If the infant has mild thrombocytopenia, however, the platelet count should be repeated in 2 to 3 days, since this type of thrombocytopenia usually reaches the nadir between days 2 and 5 after birth. If the platelet count is $<30 \times 10^9$/L, IVIG (1 g/kg, repeated if necessary) is the first line of therapy. Random donor platelets, in addition to IVIG, should be provided if the infant has evidence of active bleeding, although some experts recommend them in addition to IVIG when the platelet count is $<30 \times 10^9$/L, and provide IVIG alone for platelet counts between 30 and 50×10^9/L. Cranial imaging should be obtained in all infants with platelet counts $<50 \times 10^9$/L, to evaluate for ICH. Importantly, neonatal thrombocytopenia secondary to maternal ITP may last for weeks to months and requires long-term monitoring and sometimes a second dose of IVIG at 4 to 6 weeks of life. This is unlike NAIT, which usually resolves within 2 weeks. Recently, it has been reported that the transfer of antiplatelet antibodies (IgA type) from mothers with ITP through breastfeeding can be associated with persistent neonatal thrombocytopenia.[50]

Management

Even if the mother has severe ITP, it appears that fetal hemorrhage in utero is very rare, compared with the small but definite risk of such hemorrhage in alloimmune thrombocytopenia. Because of that, treatment of ITP during pregnancy and delivery is mostly based on the risk of maternal hemorrhage.[51] A small prospective randomized trial of low-dose betamethasone failed to prevent thrombocytopenia in newborns.[52] IVIG given prenatally to the mother with ITP has also not been clearly shown to affect the fetal platelet count.

There is in general little correlation between fetal platelet counts and maternal platelet counts, platelet antibody levels, or history of maternal splenectomy. Attempts to measure the fetal platelet count before delivery are not recommended, due to the risk associated with such attempts. The only reliable predictive measure of neonatal thrombocytopenia in a mother with ITP is a history of neonatal thrombocytopenia in a previous pregnancy.[53] In regard to the mode of delivery, there is no evidence that cesarean section is safer for the fetus with thrombocytopenia than uncomplicated vaginal delivery. Given this fact, combined with the difficulty predicting severe thrombocytopenia in neonates and the very low risk of serious hemorrhage, the 2010 International Consensus Report on the Investigation and Management of Primary Immune Thrombocytopenia concluded that the mode of delivery in ITP patients should be determined by purely obstetric indications.[51] However, interventions that increase the risk of bleeding in the fetus should be avoided, such as vacuum extraction or forceps delivery.

Congenital Thrombocytopenias

Congenital thrombocytopenias are rare conditions which most often present at birth and are therefore classified as early-onset thrombocytopenias. This is a heterogeneous group of diseases with variable clinical manifestations. Often, but not always, patients with congenital thrombocytopenia have dysmorphic features and associated abnormalities as part of a genetic syndrome. If a neonate presents with radial abnormalities, the differential diagnosis includes thrombocytopenia-absent radii (TAR) syndrome, amegakaryocytic thrombocytopenia with radio-ulnar synostosis, and Fanconi anemia. Although thrombocytopenia associated with Fanconi almost always presents later (during childhood), few isolated neonatal cases have been reported.[54] In patients with neonatal Fanconi anemia, thumb abnormalities are frequently found, and chromosomal fragility testing is nearly always diagnostic. If the infant has radial abnormalities with normal appearing thumbs, TAR syndrome should be considered.[55] The platelet count is usually $<50 \times 10^9$/L and the white cell count is elevated in >90% of TAR syndrome patients, sometimes exceeding 100×10^9/L and mimicking congenital leukemia. Infants with TAR syndrome that survive the first year of life generally do well, since the platelet count then spontaneously improves to low-normal levels that are maintained through life.[56] The inability to rotate the forearm on physical examination, in the presence of severe early-onset thrombocytopenia, suggests the rare diagnosis of congenital amegakaryocytic thrombocytopenia with proximal radio-ulnar synostosis (ATRUS). Radiologic examination of the upper extremities in these infants confirms the proximal synostosis of the radial and ulnar bones.[57] Most (but not all cases) of ATRUS are associated with Hox-A11 mutations,[58,59] and require bone marrow transplantation. Other genetic disorders associated with early-onset thrombocytopenia include trisomy 21, trisomy 18, trisomy 13, Turner syndrome, Noonan syndrome, and Jacobsen syndrome. Cases of Noonan syndrome presenting with mild dysmorphic features and very severe neonatal thrombocytopenia (mimicking congenital amegakaryocytic thrombocytopenia

[CAMT]) have been described, so genetic testing should be performed in children who present with a CAMT-like picture and no mutations in the *C-mpl* gene.[60]

In non-syndromic cases of congenital thrombocytopenia, there is usually a history of chronic thrombocytopenia in family members. These conditions belong to a heterogeneous group of diseases and due to diverse clinical manifestations and laboratory findings, the diagnosis is challenging. Often the size of the platelets helps in the differential diagnosis (Table 68.4). Myosin heavy chain-9 (MYH-9) related disorders (May-Hegglin anomaly, Fechtner syndrome, Sebastian syndrome) are inherited in autosomal dominant fashion and present with macrothrombocytopenia.[61] Other congenital thrombocytopenias presenting with large platelets include Bernard-Soulier syndrome (with autosomal recessive inheritance) and X-linked macrothrombocytopenia. Wiskott-Aldrich syndrome, in contrast, should be suspected in male neonates with severe thrombocytopenia and small platelets, particularly if they develop eczema. The diagnosis of congenital amegakaryocytic thrombocytopenia (CAMT) is challenging as it presents with normal sized platelets and may not be diagnosed in the newborn period (when it is usually confused with alloimmune thrombocytopenia) due to lack of other clinical features. A summary of congenital thrombocytopenias and their associated features is provided in Table 68.5.[62] These conditions are often paired with platelet dysfunction as well.

TABLE 68.4 Classification of Congenital Thrombocytopenias by Platelet Size

Small Platelets	Normal-Sized Platelets	Large Platelets
Wiskott-Aldrich syndrome (X-linked microthrombocytopenia)	Congenital amegakaryocytic thrombocytopenia (CAMT)	MYH9-associated thrombocytopenia (May-Hegglin anomaly, Sebastian syndrome, Fechtner syndrome, Epstein syndrome)
	Thrombocytopenia-absent radius (TAR) syndrome	Bernard-Soulier syndrome
	Thrombocytopenia associated with trisomies (13, 18, 21)	X-linked macrothrombocytopenia
		Paris-Trousseau syndrome
		Gray platelet syndrome

MYH9, Myosin heavy chain 9.
Modified from Young G. Hemostatic Disorders of the Newborn. *Avery's Diseases of the Newborn*, 9th ed. Philadelphia: Elsevier.

TABLE 68.5 Genetic and Clinical Characteristics of Congenital Thrombocytopenias

Disease	Mode of Inheritance	Degree of Thrombocytopenia	Platelet Size	Other Clinical and Pathologic Features	Genetic Defect
Congenital amegakaryocytic thrombocytopenia	AR	Severe—Type 1 Mild to moderate—Type 2	Normal	Absence of megakaryocytes in marrow. Develop bone marrow failure/pancytopenia, markedly elevated TPO levels	Mutations in *MPL* gene
Thrombocytopenia with absent radii (TAR)	AR	Severe at birth but usually less severe with age	Normal	Congenital malformations including bilateral absent radii, skeletal and cardiac abnormalities, often have cow's milk intolerance, elevated TPO levels. Decreased to absent megakaryocytes in marrow at birth, low to normal numbers later in life	Unknown. Mutation in exon junction complex subunit *RBM8A* gene. Microdeletion of chromosome 1q21.1 (not causative)
Bernard-Soulier syndrome	AR	Mild	Large	Abnormal platelet function. Decreased GPIb complex on platelets. Marrow has normal to slightly increased normal megakaryocytes.	Mutations in *GPIBA*, *GP1BB* or *GP9* genes
MYH9-related disorders	AD	Mild-Moderate	Large	All will have Dohle-like bodies in myeloid cells	Mutation in *MYH9* gene on 22q12.3-13.1, variable penetrance
May-Hegglin anomaly		Mild–Moderate	Large		

Continued

TABLE 68.5 Genetic and Clinical Characteristics of Congenital Thrombocytopenias—cont'd

Disease	Mode of Inheritance	Degree of Thrombocytopenia	Platelet Size	Other Clinical and Pathologic Features	Genetic Defect
Sebastian syndrome		Mild–Moderate	Large		
Fechtner syndrome		Mild–Moderate	Large	Nephritis, hearing loss and cataracts	
Epstein syndrome		Mild–Moderate	Large	Nephritis and hearing loss	
Familial platelet disorder with predisposition to acute myelogenous leukemia	AD	Moderate	Normal	Predisposition to develop AML or MDS. Abnormal platelet aggregation/prolonged bleeding times. Abnormal platelet function. Decreased megakaryocytes in marrow	Heterozygous RUNXI mutation on *8q22*
Mediterranean macrothrombocytopenia	AD	Mild	Large	Seen in patients from Italy and Balkan peninsula. May represent carrier state for Bernard-Soulier syndrome	Mutation in *GP1A* gene on chromosome 17
Paris-Trousseau syndrome	AD	Moderate to severe at birth but improve with age to near normal	Large	Congenital abnormalities including psychomotor retardation, trigonocephaly, facial dysmorphism, and cardiac abnormalities. Dysmegakaryopoiesis with many micromegakaryocytes and abnormal α-granules	Mutation in *FLI1* gene on 11q23.3
Gray platelet syndrome	AR	Mild–moderate	Large	Develop myelofibrosis and splenomegaly. Platelets lack α-granules and appear agranular and "gray." Variable bleeding	Unknown. Mutations in *NBEAL2* gene in AR disease
X-linked microthrombocytopenia (Wiskott-Aldrich syndrome)	X	Variable; usually mild to moderate	Small	Normal to increased megakaryocytes. Splenic destruction thought to cause small platelet size and thrombocytopenia. Patients may have eczema and T-cell lymphopenia. Increased incidence of autoimmune disorders and lymphoma	Mutation in *WAS* gene at Xp11.4-p11.21
X-linked thrombocytopenia with dyserythropoiesis (GATA1)	X	Moderate–severe	Large to normal	Usually have bleeding and bruising with abnormal platelet function. Anemia. Hypercellular marrow with dysplastic erythroid and megakaryocytic precursors	Mutation in *GATA1* gene at Xp11.23

AD, Autosomal dominant; *AML*, acute myelogenous leukemia; *AR*, autosomal recessive, *X*, X-linked, *MD*, myelodysplastic syndrome.
From Smock KJ, Perkins SL. Thrombocytopenia: an update. *Int J Lab Hematol*. 2014;36(3):269–278.

Management

Platelet Transfusions

Platelet transfusion is the primary modality of treatment for neonatal thrombocytopenia. Several studies have shown great variability in neonatal transfusion practices in the U.S. and worldwide.[24,63–65] To a large extent, this was attributed to the absence of scientific evidence in the field and the high incidence of bleeding in this population. Indeed, until recently, there was only one randomized trial that compared different platelet transfusion thresholds in neonates. This trial was limited to very low birth weight (VLBW) infants in the first week of life, and excluded patients with severe thrombocytopenia (platelet counts $<50 \times 10^9$/L).[66] The investigators found no differences in the incidence or severity of intraventricular hemorrhages (IVHs) between a group of neonates transfused for any platelet count $<150 \times 10^9$/L and a group transfused only for counts below 50×10^9/L or for clinical bleeding. Based on these findings, the investigators concluded that transfusing VLBW infants with platelet counts $>50 \times 10^9$/L does not reduce the risk of IVH.

In 2019, the much larger PlaNeT-2 multicenter trial was published. This trial randomized 660 thrombocytopenic neonates with a median gestational age of 26.6 weeks and a median birth weight of 740 g to receive platelet transfusions at a platelet count threshold of $<50 \times 10^9$/L (<50 group) or $<25 \times 10^9$/L (<25 group). Infants were randomized at any time during their NICU hospitalization when the platelet count fell below 50×10^9/L. The primary outcome was a composite of death or new major bleeding within 28 days of randomization.[67] Ninety percent of infants in the <50 group and 53% in the <25 group received at least one

platelet transfusion. Unexpectedly, infants in the <50 group had a significantly *higher* rate of mortality or major bleeding within 28 days of randomization, compared to those in the <25 group (26% vs. 19%, respectively; odds ratio 1.57, 95%CI 1.06 to 2.32). Among secondary outcomes, infants in the liberally transfused group also had a higher incidence of bronchopulmonary dysplasia. While these findings might have seemed surprising, they were consistent with a number of prior observational studies describing a poor association between severity of thrombocytopenia and bleeding risk, a lack of effectiveness of platelet transfusions to prevent bleeding in neonates, and an association between number of platelet transfusions and neonatal mortality and morbidity.[68,69]

The question of whether the benefits of the lower transfusion threshold would be limited to clinically stable infants with a low risk of bleeding and/or death was largely addressed in a secondary analysis, in which a multivariable logistic regression model was developed (incorporating factors known to influence neonatal bleeding risk and mortality) and used to predict the baseline bleeding/mortality risk of neonates enrolled in PlaNeT-2.[70] Based on their model-predicted baseline risk, 653 neonates from PlaNeT-2 were divided into four quartiles (very low, low, moderate, and high risk) and the absolute risk difference between the <50 group and the <25 group was assessed within each quartile. Interestingly, the lower transfusion threshold was associated with an absolute risk *reduction* in all four groups, varying from 4.9% in the lowest to 12.3% in the highest risk group. These results suggested that using a lower ($<25 \times 10^9$/L) prophylactic platelet transfusion threshold might be particularly beneficial in high-risk neonates, although some uncertainties remain. First, 39% of infants in PlaNeT-2 received one or more platelet transfusions prior to randomization, for unknown reasons and at non-specified platelet counts. This raises the question of whether these transfusions were given during the first few days of life, the highest risk period for IVH in preterm neonates. Second, the study required obtaining a head ultrasound within 6 hours of randomization and excluded infants with a significant IVH for 72 hours (after which they could be randomized). Thus, by design, PlaNeT-2 did not assess the effects of a restrictive versus liberal platelet transfusion threshold on the potential extension of an existing IVH. Nevertheless, the currently available evidence strongly supports the hypothesis that platelet transfusions can be harmful to neonates, and that restrictive transfusion thresholds can be safely used in the great majority of neonates. Based on this evidence, we currently recommend administering platelet transfusions to neonates according to the criteria shown in Table 68.6.

TABLE 68.6 Proposed Platelet Transfusion Recommendations

Platelet count ($\times 10^3$/mcL)	Recommendations
<25	*Transfuse all*
25–49	*Transfuse if:* Major hemorrhage in last 72 h (i.e., grade 3 or 4 IVH) Prior to surgical procedure
50–100	*Transfuse if:* Active bleeding NAIT with intracranial hemorrhage Before major neurosurgical procedures

Regarding the platelet product that should be transfused, neonates should receive 10 mL/kg of a standard platelet suspension, either a platelet concentrate ("random-donor platelets") or apheresis platelets. Each random-donor platelet unit has approximately 50 mL of volume and contains approximately 10×10^9 platelets per 10 mL.[71] There is no need to pool more than one random-donor unit for a neonatal transfusion, a practice that only increases donor exposures and induces platelet activation, without any benefit. Two additional important considerations in neonatology are the prevention of transfusion-transmitted CMV infections, and graft versus host disease (GVHD). Most blood banks provide either CMV-negative or leuko-reduced products to neonates, both of which significantly reduce (but do not completely eliminate) the risk of transfusion-transmitted CMV. Transfusion of CMV-negative and leuko-reduced blood products completely prevented transmission of CMV to VLBW infants.[72] GVHD is prevented by irradiating cellular blood products prior to transfusion. Of note, most neonatal cases of GVHD have been reported in neonates with underlying immunodeficiencies, receiving intrauterine or large volume transfusions (i.e., double exchange transfusions), or receiving blood products from a first-degree relative. Thus, these are all absolute indications for irradiating blood products.[71]

When making platelet transfusion decisions, it is important for neonatologists to be aware of the risks associated with these transfusions. In the case of platelet suspensions, the risk of bacterial contamination is higher than the combined risk of all viral infections for which platelets are routinely tested. This is because platelet suspensions are stored in the blood bank at room temperature for up to 5 days, which increases the risk of bacterial growth. In addition, platelet transfusions can induce transfusion-associated lung injury (TRALI), a process characterized by the onset of hypoxemia and bilateral pulmonary infiltrates within 6 hours of a transfusion.[73] Given that neonates have frequent episodes of respiratory decompensation due to variable causes, TRALI is likely to be under-recognized in the NICU.

Alternative Tests to Guide Platelet Transfusions

While currently platelet transfusions are administered based on platelet counts, the evidence strongly suggests that factors other than the degree of thrombocytopenia determine the bleeding risk. The PFA-100 is an *in vitro* test of primary hemostasis that provides a quantitative measurement of platelet adhesion, activation, and aggregation in whole blood.[74] Since PFA-100 closure times (CTs) represent global measurements of primary hemostasis, they are particularly attractive in neonates, in whom many factors contribute to a finely balanced hemostatic system. Two recent studies found that the CT-ADP (closure time in response to collagen and ADP) was correlated with bleeding severity in preterm neonates,[75,76] while the platelet count was not. Furthermore, changes in the CT-ADP (but not in platelet count) correlated well with changes in bleeding score.[77] Fustolo-Gunnink and collaborators recently developed a dynamic model to predict major bleeding in preterm neonates at any time-point during the first week after the onset of severe thrombocytopenia, which incorporated the variables gestational age, post-natal age, intrauterine growth retardation, NEC, sepsis, platelet count, and mechanical ventilation.[78] While not yet prospectively validated, this is a promising approach to making individualized transfusion decisions in this population.

Non-Transfusional Therapies

While severe thrombocytopenia resolves within 14 days in 80% of affected neonates, in approximately 10% it persists for

>30 days,[79] resulting in multiple platelet transfusions (>20).[80] In 2008, two thrombopoietin (TPO) mimetics, romiplostim and eltrombopag, were approved by the FDA for the treatment of adults with chronic immune thrombocytopenic purpura and, more recently, eltrombopag was approved for use in other varieties of chronic thrombocytopenia (i.e., chronic hepatitis C, aplastic anemia). In 2015,[81] eltrombopag was approved by the FDA for use in children with symptomatic chronic immune thrombocytopenia. Recent publications have reported the use of eltrombopag in adult and pediatric patients with various hematological disorders, including inherited thrombocytopenia associated with MYH9 mutations,[82,83] Wiskott-Aldrich syndrome,[84] and aplastic anemia.[85,86] Thus, it is plausible that eltrombopag would also be considered as a therapeutic alternative in neonates, infants, and children in the first year of life with different varieties of thrombocytopenia. Both agents, romiplostim and eltrombopag, begin to raise platelet counts 4 to 6 days after the initiation of the treatment, and reach peak platelet counts at 10 to 14 days. Because of these characteristics, they would only be appropriate for selected infants with thrombocytopenia expected to last longer than 10 to 14 days and severe enough to warrant treatment. Difficulties in predicting the duration of thrombocytopenia in neonates have hampered the use of any TPO mimetics in neonates and infants <12 months, which so far has been limited to sporadic reports.[87] It is likely that only a very small subset of thrombocytopenic neonates would be acceptable candidates for treatment with these medications.

Outcomes

The outcomes for patients with congenital thrombocytopenia are variable depending on the specific disorders. Patients with CAMT often develop bone marrow failure and pancytopenia and require bone marrow transplant. The thrombocytopenia in TAR patients is severe at birth, but improves over time, often reaching platelet counts >100 × 10^9/L. The hematologic outcome of MYH-9 related disorders is good, although they frequently develop nephritis, hearing loss, and cataracts later in life.

Platelet Function Disorders

Pathophysiology

Platelets play a major role in hemostasis, both by supporting the cellular structure for the primary platelet plug, and also by providing a phospholipid surface on which the plasma elements involved in coagulation can bind. Thus, a decrease in the platelet count and/or poor platelet function can result in bleeding symptoms. Platelet function disorders can be broadly categorized as congenital or acquired.

Most platelet function defects seen in the NICU are acquired, and can be due to medications, medical conditions, or medical interventions. The list of medications that can affect platelet function is extensive, but the most common medications resulting in platelet dysfunction include aspirin and other non-steroidal anti-inflammatory drugs (indomethacin and ibuprofen), prostacyclin, certain anticonvulsants (valproic acid in particular), and some antibiotics (beta-lactams). Among the medical disorders associated with platelet dysfunction, the most common and best described is uremia. Although the exact mechanism by which the platelets are affected is not clear, a prevailing theory is that the accumulation of certain substances associated with uremia disrupts the platelet phospholipid surface. Last, certain medical procedures are associated with platelet dysfunction, with the most common being the use of extracorporeal circuits (ECMO) and cardiopulmonary bypass. Therapeutic hypothermia, currently the standard of care for infants with hypoxic-ischemic encephalopathy, also causes transient platelet dysfunction.[88]

Congenital platelet function disorders occur as a result of defects or deficiencies in any of a multitude of components (functional, structural, and regulatory proteins) required for normal platelet function. The most severe platelet function defects, which can present in neonatal life, result from deficiency or absence in the glycoproteins (GP) located on the platelet surface: Bernard-Soulier syndrome, caused by deficiency of GPIb (the vWF receptor), and Glanzmann's thrombasthenia, caused by a deficiency of GPIIb/IIIa, the fibrinogen receptor.[89] Both have been reported to present with bleeding in neonatal life, although they most frequently present later in childhood.

Most of the other inherited platelet function defects are mild, and very rarely present in the newborn period. Secretory platelet disorders have defective platelet granules, and cause mild to moderate bleeding. These include δ-storage pool defects (including the common ADP secretion defect and the less common absence of dense bodies associated with Hermansky-Pudlak syndrome), and α-granules defects (gray platelet syndrome). Over the last decade, the availability of better genetic tools has led to the identification of the genetic causes of many of these platelet functional defects (i.e., mutations in NBEAL2 cause gray platelet syndrome), and of novel mutations associated with platelet dysfunction and bleeding. A thorough review of these disorders is outside of the scope of this chapter, particularly because the degree of bleeding associated with most of these conditions rarely leads to manifestations in the neonatal period.

Clinical Presentation

Patients with platelet function disorders present similar to thrombocytopenia with bleeding signs, including mucocutaneous bleeding (nose, mouth, gastrointestinal tract, genitourinary tract) and bruising. The extent, location, and nature of the bruises are generally related to birth trauma and invasive procedures. Bernard-Soulier syndrome patients present with mild thrombocytopenia, giant platelets on the blood smear (macrothrombocytopenia), and mucosal type bleeding due to platelet dysfunction. Patients with Bernard-Soulier present with gastrointestinal bleeding, bleeding after circumcision, and bleeding after cardiac catheterization in a patient with DiGeorge syndrome.[90] Glanzmann thrombasthenia is a rare autosomal recessive platelet function disorder, caused by a deficiency or abnormality of GPIIb–IIIa expression on the platelet surface. In neonates, the most common manifestations of Glanzmann thrombasthenia are generalized purpura or bleeding after circumcision, although more serious hemorrhages have also been described.[91]

Evaluation

The diagnosis of platelet function disorders in neonates is problematic, because many of the traditional tests require a large amount of blood (platelet aggregation studies), or lack reference values for neonates. The traditional assay of platelet function is platelet aggregometry. This assay uses a set concentration of platelet-rich plasma and assesses platelet aggregation via light transmission after addition of a variety of platelet agonists (ADP, epinephrine,

ristocetin, arachidonic acid, collagen, and thrombin-related activation peptide). Neonates have reduced platelet aggregation compared to older children and adults (based on cord blood values), and therefore the interpretation of this test in neonates is difficult. The amount of blood required for platelet aggregometry is 20 to 30 mL, depending on the platelet count and how many agonists will be tested. Recently, whole blood aggregometry assays have been developed and are becoming increasingly available. These assays require significantly less blood than traditional platelet aggregometry, thus making them accessible to neonates. However, the lack of neonatal reference ranges has hampered the wider use of aggregometry in this age group.

There are two other tests that can screen for platelet function disorders; however, both have significant limitations. The original screening test is the bleeding time, a test that is difficult to perform, particularly in neonates, and is rarely offered clinically. The PFA-100 is a widely used laboratory assay available in most coagulation laboratories and is a useful screening assay to evaluate for disorders of primary hemostasis. Normal ranges for the PFA have been established in cord blood,[92] and in neonatal blood.[22] The blood volume required for this test is much less than for aggregometry, and thus it can be used in neonates. Patients with severe platelet function defects such as Bernard-Soulier and Glanzmann's thrombasthenia typically have significantly abnormal results. The PFA is also often abnormal in the milder disorders such as the common ADP secretion defects; however, its sensitivity for these disorders is not sufficient to allow such defects to be ruled out if the results are normal. The PFA-100 is also abnormal in patients on aspirin and clopidogrel and ticlopidine (the collagen/ADP cartridge). Neonates on indomethacin, ibuprofen, or certain antibiotics can also have a prolonged PFA-100. The effects of other medications known to affect platelet function are not clear. Thus, the PFA is a useful screen for the platelet function defects. However, it cannot be performed when the patient is on certain medications, and it cannot rule out milder platelet function defects.

In general, it is reasonable to start with a complete blood count and a review of the peripheral blood smear. The combination of mild thrombocytopenia and large platelets (macrothrombocytopenia) is suggestive of Bernard-Soulier syndrome. Bernard-Soulier can be confirmed using flow cytometry to evaluate for the presence of GPIb on the platelet surface. Similarly, GPIIb/IIIa surface expression can be assessed to evaluate for Glanzmann's thrombasthenia. This evaluation can be done in a small blood volume and is accurate in neonatal life. Some platelet function defects also lead to easily identifiable ultrastructural changes in the platelets that can be visualized by electron microscopy. In particular, a deficiency or absence of dense bodies (δ-storage pool deficiency) or α-granules (gray platelet syndrome) can be demonstrated by electron microscopy.

More recently, a high-throughput sequencing platform targeting 63 genes relevant for bleeding and platelet disorders was generated, which allows the efficient molecular diagnosis of bleeding and platelet disorders, avoiding the need for multiple sequential tests to narrow the diagnostic possibilities. This DNA-based diagnostic platform will likely become the new approach to patients with suspected inherited platelet function disorders.[93]

Management

The management of congenital platelet function defects relies on several medications, and platelet transfusions in dire situations when the medications or local measures are ineffective. In acquired conditions, reversal of the condition that led to the platelet dysfunction will reverse the platelet defect, but this is not always possible. In such situations, the approach to management of bleeding is mostly based on platelet transfusions.

Several medications have nonspecific mechanisms whereby they can enhance hemostasis when platelet function is abnormal. These include desmopressin, antifibrinolytic agents, and recombinant activated FVII (rFVIIa). Desmopressin improves platelet function in many congenital disorders, in uremia, and during cardiopulmonary bypass. However, desmopressin is generally not used in children <2 years of age, because it can lead to vasodilatation, resulting in reductions in blood pressure sufficient to lead to clinical signs, but mostly because it is an analogue of antidiuretic hormone and can induce hyponatremic seizures. Neonates are at a particularly high risk for this complication; thus, desmopressin is not recommended in this age group.

Recombinant FVIIa was developed for the management of bleeding in patients with hemophilia and inhibitors; however, it has been shown to also be effective for managing severe bleeding in patients with severe platelet function defects. It is licensed in Europe for the management of bleeding in patients with Glanzmann thrombasthenia who are refractory to platelet transfusions. The major risk of rFVIIa is the risk of thrombosis. Thus, it is suggested that rFVIIa be used only for patients with severe bleeding in whom standard therapeutic measures have failed. The use of this agent in neonates has been reported, and it appears that neonates are at increased risk for thrombosis from this agent compared with older children.[94] There is no consensus, however, since another study evaluating the risk of thrombotic events associated with rFVIIa use in neonates showed a prevalence of 7.5% in bleeding and/or coagulopathic neonates, which was similar to those neonates who received FFP.[95] Antifibrinolytic agents might also be helpful, particularly later in life, for epistaxis and menorrhagia.

For severe bleeding that has not responded to the measures just described, a platelet transfusion should be given to provide normally functioning platelets. Although most platelet function defects are mild enough that this will never be required, for the more severe disorders such as Bernard-Soulier and Glanzmann's thrombasthenia, a platelet transfusion may be lifesaving. The risks associated with a platelet transfusion are no different from those for other patients, with one important exception: patients with Bernard-Soulier and Glanzmann thrombasthenia are at risk for alloimmunization, resulting in the formation of antibodies to GPIb and GPIIb/IIIa, respectively. Once these antibodies develop, future platelet transfusions are likely to be ineffective. Thus, it is imperative to withhold platelet transfusions for these patients except in life-threatening hemorrhage, because it may be possible to use this therapy only once in a patient's lifetime. Last, local measures are extremely important in the management of bleeding.

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69

Neonatal Erythrocyte Disorders

KATIE CARLBERG

KEY POINTS

- Erythropoiesis occurs in stages or waves during embryonic development, initiating in the yolk sac, migrating to the liver, and finally to the bone marrow. The sites of erythropoietin production also transition during development from the neuronal cells to the fetal hepatocytes, and ultimately to the renal fibroblasts.
- Fetal red blood cells (RBCs) have higher mean corpuscular volumes and mean corpuscular hemoglobins, different hemoglobin composition, and a higher oxygen affinity than adult RBCs.
- Anemia and polycythemia must be defined during the neonatal period in accordance with reference intervals appropriate for gestational and postnatal age. Anemia in the fetus or neonate can be categorized kinetically as the result of underproduction, hemorrhage, or hemolysis. Polycythemia in the fetus or neonate similarly can be attributed to either increased production or hypertransfusion.
- Areas of controversy include the use of cytomegalovirus-negative products and liberal versus restrictive transfusion thresholds for various neonatal populations.

Normal Erythrocyte Physiology in the Fetus and Newborn

Fetal Erythropoiesis

Embryonic and fetal hematopoiesis is a complex system, simultaneously adapting to and supporting the ever-changing anatomy and milieu within which it develops. Fetal erythropoiesis occurs sequentially during embryonic development in three different sites: yolk sac (extra-embryonic hematopoiesis), liver, and bone marrow.[1] The initial waves of cells originate in blood islands within the yolk sac and serve to meet the immediate needs of the growing embryo. The first types of blood cells produced are morphologically distinct, large nucleated erythrocytes[2] which have been termed "primitive erythrocytes." The yolk sac is also the source of the first wave of "definitive erythrocytes" or erythro-myeloid progenitors (EMPs) which ultimately seed the liver where they differentiate. Yolk sac formation of red blood cells (RBCs) is maximal between 2 and 10 weeks of gestation as the developing fetal liver does not become the predominant source of erythrocytes and other hematopoietic cells until after the first trimester. Bone marrow production of RBCs begins around week 18. Slowly, the liver's function transitions from hematopoiesis to metabolic and by the 30th week of fetal life, bone marrow is the major erythropoietic organ. At full term gestation, almost all RBCs are produced in the bone marrow, although a low level of hepatic erythropoiesis persists through the first few days of life. Sites of fetal erythropoiesis occasionally are reactivated in older patients with hematologic disorders such as myelofibrosis, aplastic anemia, and severe hemolytic anemia.

The growth factors and cytokines that regulate embryonic hematopoiesis remain areas of controversy,[3,4] and animal work suggests that they differ from those that regulate proliferation and differentiation of stem cells in later life.[5] RBC production in extrauterine life is controlled in part by erythropoietin (EPO), a humoral erythropoietic-stimulating factor produced primarily by the kidney. The role of erythropoietin in the developing fetus has not been completely defined, but EPO is not thought to influence the earliest stages of yolk sac erythropoiesis. Within the past decade, neural crest cells have been determined to be the first site of EPO production.[4] As production within the neural crest and neuroepithelial cells begins prior to circulation, delivery to the yolk sac does not occur until roughly day 20, with the first heartbeats. Drive of primitive erythropoiesis (prior to day 20) is therefore EPO-independent. As the sites of early hematopoiesis transition, so too do the sites of EPO production from the neuronal EPO-producing cells to the fetal hepatocytes in late embryonic stages where EPO acts in a paracrine manner, and finally a gradual transition to the fibroblasts of the kidneys (renal erythropoietin-producing cells) beginning at week 30 of gestation.

EPO is detected in fetal blood and amniotic fluid during the third trimester of pregnancy. The concentration of this hormone increases directly with the period of gestation, and thus, EPO levels in term newborns are significantly higher than in premature infants. This difference may reflect some degree of fetal hypoxia during late intrauterine life. Increased EPO titers also are seen in placental dysfunction, fetal anemia, and maternal hypoxia.[6] Fetal RBC formation is not influenced by maternal EPO. Animal studies have demonstrated that EPO does not cross the placenta.[7] In humans, transfusion-induced maternal polycythemia which decreases maternal EPO levels has no effect on fetal erythropoiesis.[8] Additionally, maternal nutritional status is not a significant factor in the regulation of fetal erythropoiesis, because iron, folate, and vitamin B_{12} are trapped by the fetus irrespective of maternal stores, to a point. Studies have demonstrated that women with severe iron deficiency bear children with normal total body hemoglobin content.[9]

The placenta has been the focus of much of the research in maternal-fetal iron homeostasis and indeed has revealed a tightly

TABLE 69.1 Mean Red Blood Cell Values During Gestation

Weeks of Gestation	Hb (g/dL)	Hct (%)	RBC (10^6/mm^3)	MCV (fL)	MCH (pg)	MCHC (g/dL)	Nucleated RBCs (% of RBCs)	Reticulocytes (%)	Diameter (μm)
12	8.0–10.0	33	1.5	180	60	34	5.0–8.0	40	10.5
16	10.0	35	2.0	140	45	33	2.0–4.0	10–25	9.5
20	11.0	37	2.5	135	44	33	1.0	10–20	9.0
24	14.0	40	3.5	123	38	31	1.0	5–10	8.8
28	14.5	45	4.0	120	40	31	0.5	5–10	8.7
34	15.0	47	4.4	118	38	32	0.2	3–10	8.5
40	16.5	51	5.25	108	34	33	0.1	3.2	8.0

Hb, Hemoglobin; *Hct*, hematocrit; *MCV*, mean corpuscular volume; *MCH*, mean corpuscular hemoglobin; *MCHC*, mean corpuscular hemoglobin concentration; *RBC*, red blood cell.
Data from Oski FA, Naiman JL. *Hematologic Problems in the Newborn*. 3rd ed. Philadelphia: Saunders; 1982.

regulated system of placental iron transport mechanisms. In the setting of maternal iron deficiency, the expression of various placental proteins is increased and include but are not limited to divalent metal transporter 1 (DMT1),[10] transferrin receptor 1 (TFR1), and ferroportin (FPN) in more severe iron deficiency.[11] Despite this intricate dance, recent studies suggest that the placental acquisition of iron is prioritized over fetal endowment.[11] This is not surprising given the well described correlation between maternal iron deficiency and adverse neonatal outcomes such as preterm birth, low birth weight, impaired immune function, and numerous neurobehavioral effects.[12–14] Additionally, parameters such as cord blood hemoglobin and cord blood ferritin may not accurately reflect the neonate's total body stores. Neonatal iron has been shown to preferentially be used for heme synthesis which maintains normal total body hemoglobin content while sacrificing brain iron endowment.[15]

Hemoglobin, hematocrit, and RBC count increase throughout fetal life (Table 69.1). Extremely large RBCs (mean corpuscular volume [MCV] of 180 fL) with an increased hemoglobin content (mean corpuscular hemoglobin [MCH] of 60 pg/cell) are produced early in fetal life. The size and hemoglobin content of these cells decrease throughout gestation, but the mean corpuscular hemoglobin concentration (MCHC) does not change significantly. Even at birth, the MCV and MCH are greater than those in older children and adults. Many nucleated RBCs and reticulocytes are present early in gestation, and the percentage of these cells also decreases as the fetus ages.

Hemoglobin production increases markedly during the last trimester of pregnancy. The actual hemoglobin concentration increases, but, more important, body weight, blood volume, and total body hemoglobin triple during this period. Fetal iron accumulation parallels the increase in total body hemoglobin content. The neonatal iron endowment at birth, therefore, is directly related to total body hemoglobin content and length of gestation. Term infants have more iron than premature infants.

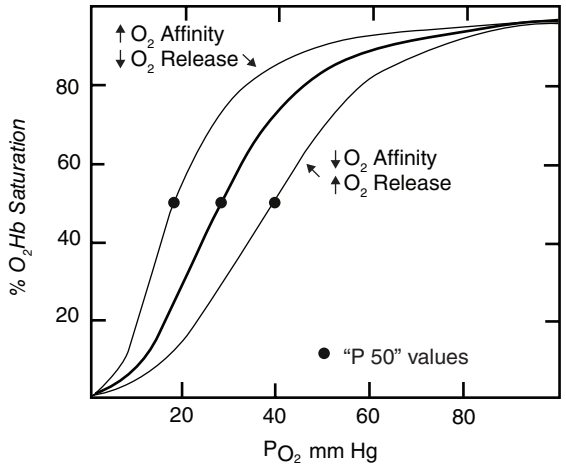
Red Blood Cell Physiology at Birth

In utero, the PO_2 in blood delivered to the tissues is only one third to one fourth the value in adults. This relative hypoxia may be responsible for the increased content of erythropoietin and signs of active erythropoiesis (nucleated RBCs, increased reticulocytes) seen in newborns at birth. When lungs become the source of oxygen, hemoglobin-oxygen saturation increases to 95% and erythropoiesis decreases. Within 72 hours after birth, erythropoietin is undetectable, nucleated RBCs disappear, and by 7 days, reticulocytes decrease to less than 1%.

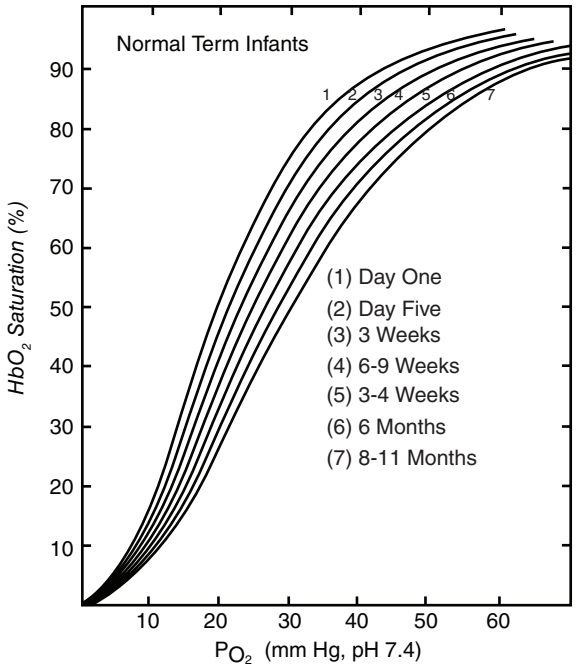
The concentration of hemoglobin during the first few hours of life increases to values greater than those in cord blood. This is both a relative increase caused by a reduction in plasma volume[16] and an absolute increase caused by placental blood transfusion.[17] The umbilical vein remains patent long after umbilical arteries have constricted, and thus transfusion of placental blood occurs when newborns are placed at a level below the placenta. The placenta contains approximately 100 mL of fetal blood (30% of the infant's blood volume). Approximately 25% of placental blood enters the newborn within 15 seconds of birth, and by one minute, 50% is transfused. The time of cord clamping is thus a direct determinant of neonatal blood volume. The blood volume in term infants (mean of 85 mL/kg) varies considerably (50 to 100 mL/kg) because of different degrees of placental transfusion.[17] These differences are readily apparent when the effects of early versus delayed cord clamping are compared at 72 hours of age: 82.3 mL/kg (early clamping) versus 92.6 mL/kg (delayed clamping).[17] These changes are largely the result of differences in RBC mass, 31 mL/kg (early clamping) versus 49 mL/kg (delayed clamping).[17] The blood volume in premature infants (89 to 105 mL/kg) is slightly greater than that in term infants, but this difference is due in large part to an increased plasma volume.[18] The RBC mass in premature infants, expressed in milliliters per kilogram, is the same as in term newborns.

Fetal and Neonatal Hemoglobin Function

A variety of hemoglobins are present during fetal and neonatal life. Fetal hemoglobin (hemoglobin F) is the major hemoglobin in utero, whereas hemoglobin A is the normal hemoglobin of extrauterine life. A single RBC may contain both hemoglobin F and hemoglobin A in varying proportions, depending on gestational



• **Fig. 69.1** The oxygen dissociation curve for normal adult hemoglobin *(bold line)*. The percent oxygen saturation (ordinate) is plotted for arterial oxygen tensions between 0 and 100 mm Hg (abscissa). As the curve shifts to the right, more oxygen is released at any given P_{O_2}. Conversely, as the curve shifts to the left, more oxygen is retained on hemoglobin at any given PO_2. The "P 50" refers to that PO_2 in which hemoglobin is 50% saturated with oxygen. This term is useful in comparing the oxygen affinities of different hemoglobins. (From Oski FA, Delivoria-Papadopoulos M. The red cell, 2,3-diphosphoglycerate, and tissue oxygen release. *J Pediatr*. 1970;77:941–956.)



• **Fig. 69.2** The oxygen affinity of blood from term infants at birth and at different postnatal ages. The gradual rightward shift of the oxygen saturation curve indicates increased oxygen release from hemoglobin as infants get older. This decreased oxygen affinity is due to a decrease in hemoglobin F and an increase in hemoglobin A. (From Oski FA, Delivoria-Papadopoulos M. The red cell, 2,3-diphosphoglycerate, and tissue oxygen release. *J Pediatr*. 1970;77:941–956.)

and postnatal age. One major difference between hemoglobins A and F is related to oxygen transport.

The transport of oxygen to peripheral tissues is regulated by several factors, including blood oxygen capacity, cardiac output, and hemoglobin-oxygen affinity. Oxygen capacity is a direct function of hemoglobin concentration (1 g hemoglobin combines with 1.34 mL oxygen). Compensatory changes in cardiac output can maintain normal oxygen delivery under conditions in which oxygen capacity is significantly reduced. The oxygen affinity of hemoglobin also influences oxygen delivery to tissues. Hemoglobin A is 95% saturated at an arterial PO_2 of 100 mm Hg, but this decreases to 70% to 75% saturation at a venous PO_2 of 4 mm Hg. The difference in O_2 content at arterial and venous oxygen tensions reflects the amount of oxygen that can be released. Changes in hemoglobin affinity for oxygen can influence oxygen delivery (Fig. 69.1).[19] At any given PO_2, more oxygen is bound to hemoglobin when oxygen affinity is increased. Stated in physiologic terms, increased hemoglobin-oxygen affinity reduces oxygen delivery, whereas decreased hemoglobin-oxygen affinity increases oxygen release to peripheral tissues.

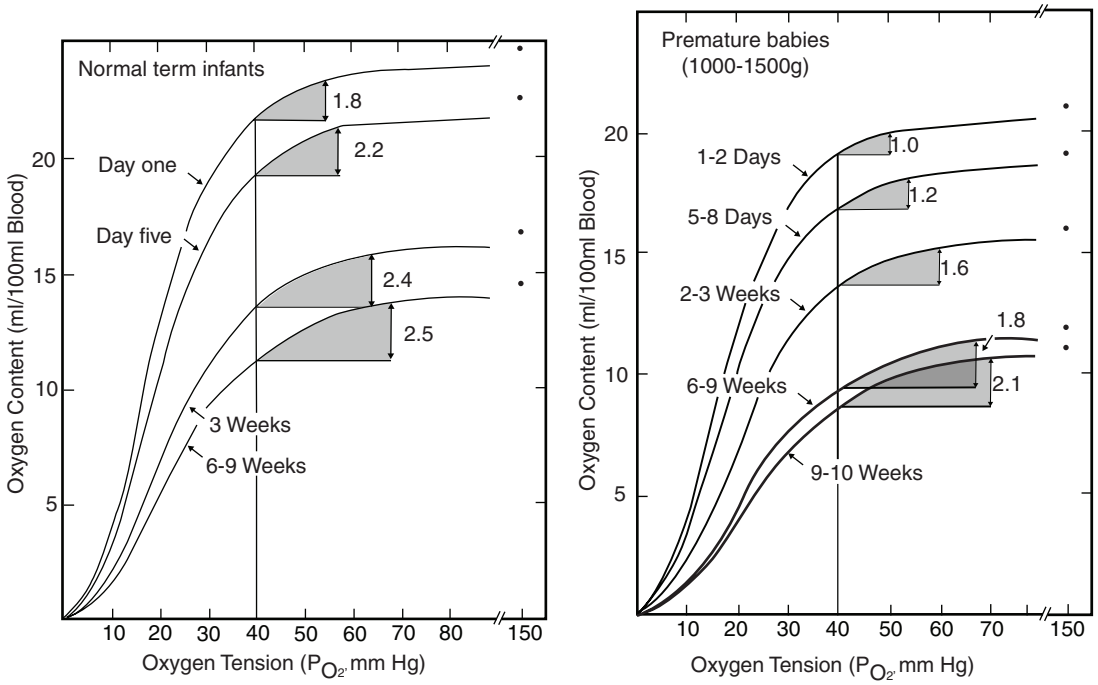
The oxygen affinity of hemoglobin A in solution is greater than that of hemoglobin F. Paradoxically, however, whole blood from normal children (hemoglobin A) has a lower oxygen affinity than that of neonatal blood (hemoglobin F).[20] This difference is related to an intermediate of RBC metabolism, 2,3-diphosphoglycerate (2,3-DPG). This organic phosphate compound interacts with hemoglobin A to decrease its affinity for oxygen, thereby enhancing O_2 release. Fetal hemoglobin does not interact with 2,3-DPG to any significant extent[21]; consequently, cells containing hemoglobin F have a higher oxygen affinity than those containing hemoglobin A. The increased oxygen affinity of fetal RBCs is advantageous for extracting oxygen from maternal blood within the placenta.

A few months after birth, infant blood acquires the same oxygen affinity as that of older children (Fig. 69.2). The postnatal decrease in oxygen affinity is due to a reduction in hemoglobin F and an increase in hemoglobin A (which interacts with 2,3-DPG). Oxygen delivery (the difference in arterial and venous O_2 content) increases while oxygen capacity (hemoglobin concentration) decreases during the first week of life (Fig. 69.3). This enhanced delivery is largely a reflection of the decreased oxygen affinity of infant blood.[22] The oxygen affinity of blood from premature infants is higher than that of term infants, and the normal postnatal changes (decrease in oxygen affinity, increase in oxygen delivery) occur much more gradually in premature infants (see Fig. 69.3).

Another role of the fetal erythrocyte that has only relatively recently been appreciated is maternal immune tolerance. In a mouse model, the immunosuppressive role of CD71+ erythroid cells both on the maternal side as well as the fetal side has been demonstrated. In pregnant mice as well as pregnant women, a higher proportion of peripheral CD71+ erythroids was found compared to their non-pregnant counterparts. This upregulation was also present locally, at the fetomaternal interface (placental tissue).[23] Through regulation of L-arginine levels and site-specific expression of PDL-1, the work of Delyea et al. suggests that fetal CD71+ erythroid cells play an integral role in the immune tolerance which is essential for a successful allogeneic pregnancy.

Anemia in the Fetus and Newborn

The cause of anemia frequently can be ascertained by medical history and physical examination. Particular focus should be given to family history (anemia, cholelithiasis, unexplained jaundice, splenomegaly), maternal medical history (especially infections), and obstetric history (previous pregnancies, length of gestation,



• **Fig. 69.3** Oxygen delivery in normal term and premature infants. Oxygen content (a function of total hemoglobin) is on the ordinate. Oxygen tension is on the abscissa. Oxygen delivery is measured by the difference in oxygen content at arterial (100 mm Hg) and venous (40 mm Hg) oxygen tensions. For both term and premature infants, oxygen delivery *(shaded areas)* increases with age. This occurs despite a decrease in oxygen content. (From Delivoria-Papadopoulos M, Roncevic NP, Oski FA. Postnatal changes in oxygen transport of term, premature, and sick infants: the role of red cell 2,3-diphosphoglycerate and adult hemoglobin. *Pediatr Res*. 1971;5:235–245.)

TABLE 69.2 Differential Approach to Anemia in the Newborn Period

Hemoglobin	Reticulocytes	Bilirubin	Direct Antiglobulin (Coombs) Test	Clinical Considerations
Decreased	Normal/decreased	Normal	Negative	Physiologic anemia of infancy and prematurity Hypoplastic anemia
Decreased	Normal/increased	Normal	Negative	Hemorrhagic anemia
Decreased	Normal/increased	Increased	Positive	Immune-mediated hemolysis
Decreased	Normal/increased	Increased	Negative	Acquired or hereditary red blood cell defects Enclosed hemorrhage with resorption of blood DAT-negative ABO incompatibility

method and difficulty of delivery). The age at which anemia becomes manifest also is of diagnostic importance. Significant anemia at birth is generally due to blood loss or alloimmune hemolysis. After 24 hours, internal hemorrhages and other causes of hemolysis are more common. Anemia that appears several weeks after birth can be caused by a variety of conditions, including abnormalities in the synthesis of hemoglobin beta chains, hypoplastic RBC disorders, and the physiologic anemia of infancy or prematurity.

Infants with anemia resulting from chronic blood loss may appear pale, without other evidence of clinical distress. Acute blood loss can produce hypovolemic shock and a clinical state similar to severe neonatal asphyxia. Newborns with hemolytic anemia frequently show a greater-than-expected degree of icterus. In addition, hemolysis often is associated with hepatosplenomegaly, and in cases resulting from congenital infection, other stigmata may be present.

Evaluation of Anemia

A simple classification of neonatal anemia based on physical examination and basic laboratory tests is presented in Table 69.2. More extensive RBC testing is discussed elsewhere.[24]

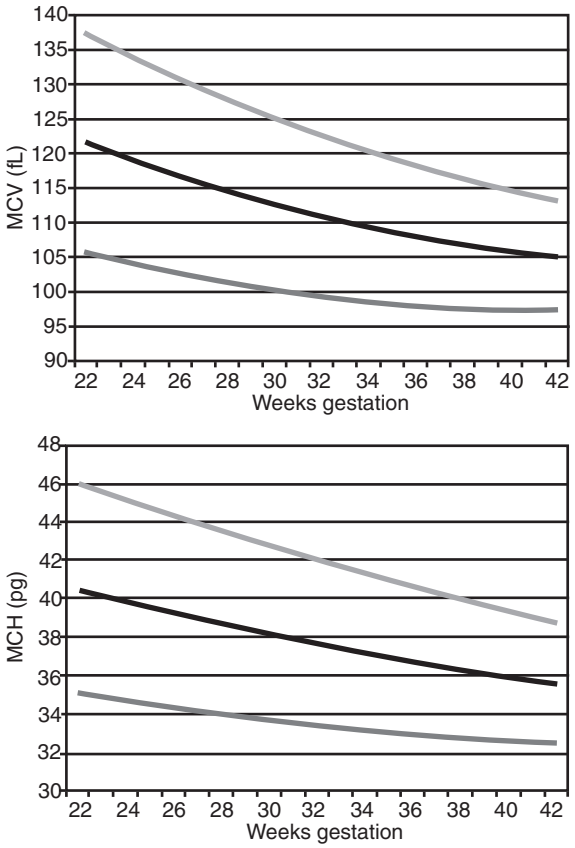
Red Blood Cell Count, Hemoglobin, Hematocrit, and Red Blood Cell Indices

RBC values during the neonatal period are more variable than at any other time of life. The diagnosis of anemia must therefore be made in terms of "normal" values for gestational and postnatal ages. The mean cord blood hemoglobin of healthy term infants ranges between 14 and 20 g/dL (Table 69.3). Shortly after birth, however, hemoglobin concentration increases. This increase is both relative (owing to a reduction of plasma volume) and absolute (owing to placental RBC transfusion).

TABLE 69.3 Red Blood Cell Values in Term and Premature Infants During the First Week of Life

	Hb (g/100 mL)	Hct (%)	Reticulocytes (%)	Nucleated RBCs (Cells/1000 RBCs)
Term				
Cord blood	17.0 (14–20)	53.0 (45–61)	<7	<1.00
Day 1	18.4	58.0	<7	<0.40
Day 3	17.8	55.0	<3	<0.01
Day 7	17.0	54.0	<1	0
Premature (Birthweight <1500 g)				
Cord blood	16.0 (13.0–18.5)	49	<10	<3.00
Day 7	14.8	45	<3	<0.01

Hb, Hemoglobin; *Hct*, hematocrit; *RBC*, red blood cell.



• **Fig. 69.4** Reference ranges for MCV *(upper panel)* and MCH *(lower panel)* for neonates on the first day after birth. The lower line shows the 5th percentile values, the middle line shows the mean values, and the upper line shows the 95th percentile values. (From Christensen RD, Henry E, Jopling J, Wiedmeier SE. The CBC: reference ranges for neonates. *Semin Perinatol*. 2009;33:3–11.)

Failure of hemoglobin to increase during the first few hours of life may be the initial sign of hemorrhagic anemia. RBC values at the end of the first week are virtually identical to those seen at birth. Anemia during the first week of life is thus defined as any hemoglobin value less than 14 g/dL. A significant hemoglobin decrease during this time is suggestive of hemorrhage or hemolysis. For example, a hemoglobin of 14.5 g/dL at 7 days of age is abnormal for a term infant whose hemoglobin was 18.5 g/dL at birth. A slight hemoglobin reduction normally occurs in premature infants during the first week of life. Beyond the first week, however, the hemoglobin concentration decreases in both term and premature infants (see Physiologic Anemia of Infancy and Anemia Prematurity, later).

The electronic equipment used for blood counts also gives statistical information regarding erythrocyte size (MCV) and hemoglobin content (MCH). The normal MCV in older children ranges from 75 to 90 fL. MCV values of less than 75 fL are considered microcytic, whereas those over 100 fL indicate macrocytosis. Normal infant RBCs are large (MCV 105 to 125 fL), and not until 8 to 10 weeks of age does cell size approach that in older children. Neonatal microcytosis is defined as an MCV of less than 95 fL at birth. The RBC hemoglobin content of neonatal cells (MCH 35 to 38 pg/cell) is greater than that seen in older children (MCH 30 to 33 pg/cell). Neonatal hypochromia is defined as an MCH of less than 34 pg/cell. Hypochromia and microcytosis generally occur together and are due to hemoglobin production defects. Neonatal hypochromic microcytosis is seen with iron deficiency and thalassemia disorders (alpha and gamma thalassemias). Both the MCV and MCH are higher in preterm infants as shown in Fig. 69.4.[25]

The site from which blood is obtained is important, because hemoglobin and hematocrit are higher in capillary blood than in simultaneously obtained central venous samples (up to 20%). This difference can be minimized by warming an extremity to obtain "arterialized capillary blood."[26] In the face of acute hemorrhage, however, central venous samples must be obtained because of marked peripheral vasoconstriction.

Reticulocyte Count

The normal reticulocyte count in children and older infants is 1% to 2% of the circulating red cells. The reticulocyte count in term infants ranges between 3% and 7% at birth, but this decreases to less than 1% by 7 days of age (see Table 69.3). In premature infants, reticulocyte values at birth are higher (6% to 10%) and may remain elevated for a longer period of time. Nucleated RBCs are seen in newborn infants, but they generally disappear by the third day of life in term infants and in 7 to 10 days in premature infants. The persistence of reticulocytosis or nucleated RBCs suggests the possibility of hemorrhage or hemolysis. Hypoxia, in the absence of anemia, also can be associated with increased release of reticulocytes and nucleated RBCs.

Peripheral Blood Smear

Examination of the peripheral blood smear is an invaluable aid in the diagnosis of anemia. The smear is evaluated for alterations in the size and shape of RBCs as well as abnormalities in leukocytes and platelets. Erythrocytes of older children are approximately the size of a small lymphocyte nucleus, whereas those of newborns are slightly larger. RBC hemoglobinization (e.g., hypochromia)

is estimated by observing the area of central pallor, which is one third the diameter of normal RBCs and more than one half the diameter of hypochromic cells. Spherocytes are detected by the complete absence of central pallor. The degree of reticulocytosis can be estimated, because these cells are larger and have a bluish coloration.

Direct Antiglobulin Test

Most cases of neonatal hemolytic anemia are due to isoimmunization. The direct antiglobulin test (DAT), previously known as the direct Coombs test, detects the presence of antibody on RBCs. The indirect antiglobulin test, previously known as the indirect Coombs test, detects anti-RBC antibodies in the plasma.

Management of Anemia

A hemoglobin of 14 g/dL corresponds to an RBC mass of 31 mL/kg. Thus, an RBC transfusion of 2 mL/kg will increase the hemoglobin concentration by approximately 1 g/dL. Packed RBCs (hematocrit approximately 67%) contain 2 mL of RBCs/3 mL of packed RBCs. Thus, the transfusion of 3 mL of packed RBCs/kg increases hemoglobin concentration by approximately 1 g/100 mL.

Packed RBCs are the product of choice when transfusion is necessary for simple anemia, as occurs in hemolysis. If anemia is accompanied by hypovolemia from acute blood loss, volume expansion must be achieved promptly, using packed RBCs and normal saline or a colloid such as 5% serum albumin (infused separately). The previously common practice of reconstituting RBCs with fresh frozen plasma to make "whole blood" is no longer acceptable because the increased donor exposure increases the risk of transmitting infectious disease. When packed RBCs need to be diluted to facilitate nonurgent transfusion, isotonic saline is the preferred diluent. Although fresh blood less than 2 days old is ideal because there is a reduced risk of hyperkalemia, this is not usually available. An acceptable substitute is packed RBCs less than 4 to 5 days old. These packed RBCs provide adequate oxygen delivery; hyperkalemia can be prevented by washing the RBCs once in saline and then reconstituting with normal saline. Washing is not required for the usually small, simple transfusions of packed RBCs, because the small volume of plasma minimizes any toxic effect of increased concentration of potassium in the plasma.

Blood currently available in most blood banks is anticoagulated with citrate-phosphate-dextrose (CPD), CPD-adenine (CPDA-1), or adenine-saline (AS-3), with a shelf life of 21, 35, or 42 days, respectively. Hematocrit usually ranges between 65% and 80% for packed RBCs. Near-normal 2,3-DPG levels are maintained for up to 12 to 14 days, which is advantageous in transfusing infants with acute hypoxia or those receiving large volumes of blood. Hematocrits range from 55% to 65%, thus facilitating flow during infusion. The newest of these preparations, AS-3, is well tolerated by newborns even after up to 42 days of storage, so long as only small-volume transfusions are given at any one time.[27] However, when larger-volume (≥20 mL/kg) transfusions are required, the theoretical concern is for fetal/neonatal renal and hepatic exposure to potentially toxic levels of the additives within these products such as adenine, mannitol, sodium chloride, dextrose, citrate, and phosphate. To date, there have been no trials assessing the safety of these additive solutions when transfusion needs increase, and many institutions utilize them in certain situations. A survey conducted in 2015 of 21 facilities across the US found that for large-volume transfusions in neonatal patients, 43% of responding centers used AS-3 RBC units, 29% used AS-1 RBCs, and 28% used CPD or CPDA RBC units.[28] Risks were mitigated using fresh units or re-washing previously radiated units so as to decrease the chance of hyperkalemia.

It is important to adopt practices that limit donor exposure in order to reduce the risk of transfusion-associated infections. Splitting and aliquoting a single red cell donation for multiple use by one neonate, using a unit through its outdate, the use of restrictive transfusion thresholds, and larger transfusion volumes are all effective ways to limit exposures.[29–32]

Preterm infants born weighing less than 1250 g are uniquely susceptible to potentially serious cytomegalovirus (CMV) infection from transfused blood, particularly if they lack immunity because their mothers are seronegative. Practices to prevent CMV infection have involved utilizing blood products from seronegative donors[33] but because approximately 40% to 60% of adults are seropositive, there is limited availability of seronegative donors. Alternatively, because CMV resides mainly in leukocytes, removal of such cells also can prevent transmission of the virus, and the use of high-efficiency leukocyte depletion filters[34] has proven effective. A potential disadvantage of using CMV-seronegative blood in CMV-positive infants receiving large amounts of blood is dilution of infant's antibody level, resulting in increased susceptibility to nursery-acquired CMV infection. Currently, a majority of neonatal services utilize leukocyte-reduced red cell products rather than relying on CMV-negative products to prevent CMV infection.[35] Although the efficacy of using leukoreduced and CMV-seronegative products was demonstrated in a recent prospective cohort study of 539 infants,[36] there remains quite a bit of controversy in terms of which products should be utilized for which patient populations. This ambiguity was highlighted when in 2016, the American Association of Blood Banks Clinical Transfusion Medicine Committee decided not to issue guidelines regarding the use of such products to reduce the risk of transfusion-transmitted CMV.[37] This controversy plays out in practice as was depicted in a US practice survey which was conducted in 2015 and published in 2018.[38]

Graft-versus-host (GVH) reaction rarely follows transfusion and occurs mainly in certain newborns at risk. For this to occur, viable lymphocytes in cellular blood products must be able to engraft and react against foreign antigens on tissues of the recipient. Infants at risk include those with congenital or acquired defects of cellular immunity, those who as fetuses received intrauterine transfusion of RBCs or platelets, newborns receiving exchange transfusion following intrauterine transfusion,[39,40] and infants receiving directed blood donations from first-degree relatives (whose genetic similarity may increase the likelihood of engraftment). Irradiation of RBCs and platelets with a minimum of 1500 rads has proved effective in preventing GVH reaction. Reports of GVH reaction after RBC transfusion in very premature infants without known risk factors[41,42] have prompted most neonatal services to irradiate all RBC blood products.[35]

Causes of Fetal and Neonatal Anemia

Hemorrhagic Anemia

Anemia frequently follows fetal blood loss, bleeding from obstetric complications, and internal hemorrhages associated with birth trauma (Box 69.1). Iatrogenic anemia due to repeated removal of blood for laboratory testing is common in premature infants. The clinical presentation of anemia depends on the magnitude and acuteness of blood loss.

BOX 69.1 Causes of Hemorrhagic Anemia in Newborns

Fetal Hemorrhage
Spontaneous fetomaternal hemorrhage
Hemorrhage following amniocentesis
Twin-twin transfusion
Nuchal cord

Placental Hemorrhage
Placenta previa
Abruptio placentae
Multilobed placenta (Vasa previa)
Velamentous insertion of cord
Placental incision during cesarean section

Umbilical Cord Bleeding
Rupture of umbilical cord with precipitous delivery
Rupture of short or entangled cord

Postpartum Neonatal Hemorrhage
Bleeding from umbilicus
Cephalohematomas, scalp hemorrhages
Hepatic rupture, splenic rupture
Retroperitoneal hemorrhage
Diffuse alveolar hemorrhage

Infants with anemia from moderate hemorrhage or chronic blood loss are generally asymptomatic. The only physical finding is pallor of the skin and mucous membranes. Laboratory studies can range from a mild normochromic normocytic anemia (hemoglobin 9 to 12 g/dL) to a more severe hypochromic microcytic anemia (hemoglobin 5 to 7 g/dL). The only therapy required for asymptomatic children is supplemental iron (3 to 4 mg elemental iron/kg once a day for 3 months). RBC replacement is indicated only if there is evidence of clinical distress (tachycardia, tachypnea, irritability, feeding difficulties). In most cases, increasing the hemoglobin to 10 to 12 g/dL removes all signs and symptoms associated with anemia. Because severely anemic infants are frequently in incipient heart failure, however, these children should be transfused very slowly (2 mL/kg/h). If signs of congestive heart failure appear, a rapid-acting diuretic (furosemide, 1 mg/kg intravenously) should be given before proceeding with the transfusion. An alternative approach is to administer a partial exchange transfusion with packed RBCs to severely anemic infants. This approach increases the hemoglobin concentration without the danger of increasing blood volume and precipitating congestive heart failure.

Infants who rapidly lose large volumes of blood appear to be in acute distress with pallor, tachycardia, tachypnea, weak pulses, hypotension, and shock. This presentation is distinct from that seen in neonatal respiratory asphyxia. Infants with respiratory problems demonstrate a marked improvement with assisted ventilation and oxygen, whereas there is little change in anemic newborns. Cyanosis is not a feature of severe anemia because the hemoglobin concentration is too low (for clinical cyanosis to be apparent, there must be at least 5 g/dL of deoxygenated hemoglobin). The hemoglobin concentration immediately after an acute hemorrhage may be normal, and a decreased hemoglobin may not be seen until the plasma volume has reexpanded several hours later. Thus, the diagnosis of acute hemorrhagic anemia is based largely on physical findings and evidence of blood loss. It is important to recognize these clinical features because immediate therapy is required. Treatment is directed at rapid expansion of the vascular space (20 mL fluid/kg) by rapid infusion of either isotonic saline or 5% albumin, followed by either type-specific, cross-matched packed RBCs. In infants in whom anemia and hypoxia are severe, non-cross-matched group O, Rh-negative RBCs are an acceptable alternative to cross-matched RBCs. Infants with hypovolemic shock caused by acute external blood loss usually show marked clinical improvement after this treatment. A poor response is seen in newborns with ongoing internal hemorrhage.

Fetal Hemorrhage

Significant bleeding into the maternal circulation occurs in approximately 8% of all pregnancies and thus represents one of the most common forms of fetal bleeding. Small amounts of fetal blood are lost in most cases, but in 1% of pregnancies, fetal blood loss may exceed 40 mL.[43] Fetomaternal hemorrhage occasionally follows amniocentesis and placental injury,[44] although anemia is seen only after unsuccessful amniocentesis or when there is evidence of a bloody tap.[45] For this reason, infants born to mothers who have had amniocentesis should be observed closely for signs of anemia. The effects of anemia resulting from fetomaternal hemorrhage are variable. Large acute hemorrhages can produce hypovolemic shock, whereas slower, more chronic blood loss results in hypochromic microcytic anemia resulting from iron deficiency. Some newborns with severe chronic fetal anemia (hemoglobin levels as low as 4 to 6 g/dL) may have minimal symptoms.

An examination of maternal blood for the presence of fetal cells is necessary to diagnose fetomaternal hemorrhage. Two techniques are available. The Kleihauer-Betke preparation involves examination of a stained specimen of maternal blood by microscopy following differential elution of hemoglobin A but not hemoglobin F from the red cells. Alternatively, flow cytometry–based techniques are probably more accurate but are less widely available. These approaches use antibodies against fetal hemoglobin (sometimes combined with antibodies against carbonic anhydrase) or against the D antigen to distinguish fetal RBCs from adult cells.[46–48]

Approximately 50 mL of fetal blood must be lost to produce significant neonatal anemia. This volume is greater than 1% of the maternal blood volume, and therefore fetal cells within the maternal circulation may be detected readily. Tests that depend on the presence of fetal hemoglobin are not valid when a maternal hemoglobinopathy with increased hemoglobin F levels coexists, such as sickle cell anemia, beta thalassemia, and hereditary persistence of fetal hemoglobin (HPFH). In addition, fetomaternal ABO incompatibility may cause rapid removal of fetal RBCs, thus obscuring any significant hemorrhage. For this reason, it is important to obtain a sample of maternal blood as soon as anemia from fetal hemorrhage is suspected.

Twin-Twin Transfusion

Transfusion of blood from one monozygous twin to another can result in anemia in the donor twin and polycythemia in the recipient. Significant hemorrhage is seen only in monochorionic monozygous twins (approximately 70% of all monozygous twins). The most common form, chronic twin-to-twin transfusion (TTTS), is seen in 10% to 15% of monochorionic pregnancies.[49,50] Bleeding occurs because of vascular anastomosis in monochorionic placentas. The anemic donor twin is usually smaller than the polycythemic recipient, with a greater than 20% difference in birthweight. Polyhydramnios is frequently seen in the recipient

twin and oligohydramnios is seen in the donor. TTTS is diagnosed when there is evidence of twin oligohydramnios (<2 cm)/polyhydramnios (>8 cm) sequence in the absence of other disorders which may lead to discordant amniotic fluid volumes. These volume thresholds differ depending on gestational age. The high rate of intrauterine mortality (approximately 63% with conservative management) has spurred attempts at fetal therapy, including decompression amniocentesis, laser coagulation of vascular anastomoses, interfetal septal disruptions, and selective feticide.[51,52]

Since first described in 1990,[53] laser coagulation for this purpose has undergone a series of technical modifications and is now considered the best option for treatment of most cases of TTTS. A systematic review from 2015 described perinatal survival of at least one twin after laser therapy in 81% to 88% of pregnancies and survival of both twins in 52% to 54% of pregnancies.[54] Despite impressive advancements made over the last 25 years, complications following laser therapy are not uncommon. Recurrent TTTS has been reported in up to 16% of cases,[55] twin anemia-polycythemia sequence (TAPS) in 2% to 13% of cases,[56] and preterm premature rupture of membranes (PPROM) in 17% to 40% of cases.[57–59]

Placental Blood Loss

Placental bleeding during pregnancy is common, but in most cases hemorrhage is from the maternal aspect of the placenta. In placenta previa, however, the thinness of the placenta overlying the cervical os frequently results in fetal blood loss. The vascular communications between multilobular placental lobes also are very fragile and are easily subjected to trauma during delivery. Vasa previa is the condition in which one of these connecting vessels overlies the cervical os and thus is prone to rupture during delivery. Abruptio placentae generally causes fetal anoxia and death, although some infants survive but can be severely anemic. Bleeding also follows inadvertent placental incision during cesarean section,[60] and thus the placenta should be inspected for injury following all cesarean sections.

Umbilical Cord Bleeding

The normal umbilical cord is resistant to minor trauma and does not bleed. The umbilical cord of premature infants, however, is weak and thus vulnerable to rupture and hemorrhage.[61] In cases of precipitous delivery, a rapid increase in cord tension can rupture the cord, causing serious acute blood loss. Short or entangled umbilical cords and abnormalities of umbilical blood vessels (velamentous insertions into the placenta) are also vulnerable to rupture and hemorrhage. Bleeding from injured umbilical cords is rapid but generally ceases after a short period of time, owing to arterial constriction. The umbilical cord should always be inspected for abnormalities or signs of injury, particularly after unattended, precipitous deliveries.

Hemorrhage After Delivery

Hemorrhagic anemia due to internal bleeding is occasionally associated with birth trauma. Characteristically, internal hemorrhages are asymptomatic during the first 24 to 48 hours of life, with signs and symptoms of anemia developing after this time. Cephalhematomas can be sufficiently large to cause anemia and hyperbilirubinemia secondary to the resorption of blood. Subgaleal hemorrhages are seen infrequently, sometimes occurring after vacuum extraction is used during delivery. In contrast to cephalhematomas, subgaleal bleeding can be extensive because hemorrhage is not limited by the periosteum. Adrenal and kidney hemorrhages occasionally follow difficult breech deliveries. Splenic rupture and hemorrhage occur most commonly in association with splenomegaly, as in erythroblastosis fetalis. Hepatic hemorrhages are generally subcapsular and may be asymptomatic. Rupture of the hepatic capsule results in hemoperitoneum and hypovolemic shock. Hepatic hemorrhages are suspected when a previously healthy infant goes into shock with clinical manifestations of an increasing right upper quadrant abdominal mass, shifting dullness on percussion, and evidence of free fluid on abdominal radiographs. In contrast to newborns with acute blood loss from fetomaternal or umbilical vessel bleeding, infants with hepatic hemorrhage generally demonstrate a poor clinical response to blood replacement. Diffuse alveolar hemorrhage is a rare but life-threatening cause of neonatal anemia, the etiology of which can be broadly divided into three categories: immune disorders, cardiovascular causes, and non-immune, non-cardiovascular origins.[62] The impact of iatrogenic blood loss has been well described, especially in premature neonates as the phlebotomy requirements are noted to increase with decreasing gestational age and increasing illness severity. The total blood volume removed from a preterm infant over the first weeks to months of life frequently exceeds their total blood volume.[63]

Hemolytic Anemia

RBCs from children and adults normally circulate for 100 to 120 days. Erythrocyte survival in newborns is shorter at 70 to 90 days in term infants, and 50 to 80 days in premature infants.[64] Hemolytic anemia, which further shortens RBC survival, may arise for many reasons (Box 69.2). Red cell destruction can occur by macrophage recognition of abnormal RBC membrane properties (extravascular hemolysis) or, alternatively, RBC destruction can occur by direct damage of the RBC in the circulation with the resultant release of hemoglobin (intravascular hemolysis). In most cases some degree of both types of hemolysis occurs.

In older infants and children, the usual response to increased RBC destruction is enhanced erythropoiesis, and there may be little or no anemia if the rate of production matches the accelerated rate of destruction. In these cases of well-compensated hemolysis, the major manifestations are due to increased erythrocyte destruction (hyperbilirubinemia) and augmented erythropoiesis (reticulocytosis). During the early neonatal period, however, the

BOX 69.2 Causes of Hemolytic Anemia During the Newborn Period

Immune Disorders

Isoimmune: Rh and ABO incompatibility
Maternal immune disease: autoimmune hemolytic anemia, systemic lupus erythematous
Drug-induced: penicillin

Acquired Red Blood Cell (RBC Disorders)

Infection: cytomegalovirus, toxoplasmosis, syphilis, bacterial sepsis
Disseminated and localized intravascular coagulation, respiratory distress syndrome

Hereditary RBC Disorders

Membrane defects: hereditary spherocytosis, hereditary elliptocytosis
Enzyme abnormalities: glucose-6-phosphate dehydrogenase, pyruvate kinase
Hemoglobinopathies: α-thalassemia syndromes, γ/β-thalassemia

increased oxygen-carrying capacity of blood (see Physiologic Anemia of Infancy and Anemia Prematurity, later) may blunt any compensatory erythropoietic activity in cases of mild hemolysis. Consequently, hyperbilirubinemia in excess of normal neonatal levels may be the only apparent manifestation of hemolysis. In most cases of significant hemolysis, however, some degree of reticulocytosis is usually present.

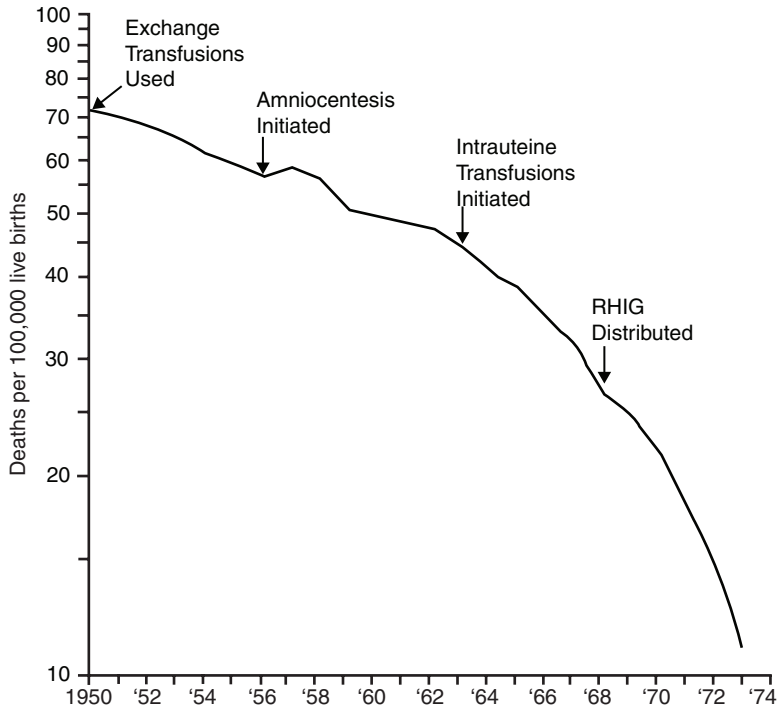
Immune Hemolysis

Placental transfer of maternal antibodies directed against fetal RBC antigens is the most common cause of neonatal hemolysis. This phenomenon is a consequence of maternal sensitization to fetal RBC antigens inherited from the father. Hemolysis occurs only in the fetus. The spectrum of clinical problems ranges from minimal anemia and hyperbilirubinemia to severe anemia with hydrops fetalis. At one time, before effective prevention of Rh sensitization was available, hemolytic disease of the newborn was responsible for more than 10,000 deaths annually in the United States.[65] In 1968, IgG and anti-Rh(D) gained regulatory approval and licensure for routine postpartum prophylaxis in RhD-negative women. Globally, this shift led to a decrease in the rate of RhD alloimmunization from 16% to roughly 2%[66] and ultimately to <0.5% upon additional antenatal administration in 1977 (Fig. 69.5).[67,68] Nevertheless, Rh incompatibility still occurs in areas where immune prophylaxis is not readily available. In the United States, most cases of alloimmune hemolysis are due to ABO maternal-fetal incompatibility with a smaller fraction resulting from sensitization to Kell, Duffy, Kidd, and other Rh antigens such as c and E.

Rh Hemolytic Disease: Erythroblastosis Fetalis

The role of Rh antibodies in classic erythroblastosis fetalis was first elucidated by Levine and Katzen in 1941.[69] There are over 50 (serologically defined) antigens within the Rh blood group, all of which are encoded by *RHD* and *RHCE*. These two genes are located on chromosome one, share 92% nucleotide sequence homology and 96% amino acid sequence similarity, and are organized in opposite orientation.[70–72] The *RHCE* gene encodes both the C/c and E/e proteins and their variants while the *RHD* gene encodes the RhD protein and its variants. The most common isoforms are D, C, c, E, and e. Rh-positive RBCs are those that possess the D antigen. This phenotype may result from homozygosity (DD) or heterozygosity (Dd) for the D antigen. In Rh-positive whites, approximately 44% are homozygous (DD) and 56% are heterozygous (Dd). There is no separate isoform for this antigen (as there is for C, c, E, and e), but rather a lowercase d is used to denote the absence of D, or Rh-negative status. As there is no "d" antigen, no "anti-d" serum has been identified. The majority of people with an Rh-negative phenotype have the *RHD* gene deleted from both chromosomes. The frequency of Rh negativity varies in different ethnic groups. It is high in whites (15%), lower in blacks (5%), and virtually nonexistent in Asians.[73] Interestingly, the genotype of Rh negativity also varies by ethnicity. In black populations, only 18% of RhD-negative individuals are homozygous for the deletion. Instead, *RHD* variants are more common which either lead to the expression of an incomplete or hybrid D antigen (*RHD* pseudogene) or complete lack of expression (phenotypically D-negative). The former can result in "weak D" (previously "D^u positive") antibody testing.[74] These are relevant scenarios for obstetrical care as the genotype can predict which women can be treated as D-positive versus those that should be managed with anti-D immunoglobulin.[72,75,76]

The pathophysiology of alloimmune hemolysis resulting from Rh incompatibility includes the following: an Rh-negative mother, an Rh-positive fetus where the D antigen is expressed on fetal RBCs as early as 38 days of gestation,[77] leakage of fetal RBCs into maternal circulation, maternal sensitization to D antigen on fetal RBCs, production and transplacental passage of maternal anti-D antibodies into fetal circulation, attachment of maternal antibodies to Rh-positive fetal RBCs, and



• **Fig. 69.5** Infant death rates from hemolytic disease of the newborn, United States, 1950 to 1973.

destruction of antibody-coated fetal RBCs. Rh hemolytic disease occurring during the first pregnancy is rare (occurring in 1% of cases) but increases significantly with each subsequent pregnancy. Significant hemolysis occurring in the first pregnancy indicates prior maternal exposure to Rh-positive RBCs. On occasion the sensitization may be a consequence of an earlier transfusion in which Rh-positive RBCs were administered by mistake or in which some other blood component (e.g., platelets) containing Rh(D) RBCs was transfused. Small volumes of fetal RBCs enter the maternal circulation throughout gestation, although the major fetomaternal bleeding responsible for sensitization occurs during delivery[44] with certain factors increasing the volume of hemorrhage at that time such as cesarean delivery, multifetal gestation, bleeding placenta previa or abruption, manual removal of placenta, etc. Fetomaternal bleeding resulting from various other antepartum events such as abortion (spontaneous or induced), invasive procedures, maternal abdominal trauma, ectopic pregnancy, etc. account for only 1% to 2% of Rh alloimmunization.[78]

Once sensitization has occurred, reexposure to Rh(D) RBCs in subsequent pregnancies leads to an anamnestic response, with an increase in the maternal anti-D titer. In this circumstance, anti-D immune globulin is no longer effective at preventing or reducing the severity of consequent hemolysis in the fetus. If future pregnancies are desired, a woman has three options which allow for utilization of her own gamete: use of a gestational carrier, preimplantation genetic testing where an RHD negative embryo is selected and fertilized in vitro if the father is heterozygous for RHD, and insemination with sperm from a D-negative donor if the father is homozygous for RHD.

The major factor responsible for the reduced death rate related to Rh alloimmunization is the development of Rh immune globulin to prevent maternal sensitization. Important early observations were that fetomaternal RBC transfer (and thereby sensitization) occurs primarily during delivery and that the frequency of Rh immune hemolytic disease was much lower in ABO-incompatible pregnancies (maternal RBC type O, fetal RBC type A or B). The apparent beneficial effect of ABO incompatibility is because maternal anti-A and anti-B antibodies recognize the corresponding A and B fetal RBCs, leading to their destruction before sensitization can occur.

It is recommended that all women have their blood typed (ABO and Rh) along with an antibody screen at their first prenatal visit. For women who are Rh negative with a fetus that is or could be Rh positive, it is recommended that they receive anti D-immune globulin at 28 weeks' gestation, within 72 hours of delivery of a D positive baby, as well as after an antepartum event which could increase the risk of fetomaternal bleeding (see above). The antibody screen should be repeated at 28 weeks, prior to receiving immune globulin given the risk, albeit small, that the mother has become sensitized in that window. Although commercially available and highly sensitive (99.3%) and specific (98.4%),[79] noninvasive methods which utilize fetal cell free DNA in maternal plasma is not yet accepted as standard of care for determining the fetal Rh status. Prior to administering the anti D-immune globulin at delivery, a rosette test is done to determine if the routine dose is sufficient.[80] This test screens for excessive fetomaternal bleeding and if positive, should be followed by a quantitative test such as the Kleihauer-Betke test[81] or flow cytometry. In suspicious cases (e.g., with placental abruption or neonatal anemia), the volume of fetal hemorrhage can be quantified using the Kleihauer-Betke procedure.

As noted previously, pregnant Rh-negative women previously sensitized to Rh(D) should not receive anti D-immune globulin. In this circumstance, if the fetus is indeed at risk (Rh positive), the transplacental passage of maternal anti-D leads to a positive DAT on Rh(D) fetal RBCs. Depending on the amount of anti-D absorbed, a variable degree of fetal hemolysis occurs, thereby leading to anemia, hepatosplenomegaly, and increased bilirubin formation. In utero, bilirubin is removed by transfer across the placenta into the maternal circulation; therefore, hyperbilirubinemia is not a problem until after delivery, when levels may increase because of immaturity of hepatic conjugating enzymes as well as increased enterohepatic circulation. The major threat to the fetus is severe anemia leading to hydrops fetalis and intrauterine death. Typically, maternal anti-D titers are followed serially until they reach a "critical titer" at which point the fetus is felt to be at risk for these outcomes. As this is only a screening test and can actually increase even in cases where the fetus is Rh negative, once the critical titer is reached, the presence of fetal anemia can be assessed with various methods such as the middle cerebral artery peak systolic velocity (MCA-PSV).

Mild hemolytic disease is most common, manifested by a positive DAT with minimal hemolysis, little or no anemia (cord blood hemoglobin greater than 14 g/dL), and minimal hyperbilirubinemia (cord blood bilirubin less than 4 mg/dL). Aside from early phototherapy, these newborns generally require no therapy unless the postnatal rate of rise in bilirubin is greater than expected. Infants who do not become sufficiently jaundiced to require exchange transfusion are at risk of development of severe late anemia associated with a low reticulocyte count, usually at 3 to 6 weeks of age; thus, it is important to closely monitor hemoglobin levels after hospital discharge.

Moderate hemolytic disease is found in a smaller proportion of affected infants. This form is characterized by hemolysis, moderate anemia (cord blood hemoglobin less than 14 g/dL), and increased cord blood bilirubin levels (greater than 4 mg/dL). The peripheral blood may reveal numerous nucleated RBCs, decreased numbers of platelets, and occasionally a leukemoid reaction with large numbers of immature granulocytes. Infants with Rh disease also may exhibit marked hepatosplenomegaly, a consequence of extramedullary hematopoiesis and sequestration of antibody-coated RBCs. The risk of development of bilirubin encephalopathy is high if these neonates do not receive treatment. Thus, early exchange transfusion with type O, Rh-, and S-negative fresh RBCs (less than 5 days old) is usually necessary, in conjunction with intensive phototherapy. This approach has been responsible for the favorable outcome for most infants with moderate alloimmune hemolysis. Exchange transfusion is a procedure with which fewer and fewer providers have familiarity given the successful implementation of anti-Rh(D) prophylaxis in RhD-negative women. Significant complications of an exchange include infectious, catheter-related, metabolic, cardiovascular, and hematologic.[82–84] It is common for newborns who receive exchange transfusion to demonstrate a lower-than-normal hemoglobin concentration at the nadir of their "physiologic" anemia. Therefore, follow-up evaluation of hemoglobin for at least 2 months is important. The decrease in hemoglobin may be due in part to persistence of some anti-D antibody and destruction of the patient's own Rh(D)-positive RBCs. Also, this low hemoglobin measurement may reflect the decreased oxygen affinity and enhanced oxygen delivery of adult RBCs used for the exchange process, thereby blunting the expected erythropoietic response to hypoxia. Some studies suggest that the administration of recombinant human erythropoietin (rHuEPO) may

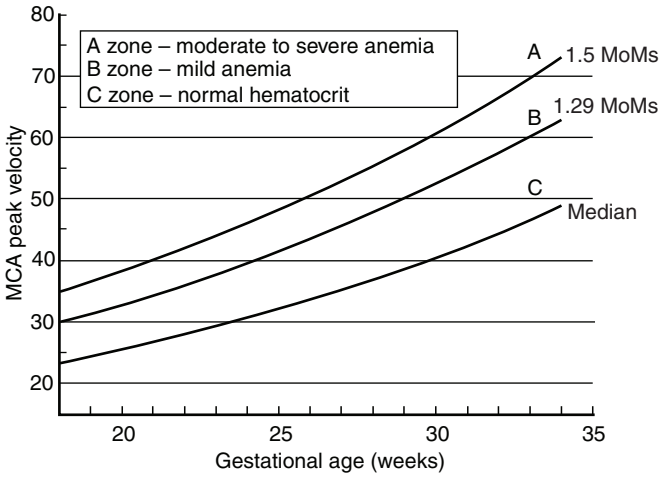
minimize this late anemia of Rh hemolytic disease,[85,86] although it is not always effective.[87]

Severe hemolytic disease is seen in approximately 25% of affected infants, who are either stillborn or hydropic at birth. Understanding of hydrops fetalis, originally attributed to high-output cardiac failure secondary to severe anemia, is incomplete. Two other consequences of anemia also may contribute to the edema of hydrops. One of these is low colloid osmotic pressure resulting from hypoalbuminemia, a consequence of hepatic dysfunction. The second is a capillary leak syndrome secondary to tissue hypoxia. Management of seriously affected fetuses is directed at the prevention of severe anemia and death. To accomplish this, it first is necessary to identify those fetuses at risk. An increase in the maternal anti-D titer in a previously sensitized Rh-negative woman is a good serologic measure of a fetus in potential jeopardy. Moreover, a previous history of neonatal hemolytic disease resulting from anti-D antibodies suggests that the current fetus also may be at risk. In this regard it may be useful to know the fetal Rh blood type because this identifies those Rh-negative infants who are not at risk. In many cases this can be accomplished by direct Rh typing of fetal RBCs obtained via cordocentesis. Alternatively, molecular biologic techniques can be used to determine the Rh genotype in DNA obtained from amniocytes, chorionic villus samples, or cell free fetal DNA in maternal circulation.[88–90] When the fetus is found to be Rh-negative, no further maternal monitoring or fetal blood studies are necessary.

An increase in the maternal titer of immunoglobulin G (IgG) anti-D indicates maternal sensitization but does not accurately predict the potential severity of fetal hemolysis. Previously, when the indirect antiglobulin test exceeded a critical threshold level (1:16 to 1:32), amniocentesis was done to perform spectrophotometric estimation of bile pigment in amniotic fluid as measured by the deviation in optical density (OD) at 450 nm. Plotting the "ΔOD450nm" against fetal age provided a good correlation with the severity of fetal hemolysis during the third trimester, and the trend of two or more values is a more reliable predictor of severity of fetal disease.[91]

Efforts have been made to use noninvasive detection of fetal anemia. Ultrasonography signs of hydrops fetalis represent a relatively late sign of fetal anemia, often not developing until Hgb values are more than 7 g/dL below gestational age norm. However, Doppler assessment of peak velocity in the fetal MCA, reflecting the lower viscosity associated with more severe anemia, has become the standard of care at most centers. MCA Doppler has been compared to the previous gold standard of the amniocentesis for "ΔOD450nm."[92] In one study, 45% of infants had severe anemia at cordocentesis, defined as a hemoglobin level at least five standard deviations below the mean for gestational age. The MCA Doppler was both more accurate than the ΔOD450nm (85% as compared with 76%) and more sensitive (88% as compared with 76%). It was suggested by the authors that more than 50% of invasive procedures might be avoided using this technique.

In severely affected fetuses, hydrops may occur as early as 20 to 22 weeks' gestation. Measurement of the peak MCA Doppler can begin as early as 18 weeks but more commonly is initiated at approximately 24 weeks, then repeated every 1 to 2 weeks, with adjustment in normal velocity with advancing gestation age to define the risk of severe anemia (Fig. 69.6). When the MCA Doppler exceeds 1.5 multiples of the median (A Zone) between 24 and 35 weeks' gestation, cordocentesis is required for direct determination of hematocrit as well as fetal blood type, DAT, reticulocyte count, and total bilirubin.[93] The rate of fetal trauma and morbidity associated with cordocentesis is less than



• **Fig. 69.6** Middle cerebral artery (MCA) Doppler graph based on gestational age. (From Moise KJ. Management of rhesus alloimmunization in pregnancy. *Obstet Gynecol*. 2008b;112:164–176.)

2%.[94,95] Cordocentesis should be performed with blood available for intravascular intrauterine transfusion if necessary. Such blood should be type O, RhD and S negative, cytomegalovirus negative, and less than 72 hours from collection; extended crossmatch is often performed with maternal blood type. Irradiation is mandatory, and many centers also perform leukoreduction. The transfusion is generally administered at approximately 20 mL/kg estimated fetal weight with a target of 40% to 50% hematocrit. Beyond 35 weeks, amniocentesis to determine both fetal lung maturity and the ΔOD450nm should be performed. Induction of delivery should be considered if the lungs are mature in order to decrease the risk of needing subsequent intrauterine transfusion with their attendant risks, or postnatal exchange transfusion for hyperbilirubinemia. For a full discussion and algorithm for management of pregnant patients with RhD alloimmunization, see Moise, 2008.[93]

Hyperbilirubinemia (with elevation of the conjugated fraction) often develops in newborns who have received intrauterine RBC transfusions and often predicts the need for exchange transfusions. This hyperbilirubinemia reflects the severity of hemolysis and its effects on the fetal liver. In some cases, anemia may be minimal or absent, and the DAT may be negative if the Rh-negative RBCs transfused prenatally still predominate. In such cases the infant may not require exchange transfusion. Neonatal exchange transfusion, amniocentesis, selective early induction of delivery, and intrauterine fetal blood transfusions all have contributed to the declining neonatal death rate from Rh incompatibility.

ABO Incompatibility

Hemolysis associated with ABO incompatibility is similar to Rh hemolytic disease in that maternal anti-A or anti-B antibodies enter the fetal circulation and react with A or B antigens on the erythrocyte surface (Table 69.4). In persons with type A and type B blood, naturally occurring anti-B and anti-A isoantibodies largely are IgM molecules that do not cross the placenta. In contrast, the alloantibodies present in persons with type O blood also include IgG antibodies that can traverse the placenta.[96] For this reason, ABO incompatibility is largely limited to type O mothers with type A or B fetuses. The presence of IgG anti-A or anti-B antibodies in type O mothers also explains why hemolysis caused

TABLE 69.4 Clinical and Laboratory Features of Immune Hemolysis Due to Rh Disease and ABO Incompatibility

	Rh Disease	ABO Incompatibility
Clinical Features		
Frequency	Unusual	Common
Pallor	Marked	Minimal
Jaundice	Marked	Minimal to moderate
Hydrops	Common	Rare
Hepatosplenomegaly	Marked	Minimal
Laboratory Features		
Blood type		
Mother	Rh(−)	O
Infant	Rh(+)	A or B
Anemia	Marked	Minimal
Direct antiglobulin test	Positive	Frequently negative
Indirect antiglobulin test	Positive	Usually positive
Hyperbilirubinemia	Marked	Variable
Red blood cell (RBC) morphology	Nucleated RBCs	Spherocytes

by ABO incompatibility frequently occurs during the first pregnancy without prior "sensitization." ABO incompatibility is present in approximately 12% of pregnancies, although evidence of fetal RBC sensitization (i.e., positive result on DAT) is found in only 3% of births, and less than 1% of live births are associated with significant hemolysis.[44,97] The relative mildness of neonatal ABO hemolytic disease contrasts sharply with the findings in Rh incompatibility. In large part, this is because A and B antigens are present in many tissues besides RBCs; consequently, only a small fraction of anti-A or anti-B antibody that crosses the placenta actually binds to erythrocytes, the remainder being absorbed by other tissues and soluble A and B substances in plasma.

Although hemolytic disease resulting from ABO incompatibility is milder than Rh disease, severe hemolysis occasionally occurs. In suspected cases of ABO incompatibility, it is essential to exclude other antibodies and other nonimmune causes of hemolysis such as glucose-6-phosphate dehydrogenase (G6PD) deficiency or hereditary spherocytosis. In most cases, pallor and jaundice are minimal (see Table 69.4). Hepatosplenomegaly is uncommon. Laboratory features include evidence of minimal to moderate hyperbilirubinemia and, occasionally, some degree of anemia. The DAT sometimes is negative, although the indirect antiglobulin test (neonatal serum plus adult group A or B RBCs) more commonly is positive. This paradox is related to the fact that fetal RBCs, compared with adult erythrocytes, have less type-specific antigen on their surface.[98] The peripheral blood smear is characterized by marked spherocytosis that is indistinguishable from that seen in hereditary spherocytosis (see Hereditary Spherocytosis, later).

Hemolysis in ABO incompatibility is usually mild, presenting with some degree of hyperbilirubinemia. Some infants with ABO incompatibility may be discharged home from medical establishments before clinical jaundice is evident. It is critical that infants with ABO incompatibility be monitored closely for evolving jaundice and hyperbilirubinemia in the first few days of life. In most cases, hyperbilirubinemia is readily controlled by phototherapy. In the minority of cases not controlled by phototherapy, exchange transfusion with group O, Rh-, S-compatible RBCs is utilized. Additional follow-up at 2 to 3 weeks of age to check for anemia in these infants is essential.

Minor Blood Group Incompatibility

With the sharp decline of hemolytic disease caused by Rh incompatibility, the proportion of cases caused by Rh c, Rh E, Kell, Duffy, and Kidd incompatibility has increased from the previous estimates of 1% to 3%, to as high as 20% for Kell sensitization.[99,100] The pathophysiology of these disorders is similar to that of Rh and ABO incompatibility. The infrequency of minor group incompatibility is primarily a reflection of the lower antigenicity of these RBC antigens. Diagnosis of minor group incompatibility is suggested by hemolytic anemia with a positive DAT in the absence of ABO or Rh incompatibility and with a negative maternal DAT. Definitive diagnosis requires identification of the specific antibody in neonatal serum or an eluate from neonatal RBCs. This is readily accomplished by testing maternal serum against a variety of known RBC antigens. With some antibodies such as Kell, antibody titer and amniocentesis findings may underestimate the severity of fetal hemolysis.

Immune Hemolytic Anemia Due to Maternal Disease

Maternal autoimmune hemolytic anemia or lupus erythematosus during pregnancy may be associated with passive transfer of IgG antibody to the fetus. The diagnosis is suggested by the presence of neonatal hemolytic disease, a positive DAT, absence of Rh or ABO incompatibility, and antiglobulin-positive hemolysis in the mother. Treatment with prednisone in the mother may reduce both maternal hemolysis and the risk of neonatal morbidity. As in other cases of neonatal hemolysis, treatment is focused on prevention of severe hyperbilirubinemia and kernicterus.

Nonimmune Acquired Hemolytic Disease

Infection

Cytomegalic inclusion disease, toxoplasmosis, syphilis, and bacterial sepsis all can be associated with hemolytic anemia. In most of these conditions, some degree of thrombocytopenia also exists. Generally, hepatosplenomegaly is present. In cases of bacterial sepsis, both the direct and indirect bilirubin levels may be elevated. The mechanism of hemolysis associated with infection is not clearly defined. Documentation of infection as the cause of hemolysis is made by the presence of other clinical and laboratory evidence of neonatal infections. Hemolysis caused by infections may present early in the neonatal period, or it can be delayed for several weeks.

Coronavirus-2019 (COVID-19) infection has been associated with anemia. The anemia seen with COVID-19 is multifactorial but in large part can be attributed to immune destruction. Autoimmune hemolytic anemia has been described in asymptomatic patients,[101] symptomatic patients with severe acute respiratory syndrome, as well as in children with multisystem inflammatory syndrome (MIS-C). Both warm[101] and cold[102] agglutinin disease

have been described.[103] Neonatal infection is thought to occur in one of three ways: transplacental hematogenous spread or viral particles in the amniotic fluid, intrapartum transmission after exposure to maternal infected fluids (secretions or feces), and postpartum transmission from an infected contact (any caregiver). When postpartum transmission from an infected mother is suspected, the mode is felt more likely to be respiratory secretions rather than breast milk[104,105] and various organizations including the American Academy of Pediatrics and World Health Organization have strongly supported and encouraged the protection of breastfeeding during the pandemic in various forums.[106] Anemia in the neonate infected with COVID-19 has not been widely described but one case report describes an 8-week-old who developed MIS-C with diarrhea and anemia after having been exposed to a positive family member at 2 weeks of age.[107] Other causes of anemia in neonates with COVID-19 may be more related to the conservative management employed by providers in the early stages of the pandemic such as mother-infant separation, early cord clamping, etc. that may have predisposed the infant to iron-deficiency anemia.

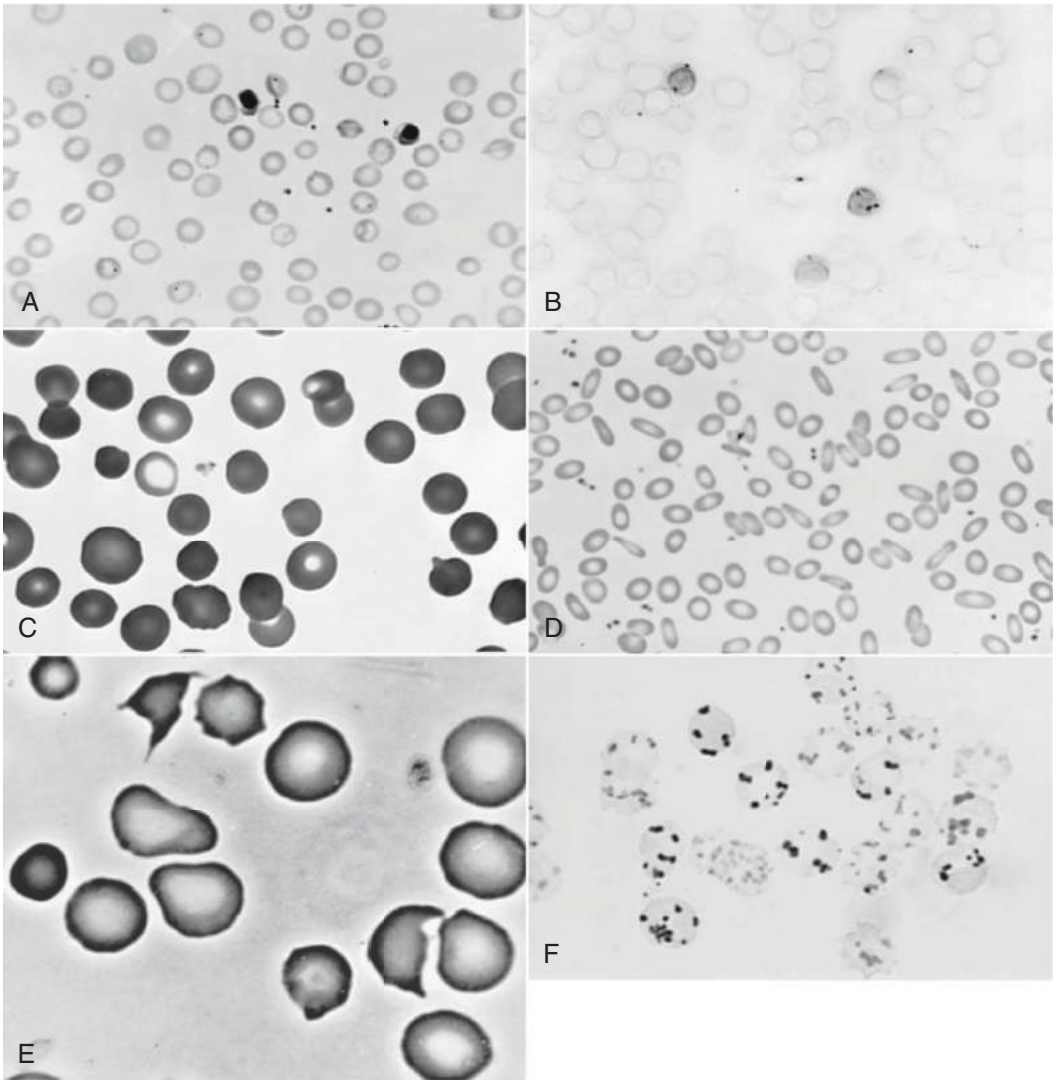
Schistocytic Anemias

Disseminated intravascular coagulation (DIC) is discussed elsewhere in this text. The hemolytic component of this disorder is secondary to the deposition of fibrin within the vascular walls. When erythrocytes interact with fibrin, fragments of RBCs are broken off, producing fragile, deformed RBCs, or schistocytes. Abnormalities of the placental microcirculation or macrovascular anomalies such as an umbilical vein varix are rare causes of congenital schistocytic anemia.[108]

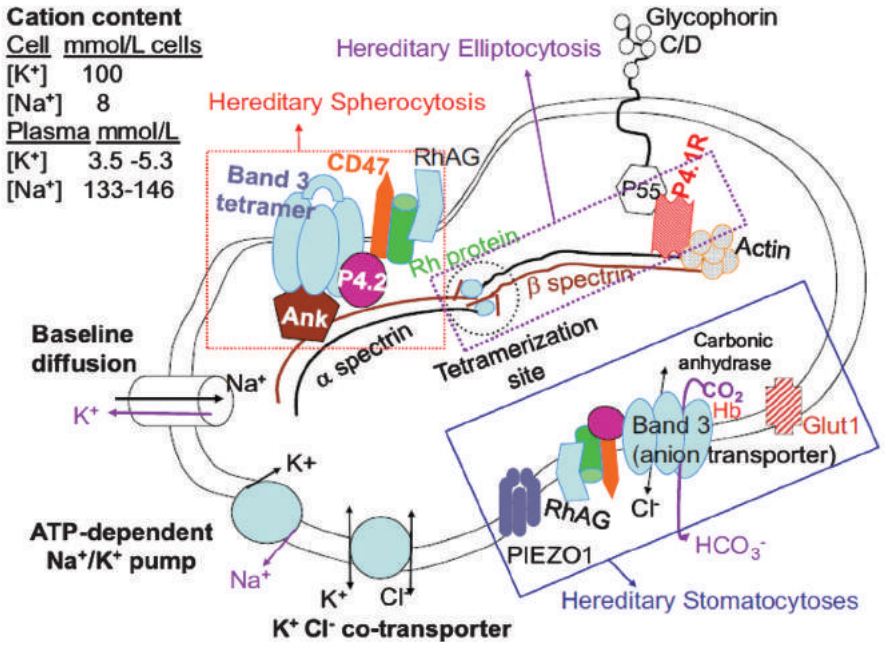
Hereditary Red Blood Cell Disorders

Membrane Defects

Common findings in RBC membrane disorders are the presence of dominant inheritance and abnormal RBC morphology (Fig. 69.7). The ultrastructure of the RBC is critical to its function and deformability and is dependent upon both structural support of the lipid bilayer as well as the maintenance of a cation gradient between the RBC and its environment which establishes the RBC



• **Fig. 69.7** (A) Hypochromic-microcytic red blood cells (RBCs) secondary to chronic fetal blood loss. (B) Fetal RBCs in the maternal blood after a fetomaternal hemorrhage (acid-elution technique). (C) Hereditary spherocytosis. (D) Hereditary elliptocytosis. (E) Glucose-6-phosphate dehydrogenase (G6PD)-deficient RBCs during acute hemolytic episode. (F) Heinz bodies from a patient with G6PD-deficient hemolysis (stained with supravital dye).



• **Fig. 69.8** Structural organization and functions of various red cell membrane proteins in health and disease. (From King MJ, Garcon L, Hoyer JD, et al. ICSH guidelines for the laboratory diagnosis of nonimmune hereditary red cell membrane disorders. *Int J Lab Hematol*. 2015;37[3]:304–325.)

surface-to-volume ratio (Fig. 69.8).[109] Disorders which alter the membrane structural organization such as hereditary spherocytosis, hereditary elliptocytosis, hereditary pyropoikilocytosis, and Southeast Asian ovalocytosis or the membrane transport function such as overhydrated hereditary stomatocytosis, dehydrated hereditary stomatocytosis or xerocytosis. familial pseudohyperkalemia, and cryohydrocytosis therefore result in deviation from the typical biconcave shape, and ultimately, to hemolysis. Aside from hereditary spherocytosis, these disorders are uncommon.

Hereditary Spherocytosis

The hallmark of hereditary spherocytosis (HS) is the presence of spherocytes in the peripheral blood smear (see Fig. 69.7). Causal mutations have been identified in six genes which encode the vertical components of the membrane cytoskeleton: spectrin, ankyrin, band 3, or band 4.2. A large majority of these mutations are autosomal dominant. The phenotype manifests when, because of either quantitative or qualitative defects in these proteins, the stability of the interactions between the cytoskeleton and the membrane lipid bilayer is weakened, promoting vesiculation and loss of bits of the bilayer, thus leading to progressive loss of membrane surface area.[110] As RBCs become more spherical, they lose flexibility and become vulnerable to entrapment in the spleen and attack by macrophages, leading to hemolysis. Removal of the spleen after 5 years of age allows spherocytes to have a near-normal life span despite their cytoskeletal defects and abnormal shape.

The clinical manifestations of HS in neonates range from the very rare presentation of hydrops fetalis with fetal death[111] to fully compensated, lifelong hemolysis marked by reticulocytosis but no anemia. Anemia at birth (Hgb < 15 g/dL) is present in 28% to 43% of infants.[112] Neonatal hemolysis or hyperbilirubinemia appears in approximately half of all affected infants.[113] It is often the exaggerated neonatal hyperbilirubinemia that raises the suspicion of HS. In many cases, other family members are affected in a pattern consistent with autosomal dominant inheritance. In 20% to 30% of cases, recessive inheritance is noted, but only homozygotes express the clinical features of spherocytosis. Occasionally, the appearance of a new case of spherocytosis in a previously unaffected family is due to spontaneous mutation.

The diagnosis of HS is suspected when spherocytes are seen on the blood smear of an infant with hyperbilirubinemia or other laboratory evidence of hemolysis. Spherocytes also are seen in ABO incompatibility. Blood typing and a DAT may support the diagnosis of ABO incompatibility, but the DAT occasionally can be negative and thus misleading. In this situation, evaluation of the family for other persons affected with spherocytosis may point to a hereditary rather than an acquired cause. Historically, it had been necessary to wait until the infant was at least 3 months of age to obtain a definitive laboratory diagnosis of HS, because, by this age, the confounding effects of maternal antibody and fetal RBCs are no longer present and for many years, the "gold standard" for testing had been the osmotic fragility test. More recently, flow cytometric analysis of eosin-5′-maleimide (EMA)-labeled RBCs has become the preferred diagnostic test. EMA binds various RBC membrane proteins, including band 3 and Rh-related proteins. The fluorescence levels in patients with hereditary spherocytosis is typically two thirds that found in normal controls.[114,115] For the neonate, the advantages of this test are its short turnaround time, the fact that it can be performed on a small amount of blood (a few microliters), ability to run the test even after a patient has received a blood transfusion,[116] and that results are reliable even within the newborn period as long as they are compared to samples from age-matched controls. The test has a high sensitivity and specificity at 96.4% and 94.2%, respectively[117] but false positives can occur in the presence of other inherited RBC disorders such as hereditary pyropoikilocytosis, Southeast Asian ovalocytosis, and congenital dyserythropoietic anemia type II as well as in cases of autoimmune hemolytic anemia.[117]

The mainstay of treatment of HS during the newborn period is directed toward management of hyperbilirubinemia, which often is present and may not peak until the infant is several

days of age. Occasionally, RBC transfusions are required in the neonatal period for management of symptomatic anemia.[111] A common occurrence is the appearance of a transient but severe anemia during the first 20 days of life due to underproduction of erythropoietin in the face of continuing hemolysis.[118] Careful monitoring of affected infants after discharge from the nursery is warranted. Splenectomy is the definitive treatment for HS, but this is not recommended before 5 years of age because of the increased risk of overwhelming sepsis with encapsulated organisms such as *Streptococcus pneumoniae*[119] that occurs following splenectomy in infants and young children. Guidelines regarding the indications for total or partial splenectomy have been reviewed.[120,121]

Hereditary Elliptocytosis

Hereditary elliptocytosis is an autosomal dominant, clinically heterogeneous group of disorders that are caused by mutations of the RBC membrane cytoskeletal proteins, usually spectrin or protein 4.1, that weaken skeletal protein interactions and increase RBC mechanical fragility.[110] Heterozygotes usually exhibit elliptocytes on the blood smear, but in most instances hemolysis is absent. Homozygotes or compound heterozygotes may have sufficient weakening of the cytoskeleton to cause significant hemolysis accompanied by striking abnormalities in RBC morphology such as homozygous hereditary elliptocytosis or hereditary pyropoikilocytosis.

Transient poikilocytosis and hemolysis may occur during the newborn period in infants destined ultimately to have asymptomatic elliptocytosis.[122] RBC membrane mechanical fragility is strikingly abnormal in these infants, probably as a consequence of the destabilizing influence of large amounts of free intraerythrocytic 2,3-DPG, a byproduct of the presence of fetal hemoglobin.[123] As fetal hemoglobin levels decline postnatally in affected infants, membrane mechanical fragility lessens, hemolysis disappears, and RBC morphology undergoes a transition from poikilocytosis to elliptocytosis. Without knowledge of the membrane protein mutations present in the infant and the infant's family, it is difficult to predict at birth who will have transient poikilocytosis with ultimate recovery and who is destined to have lifelong pyropoikilocytosis with hemolysis. A practical approach to this problem is to wait 4 to 6 months to see if common HE evolves or chronic hemolysis with abnormally shaped RBCs persists.

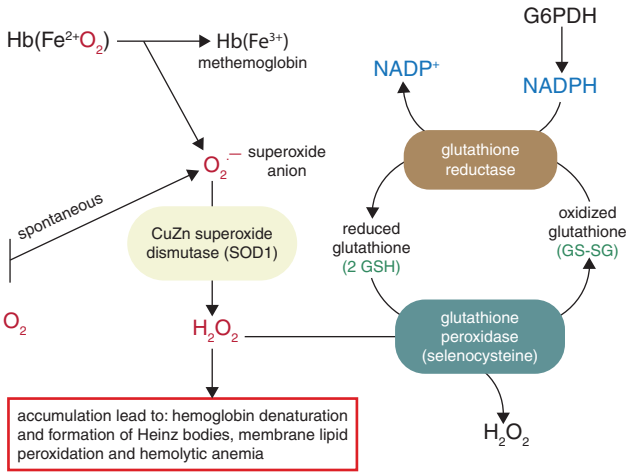
Red Blood Cell Enzyme Abnormalities

Hyperbilirubinemia, anemia, and hydrops fetalis can be the result of inherited RBC enzymopathies. The two most encountered red cell enzyme disorders are glucose-6-phosphate dehydrogenase (G6PD) deficiency and pyruvate kinase (PK) deficiency. Overviews of these two disorders, as well as other more esoteric enzyme deficiencies, are available elsewhere,[110,124] and the special features of RBC enzymopathies in the newborn period have also been summarized.[125]

Glucose-6-Phosphate Dehydrogenase Deficiency

G6PD deficiency is a sex-linked disorder that affects millions of people throughout the world, particularly in Mediterranean countries, the Middle East, Africa, and Asia. Like sickle cell trait and thalassemia, G6PD deficiency is thought to have become common because it provides a measure of protection against malaria.[126–128] In most G6PD-deficient persons, hemolysis and anemia are present only episodically. Precipitating factors can include infection, exposure to medications that are potent oxidants, or to other agents such as fava beans, naphthalene, or certain petrochemical-derived substances. Rarely, hemolytic anemia is chronic rather than episodic and is present even in the absence of obvious exposure to oxidant stress. Over 400 variants have been identified within *G6PD*, not all of which are clinically significant. The WHO has developed a classification system based on the degree of enzyme deficiency and the severity of hemolysis. Most mutations are missense point mutations that lead to altered enzyme function.[129,130] Normal RBCs contain abundant amounts of reduced glutathione (GSH), a sulfhydryl-containing tripeptide that serves as an intracellular antioxidant, neutralizing oxidant drug metabolites and activated oxygen species released during phagocyte activation. Because of the enzyme deficiency, G6PD-deficient RBCs have a limited capacity to regenerate GSH from oxidized glutathione (Fig. 69.9).[131] In the absence of GSH,[131] RBCs are vulnerable to oxidant injury of hemoglobin. Denatured globin precipitates termed *Heinz bodies* bind to the cell membrane, unfavorably altering its structure and function. Membrane lipid peroxidation may contribute to altered function. The ultimate result of these insults is hemolysis.

The variant G6PD A− is responsible for nearly all the G6PD deficiency seen in Africans and is present in approximately 10% of African Americans. G6PD A− was previously considered to be a single biochemical disorder but is now known to be due to three different genotypes and are classified as class III variants. G6PD A− affects the stability of the enzyme, causing an accelerated decline in activity during the life span of the RBC. Only in the oldest RBCs does enzyme activity reach low enough levels to create vulnerability to oxidant hemolysis. For this reason, hemolysis, if it occurs, is usually mild and self-limited. In contrast, in Asians and persons of Mediterranean or Middle Eastern descent, G6PD deficiency decreases the enzyme activity in young and old RBCs alike. Hemolysis is usually more severe and can be life threatening. G6PD variants found throughout the Mediterranean and Middle East, once thought to be biochemically different, are now recognized to be due to one common single genotype. In these ethnic groups, some G6PD-deficient persons are susceptible to severe and even fatal episodes of hemolysis following exposure to fava beans (favism). The pathophysiology of favism has been elucidated[132] and reviewed elsewhere.[133] Formation of reactive oxygen species results from high concentrations of divicine and isouramil in the fava bean. Favism is only very rarely seen in African Americans



• **Fig. 69.9** Pentose phosphate pathway and G6PD deficiency. (Used with permission of themedicalbiochemistrypage.org. From Harcke SJ, Rizzolo D, Harcke HT. G6PD deficiency: an update. *JAAPA*. 2019;32:21–26.)

with G6PD A−.[134] For pregnant and lactating women, some inciting agents can be transmitted to the fetus across the placenta or to the neonates via breast milk and cause hemolysis in an affected fetus or neonate.[135,136]

The gene for G6PD is located on the X chromosome. All of the RBCs of G6PD-deficient males are affected by the enzyme deficiency, whereas a variable fraction of RBCs of G6PD-deficient females is enzyme deficient, depending on the degree of lyonization.[137] Because of these differences, hemolysis due to G6PD deficiency occurs mainly in males and is much less common in females.

The diagnosis of G6PD deficiency is suggested by the appearance of a DAT-negative hemolytic anemia in association with infection or the administration of drugs. Cells that appear as if a bite had been taken from them as a result of splenic removal of Heinz bodies are occasionally seen on the peripheral blood smear. Supravital stains of the peripheral blood with crystal violet may reveal Heinz bodies during hemolytic episodes. Although screening tests are available, they are not as sensitive as direct assay of RBC G6PD activity. Measurement of enzyme activity may not reveal the deficiency in African Americans immediately after a hemolytic episode, because the population of enzyme-deficient RBCs has been eliminated. Repeating the assay later is often necessary. Identification of specific G6PD mutations by DNA analysis is available, but rarely used for diagnostic purposes.[129,130]

In the neonatal period the major manifestation of G6PD deficiency is hyperbilirubinemia. There usually is more jaundice than anemia, and the anemia is rarely severe. Also, in contrast to classic Rh-related neonatal hyperbilirubinemia, neonatal jaundice due to G6PD deficiency rarely is present at birth, with clinical onset usually occurring between days 2 and 3.[138] Most infants with hyperbilirubinemia due to G6PD deficiency are of Mediterranean, Middle Eastern, or Asian descent.

In most cases of neonatal hyperbilirubinemia due to G6PD deficiency, there is no obvious exposure to external oxidants. The degree of hyperbilirubinemia reflects both the increased bilirubin load presented to the liver by hemolysis of G6PD-deficient RBCs and the presence or absence of the variant form of UGT responsible for Gilbert syndrome.[139] The relative importance of the latter is underscored by the observations that most jaundiced G6PD-deficient neonates are not anemic and that often, evidence for increased bilirubin production secondary to hemolysis is lacking.[140,141]

The severity of jaundice in G6PD deficient varies widely, from being subclinical to causing kernicterus if not treated.[142] Data from the USA Kernicterus Registry from 1992 to 2004 indicate that more than 30% of kernicterus cases were associated with G6PD deficiency. These observations have raised the question of whether testing for G6PD deficiency should be included in newborn screening programs worldwide.[143] Neonatal screening for G6PD deficiency has been very effective in reducing the incidence of favism later in life in Sardinia[144] and in other regions where this potentially fatal complication is common. Currently, in the United States only the District of Columbia and Pennsylvania require newborn screening for G6PD deficiency.

Therapy for neonatal hemolysis and hyperbilirubinemia resulting from G6PD deficiency includes (1) phototherapy or exchange transfusion to prevent kernicterus, (2) RBC transfusion for symptomatic anemia, (3) removal of potential oxidants that may be contributing to hemolysis, and (4) treatment of infections using agents that do not themselves initiate hemolysis. In infants known to be G6PD deficient, prevention of severe hyperbilirubinemia by administration of a single intramuscular dose of tin-mesoporphyrin, an inhibitor of heme oxygenase, has been advocated.[145,146]

Pyruvate Kinase Deficiency

Pyruvate kinase (PK) deficiency is an autosomal recessive disorder that occurs in all ethnic groups. Although it is the most common of the Embden-Meyerhof glycolytic pathway defects, it is rare in comparison with G6PD deficiency. Hundreds of cases, mostly in northern Europeans, have been described in the literature, although many more unpublished cases also occur.[147] PK is one of the two enzymes that generate adenosine triphosphate (ATP) in RBCs. Because nonerythroid tissues have alternative means of generating ATP, clinical abnormalities in PK deficiency are limited to RBCs. The number of pathogenic variants is continuously growing and currently is well over 290.[148,149] Reflecting this genetic diversity, the hemolytic anemia that characterizes PK deficiency varies considerably in severity from family to family. Most patients are diagnosed within the first months of life with approximately 90% of PK-deficient babies experiencing hyperbilirubinemia and 46% requiring exchange transfusions.[150] Death or kernicterus may occur. Severe intrauterine anemia and hydrops fetalis have been reported.[147] In two cases, newborns with PK deficiency presented with neonatal cholestasis and developed progressive liver dysfunction leading to fatal hepatic failure.[151]

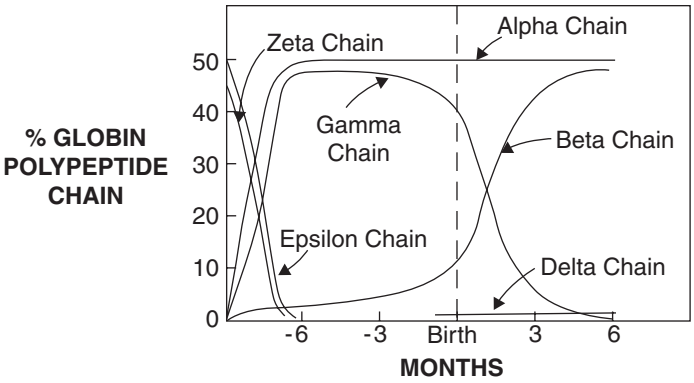
The diagnosis of PK deficiency should be considered in a jaundiced newborn with evidence of nonimmune hemolysis in the absence of infection or exposure to hemolytic agents. Hemoglobinopathies and membrane disorders should be ruled out by examination of the blood smear and other appropriate diagnostic tests before proceeding to assay of RBC PK activity. RBC morphology is normal in PK deficiency, although a few dense cells with irregular margins (echinocytes) are occasionally seen. PK heterozygotes are clinically and hematologically normal but usually have roughly half the normal amount of RBC PK activity. When infants have been transfused before confirmation of diagnosis, the determination of RBC PK activity in parents may detect the heterozygous state and help lead to the diagnosis.[152]

Treatment of hyperbilirubinemia by phototherapy and exchange transfusion if necessary is usually the only therapy necessary in the newborn period. RBC transfusions for anemia occasionally are required. Splenectomy may reduce the rate of hemolysis but should be avoided in infancy and early childhood because of the high risk of infection after splenectomy.

Treatment has been largely supportive with many patients requiring RBC transfusions and splenectomy. Data from a large cohort demonstrated that 84% of 250 patients had received at least one transfusion while roughly 50% (25 of 52) patients 5 years or younger received regular transfusions.[150] Transfusion dependence lessens and can even resolve with age. Complications are related to iron overload secondary to ineffective erythropoiesis and RBC transfusions as well as chronic hemolysis.

To date 18 patients have been reported in the literature to have received hematopoietic stem cell transplantation (HSCT), the indications protocols and outcomes for 16 of whom are described by van Straaten et al.[153]

AG-348 is an oral allosteric activator of PKR which acts by increasing PK activity and ATP levels. Phase II studies have demonstrated a hemoglobin response (rise in hemoglobin from baseline of greater than 1 g/dL) in a large majority of the patients with PK deficiency.[154] Further investigation has shown that clinical response is, at least to some degree, genotype dependent.[155] A lentiviral vector for delivery of a wild type *PKR* has demonstrated



• **Fig. 69.10** Fetal and neonatal hemoglobin production.

successful correction of the disease phenotype in a murine model,[156] and phase II gene therapy study is underway.[157]

Hemoglobin Disorders

Beyond infancy the predominant hemoglobin tetramer is hemoglobin A (Hb A), composed of two alpha globin chains and two beta globin chains (α2β2). To appreciate the hemoglobinopathies that occur in newborns, however, it is necessary to understand the normal developmental changes that occur in globin synthesis during fetal and neonatal life (Fig. 69.10). Embryonic hemoglobins are composed of zeta (alpha-like) and epsilon (beta-like) chains. Gower1 ($\zeta_2\varepsilon_2$), Gower2 ($\alpha_2\varepsilon_2$), and Portland ($\zeta_2\gamma_2$) comprise 50%, 25%, and 25% of embryonic hemoglobin, respectively (Table 69.5). Under normal circumstances, the transitions from zeta to alpha globin chains and epsilon to gamma globin chains are complete by the end of the first trimester. Epsilon chain expression wanes and is replaced by gamma chain expression, allowing fetal hemoglobin, Hb F (α2γ2), to become the predominant hemoglobin in the fetus by 8 weeks of gestation. Hb F percent peaks at 90% by 28 weeks' gestation, after which point it slowly trends downward as beta globin expression is induced. At birth, roughly 75% of the hemoglobin is Hb F. As RBCs made before birth are replaced postnatally, the percentage of Hb F declines rapidly such that by 6 months of age it has typically fallen to 1% to 5% (Fig. 69.11).

Only trace amounts of the minor adult hemoglobin, hemoglobin A2 (α2δ2), and Hb Barts (γ4) are present in cord blood. Hb Barts quickly disappears, whereas the hemoglobin A2 level increases gradually to the adult level of 2% to 3% by 1 year of age.

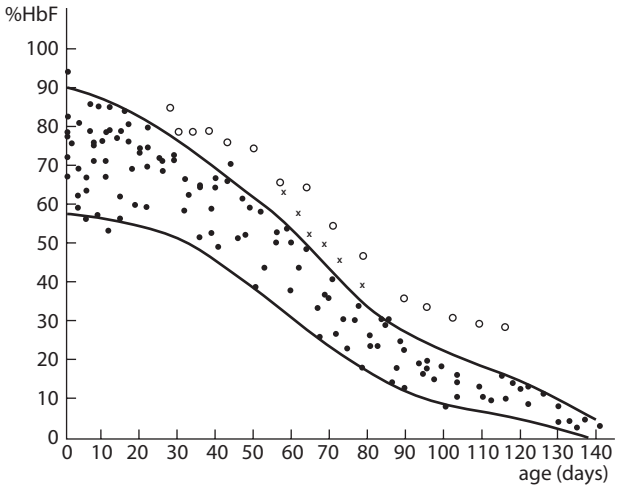
Beta globin disorders such as sickle cell disease or beta thalassemia major are not clinically apparent until several months of age, when the switch from Hb F to Hb A synthesis reveals the defect. In contrast, gamma globin mutations are most evident in fetal and neonatal life and then disappear by approximately 3 months of age as gamma globin synthesis wanes. Alpha globin disorders are evident at all stages of development from fetal to adult.

Thalassemia Syndromes

Thalassemias are due to absent or deficient synthesis of one or more of the normal globin chains, leading to a relative excess of the complementary or partner chain. For example, alpha thalassemias are due to diminished synthesis of alpha globin chains, leading to an excess of beta chains (or, in the fetus, of gamma chains). The excess beta chains form tetramers (β4 or hemoglobin H) that are unstable and can lead to hemolysis beyond infancy. The excess gamma chains also form tetramers, Hb Barts (γ4), that have

TABLE 69.5 Hemoglobin Composition of Cord Blood

	Hemoglobin	Globin Polypeptides	% in Cord Blood
Embryonic	Gower-1	Zeta-2, Epsilon-2 ($\zeta_2\varepsilon_2$)	0
	Gower-2	Alpha-2, Epsilon-2 ($\alpha_2\varepsilon_2$)	0
	Portland	Zeta-2, Gamma-2 ($\zeta_2\gamma_2$)	0
Fetal	Barts	Gamma-4 (γ_4)	<1%
	Hgb F	Alpha-2, Gamma-2 ($\alpha_2\gamma_2$)	60%–85%
Adult	Hgb A	Alpha-2, Beta-2 ($\alpha_2\beta_2$)	15%–40%
	Hgb A_2	Alpha-2, Delta-2 ($\alpha_{2\delta2}$)	<1%



• **Fig. 69.11** Decreasing concentration of fetal hemoglobin after birth. (From Garby L, Sjöhn S, Vuille JC. Studies of erythro-kinetics in infancy. II. The relative rates of synthesis of haemoglobin F and haemoglobin A during the first months of life. *Acta Paediatr*. 1962;51:245–254.)

an increased affinity for oxygen but do not cause hemolysis. In beta thalassemia, excess alpha globin chains accumulate, forming aggregates that injure the cell membrane, leading to hemolysis. In addition, the decrease in overall production of hemoglobin produces small RBCs (microcytosis) that are often filled with less than the normal amount of hemoglobin (hypochromia).

In the United States, changing immigration patterns have markedly increased the numbers of infants born with significant hemoglobinopathies. In California, analysis of newborn screening results between 1998 and 2006 demonstrated clinically significant hemoglobin genotypes in 0.05% of newborns; of these, the prevalence of alpha and beta thalassemia syndromes combined (28%) was almost as high as the prevalence of sickle cell disease (32%).[158]

Alpha Thalassemia

Alpha thalassemia is of particular importance to neonatologists because its clinical manifestations are present in utero and at birth. The molecular basis for alpha thalassemia is usually deletion of one or more of the four alpha globin genes as opposed to point mutations which are more commonly seen in the pathologic variants in beta thalassemia. A thalassemic hemoglobinopathy involving the abnormal hemoglobin Constant Spring also may behave functionally as a mild form of alpha thalassemia. Clinical severity is dictated by how many alpha globin genes are absent or nonfunctional. An infant can inherit no, one, or two variants or deletions from each parent, giving rise to the following four clinical syndromes:

1. *Silent carrier state*. Deletion or nonfunction of a single alpha globin gene is not accompanied by any clinical or hematologic abnormalities.
2. *Alpha thalassemia trait*. Deletion or nonfunction of two alpha globin genes, in *cis* (more common in people of Asian descent) or *trans* (more common in people of African descent), is associated with mild microcytic anemia, without hemolysis or reticulocytosis.
3. *Hemoglobin H disease*. When three of four alpha globin genes are deleted or nonfunctional, a mild to moderate hemolytic anemia is found, often aggravated by oxidant stresses just as in G6PD deficiency. The RBCs are hypochromic and microcytic and contain inclusions of hemoglobin H when appropriate staining is performed. Hemoglobin H Constant Spring (two deletions in cis in combination with the non-deletional variant, Constant Spring, on the other chromosome) can be a particularly severe syndrome, with up to one third requiring regular transfusions.[159,160]
4. *Hemoglobin Barts Hydrops Fetalis (alpha thalassemia major)*. Lack of all four alpha globin genes is associated with a severe intrauterine hemolytic anemia and hydrops fetalis, with massive hepatosplenomegaly, and, in most instances, fetal demise. The RBCs are very hypochromic, fragmented, and bizarre in shape. Erythroblastosis is present.[161] Timing of fetal loss depends upon the size of the causative deletions. For the larger deletions (i.e., FIL and THAI), not only are the alpha genes deleted, but so too are the embryonic, alpha-like genes which encode zeta globin. When this is the case, the pregnancy is typically lost around 6 to 8 weeks' gestation as there are no alpha-like globin chains with which the epsilon or gamma chains can bind to form functional tetramers, whereas when the deletion leaves the zeta globin gene intact (i.e., SEA), a low level of zeta globin gene expression continues in definitive erythroid cells which can combine with gamma chains and produce small amounts of Hb Portland ($\zeta_2\gamma_2$). Unlike the Hb Barts ($\gamma 4$), Hb Portland is a functional form of hemoglobin that allows the fetus to survive up to the second or third trimester. Untreated, however, all affected individuals die at these stages of development.

No treatment is needed for the silent carrier state or for alpha thalassemia trait, but studies to determine the thalassemia status of other family members, particularly those in their reproductive years, are recommended so that genetic counseling and prenatal diagnosis if indicated can be provided. Parents of infants who have hemoglobin H disease should be instructed to avoid oxidant agents that can cause hemolysis (the same list that is given to patients with G6PD deficiency). Although these infants are usually only mildly anemic, they may experience severe episodes of hemolysis during infections or with exposure to oxidant agents.

The diagnosis of the alpha thalassemia syndromes is easily made during the newborn period by correlation of the clinical and hematologic appearance of the child with the amount of Hb Barts (γ_4) present in the RBCs (Table 69.6).[162] Screening of all newborns for hemoglobin H disease is justified in populations with a substantial number of at-risk pregnancies.[158,162]

The large amount of Hb Barts found in the RBCs of patients with Hb H or alpha thalassemia major contributes to the clinical severity of the syndrome because the increased oxygen affinity of this hemoglobin impairs oxygen release to the tissues. DNA-based diagnostic tests are available for prenatal diagnosis which is often carried out when a pregnancy at risk for a fetus with alpha thalassemia major is identified.[161] DNA samples for fetal testing historically have been obtained via invasive methods such as chorionic villus sampling (between 10 and 12-weeks' gestation) or amniocentesis (after 15 weeks' gestation). There exists proof of concept and principal for noninvasive methods involving cell free fetal DNA circulating in maternal plasma, but validation of this testing is ongoing.[163]

Aside from the less invasive nature of such testing, with the technology and methodology currently in use, a diagnosis made from maternal plasma can be made earlier in gestation compared to traditional invasive methods. Earlier diagnosis creates a new, earlier therapeutic window into which various novel therapies may be implemented. Over the last decade, much attention has been given to the concept of in utero HSCT for hemoglobinopathies. Utilizing maternal cells, the goal of infusing haploidentical stem cells is to induce fetal tolerance, not necessarily to achieve donor engraftment or cure. The thought is that curative transplant could then be pursued postnatally without the need for myeloablative conditioning. This elegant concept has been successfully achieved in animal models and clinical trials in humans are underway.[164,165]

In the meantime, for families who chose to continue a pregnancy with a fetus diagnosed with alpha thalassemia, intrauterine RBC transfusions may be administered at a regular interval in order to support the growing and developing fetus. Transfusions must then be continued postnatally. The long-term outcome of these survivors has been a topic of much debate. Some reports depict favorable neurocognitive outcomes[166] while others raise concern about growth retardation and neurodevelopmental delays.[167] The largest study to date includes 69 individuals with alpha thalassemia who survived until birth either naturally ($n = 28$) or with intrauterine interventions such as straight transfusions, exchange transfusions, or hematopoietic stem cell infusions ($n = 41$). Thirty-nine of the 69 were over 5 years of age at the time of data collection. The authors concluded that although intrauterine interventions had some positive impact during the neonatal period such as prolonged gestation, improved Apgar scores, and

TABLE 69.6 Alpha Thalassemia Syndromes

		Anemia	Hemolysis	α:β Chain Synthesis	Abnormal Hemoglobins	
					Cord Blood	Adult Blood
Normal	α/α α/α	None	None	0.95–1.10	0%–1% γ_4	—
Silent carrier	α/— α/α	None	None	0.85–0.95	1%–2% γ_4	—
Alpha-thalassemia trait	—/— α/α	Mild hypochromic Microcytic	None	0.72–0.82	5%–6% γ_4	—
Hemoglobin "H" disease	—/— α/—	Moderate hypochromic Microcytic	Moderate	0.30–0.52	20%–40% $\gamma_4$0%–5% β_4	20%–40% β_4
Homozygous alpha-thalassemia ("hydrops")	—/— —/—	Severe hypochromic Microcytic	Severe	0	70%–80% $\gamma_4$15%–20% $\beta_4$0%–10% $\zeta_2\gamma$	

shortened duration of neonatal mechanical ventilation, the risks for later adverse outcomes such as growth retardation and neurodevelopmental delays were not entirely obviated.

Beta Thalassemia

Like alpha thalassemia, beta thalassemia is found in regions of the world where malaria was formerly endemic: Southeast Asia, India, Africa, and the Mediterranean basin. Although deletions of the beta globin locus occasionally cause beta thalassemia, most cases are caused by point mutations that affect transcription, messenger RNA (mRNA) processing, or translation.[168–170] Two general types of beta thalassemia are recognized. In beta0 thalassemia, no beta globin is produced by the thalassemic locus, whereas in beta+ thalassemia, there is reduced but measurable output of beta globin. The severity of homozygous beta thalassemia (formerly beta thalassemia major) is greatest when two beta0 thalassemia genes are inherited; clinical disease usually is much milder when two beta+ thalassemia genes are inherited. By contrast, the inheritance of one thalassemia gene (beta thalassemia trait) is characterized by a mild microcytic anemia that needs to be distinguished from alpha thalassemia and iron deficiency. Severe beta thalassemia is associated with lifelong hemolytic anemia, dependence on regular RBC transfusions for survival, and the gradual development of transfusion-associated hemosiderosis.[170] Survival has improved, however, with improvements in iron chelation and with the use of HSCT for patients with available matched donors.[171,172]

The clinical abnormalities of beta thalassemia are not evident at birth and first manifest only after 3 months of age, when beta globin normally becomes the dominant form of non-alpha globin synthesized. Although affected newborns appear clinically normal, the diagnosis of beta0 thalassemia can be made at birth by detecting a complete absence of Hb A, using hemoglobin electrophoresis or similar techniques. Definitive diagnosis of beta+ thalassemia by these techniques, however, is not possible in the newborn period, because the reduced amount of Hb A produced overlaps the range for normal babies. Direct identification of beta thalassemia mutations by DNA diagnostic techniques is increasingly available and allows the identification at birth of all infants with more severe beta thalassemias. These techniques, however, are more commonly used for prenatal diagnosis of beta thalassemia syndromes. DNA may be obtained using the same methods as for alpha thalassemia, allowing families to make informed decisions regarding termination of pregnancy.[173] The implementation of a strategy of carrier detection, genetic counseling, and prenatal diagnosis in countries where beta thalassemia is common has led to a striking reduction in the number of births of infants with beta thalassemia major.[174] More recent advances have included preimplantation genetic diagnosis[175] and noninvasive prenatal testing.[163]

Hemoglobin E/Beta Thalassemia

Hb E is a structurally abnormal hemoglobin that results from an amino acid substitution (lysine for glutamine) at the number 26 amino acid of beta globin, counting from the N terminus. Because this mutation also adversely affects mRNA processing, there is reduced output of beta globin mRNA. Hb E trait is therefore an example of a thalassemic hemoglobinopathy. Hb E carriers are microcytic but not anemic. Even those who are homozygous for Hb E have little or no anemia. However, coinheritance of Hb E trait and beta0 thalassemia trait can give rise to a transfusion-dependent form of beta thalassemia.[176] As with other types of transfusion-dependent beta thalassemia, clinical abnormalities are not seen until the infant is 3 to 6 months of age. However, the presence of Hb E is easily detected at birth by hemoglobin electrophoresis or related techniques. Infants found to have Hb E need careful follow-up evaluation to exclude the possibility of Hb E/beta thalassemia. DNA-based detection of the Hb E mutation is feasible[177] and has been applied to both prenatal and neonatal diagnosis. Infants born to mothers with Hb E/beta thalassemia have a higher risk of preterm birth, low birthweight, and fetal growth restriction.[178]

Other Variants Within the Hemoglobin Beta Gene Cluster

Many other variants within the hemoglobin beta (HBB) gene cluster exist and include large deletions as well as gene rearrangements

which result in fusion proteins. These variants result in phenotypes which are broadly and commonly referred to as deletional and non-deletional hereditary persistence of fetal hemoglobin (HPFH), delta-beta thalassemia, gamma-delta-beta thalassemia, or fusion proteins (i.e., hemoglobin Lepore). The clinical phenotype of heterozygous individuals is typically quite mild, appearing similar to a beta thalassemia trait. However, the picture becomes quite complicated with potentially severe morbidity for those who are homozygous or compound heterozygous, depending on the genes affected and oftentimes the amount of Hb F. The variability in clinical and laboratory findings of hemoglobin fractions and RBC indices depend not only on the thalassemic genotype but also on quantitative trait loci, various epigenetic factors (regulatory regions, enhancers, etc.), and possible co-inheritance of alpha thalassemia. These complexities emphasize the need for molecular characterization of the underlying defect(s) in those patients for whom a more obvious diagnosis does not seem to fit.

Sickle Cell Disease

The sickling hemoglobinopathies are beta globin mutations that, as with beta thalassemia, do not become clinically evident until the infant reaches several months of age. Sickle cell anemia, the most severe of the sickle cell diseases (SCDs), is the result of inheritance of two betaS mutations (substitution of valine for glutamic acid at the sixth amino acid on the beta globin chain), one from each parent. Sickle/beta0 thalassemia, phenotypically identical to sickle cell anemia, is caused by inheritance of one betaS and one beta thalassemia mutation. A third common form of sickle cell disease, hemoglobin S-C disease, is somewhat milder than sickle cell anemia or sickle-beta0 thalassemia. It is the consequence of inheritance of one betaS mutation and one betaC mutation (the substitution of lysine for glutamic acid at the sixth amino acid on the beta globin chain). Although no clinical abnormalities are present at birth, early diagnosis is important, because two potentially fatal but largely preventable complications may occur during the first year of life. The first is the splenic sequestration crisis, an unpredictable pooling of large numbers of RBCs in the spleen, which leads to a rapid decrease in hematocrit and, in the most severe cases, cardiovascular collapse and death. The second is overwhelming septicemia, usually caused by *S. pneumoniae*. The unusually high susceptibility to infection with encapsulated organisms such as *S. pneumoniae* is the consequence of functional asplenia, which commonly appears by 1 year of age in infants with sickle cell anemia or sickle-beta0 thalassemia and later in persons with hemoglobin S-C disease. Prompt treatment of splenic sequestration with RBC transfusions is lifesaving, so parents are taught to recognize early manifestations such as splenic enlargement, lethargy, and pallor. Overwhelming sepsis can be prevented in most instances by early immunization with *Haemophilus influenzae* and conjugated pneumococcal vaccines, and by institution of daily prophylactic penicillin at a dose of 125 mg twice daily,[179] beginning at 2 months of age. The need to institute these prophylactic measures within the first 1 to 2 months of life provided compelling rationale for neonatal screening and diagnosis of the sickling disorders. Data regarding the impact of these interventions have confirmed a 68% reduction in mortality from sickle cell disease for children ages zero to three, between 1983 and 1986 and between 1999 and 2002.[180] With this, the National Heart, Lung, and Blood Institute within the NIH formally recommended universal newborn screening for SCD in 1987. In 2006, with the publication of the Recommended Uniform Screening Panel (RUSP), New Hampshire became the last of the 50 states to adopt newborn sickle cell screening. An excellent overview of issues related to newborn screening for sickle cell disease has been published by Wethers et al.[181] The importance of early meetings with an experienced SCD clinician cannot be overstated and should occur between 6 and 8 weeks of life. The goal of these meetings should be to provide care givers vital information regarding management of vaso-occlusive episodes (VOEs), fever plan, spleen palpation, penicillin prophylaxis, immunization, results of blood counts, genetic counselling, and possible complications of the disease.

For infants who have inherited SCD, the reported age of first VOE is quite varied and is thought to depend on factors such as genotype, coinheritance of alpha thalassemia or other RBC abnormalities such as G6PD, environmental (altitude, nutrition, etc.), and provider awareness. In one large, multinational cross-sectional retrospective questionnaire, over 50% of patients had their first VOE before the age of 5.[182] Hydroxyurea remains one of the only disease-modifying agents for patients with SCD and was approved by the FDA for use in this patient population in 1998. It has been shown to have significant effect on rates of VOE, acute chest syndrome, stroke, transfusion needs, and overall survival, among others. Since 2014, NHLBI has recommended it for children down to 9 months of age, rather than waiting to start therapy until symptoms develop. The safety and efficacy of this approach was provided in large part by the BABY HUG study.[183] More recently studies, looking at personalized, PK-based dosing, suggest extending the age of initiation down to 6 months.[184,185]

Infants without a hemoglobinopathy born to mothers with SCD present more of a clinical problem during gestation and the neonatal period than is the case with infants who have SCD. In a large systematic review and meta-analysis of observational studies, looking at greater than 26,000 pregnancies in women with SCD, the odds ratios for stillbirth, preterm delivery, and small for gestational age were 4.0, 2.2, and 4.0, respectively, for women with hemoglobin SS disease.[186] These problems may in part be attributed to the increased metabolic demands and physiologic hypercoagulability of pregnancy which, in the setting of SCD, result in abnormalities of the placenta such as small size, infarction, and an increased incidence of placenta previa and abruptio placentae. These problems are not caused by the presence of the sickle trait, beta thalassemia trait, or hemoglobin C trait in the infant, because no hematologic disease is associated with the carrier state for these mutations, even in adult life when they are fully expressed, except under conditions of extreme hypoxia. One caveat regarding sickle trait blood is that blood from an adult donor who has sickle trait should not be used for exchange transfusions in the newborn, particularly if hypoxemia is present, because use of sickle trait RBCs in this setting may contribute to a fatal outcome.[187]

Hypoplastic Anemia

Hypoplastic anemias in the neonatal period can be caused by inherited disorders, infections, and drugs. This section will focus primarily on the hereditary causes which are likely to present within this timeframe. As bone marrow failure syndromes are rare, a high index of suspicion is oftentimes needed for the correct diagnosis to be made. Critical to the workup is an assessment of any congenital anomalies (skeletal, renal, cardiac, craniofacial, etc.), obtaining a thorough family history of any cancers, hearing disorders, renal dysfunction, etc., and various high yield labs (reticulocyte count, CBCd, eADA, sometimes Hgb F).

Diamond-Blackfan Anemia

The two major causes of RBC aplasia in children are Diamond-Blackfan anemia (DBA) and transient erythroblastopenia of childhood (TEC). The latter condition, TEC, is a disease that rarely occurs before 6 months of age,[188,189] and most children with this disorder are older infants or young children. In contrast, many infants with DBA are anemic at birth or become so in the first months of life with the median age of diagnosis being 2 months.

Originally termed congenital hypoplastic anemia, DBA is a red cell aplasia characterized by the absence of recognizable erythroid precursor cells in the bone marrow.[190] It is a disorder of ribosome biogenesis[191] that results in profound erythroid hypoplasia because erythroid progenitors and precursors are highly sensitive to apoptotic cell death. Although many cases are sporadic, familial, autosomal-dominant DBA is estimated to account for up to 45% of cases.[192] Over 220 distinct, pathologic mutations have been described, most of which occur within the genes encoding one of 19 (of the 79 total) ribosomal proteins.[193] *RPS19* and *RPL5* are the most commonly affected genes, variants within which account for roughly 25% and 9% to 21% of cases, respectively.[193] Recently, causative mutations have also been found in *GATA1*, *TSR2*, *ADA2*, and *EPO*.[194] Accumulation of p53 and consequent cell cycle arrest has been shown to be erythroid lineage-specific, sparing the myeloid and megakaryocyte lineage and accounting for the phenotype of DBA.[195,196] Apoptosis follows cell cycle arrest and ultimately leads to anemia. Some of the ribosomal proteins have extra-ribosomal functions which are thought to contribute to the various other manifestations of DBA including poor growth and congenital malformations.

Anemia in DBA is lifelong, but the onset of the disease is variable. Penetrance is incomplete and expressivity is widely variable, even for members of the same family. Many affected infants are severely anemic in the newborn period, and pallor at birth or soon thereafter has been a feature of the disease in most cases. Pre- and postnatal growth failure, thumb abnormalities, or other congenital anomalies including craniofacial, ophthalmologic, as well as structural renal and heart defects are seen in half of patients,[197] which can be helpful when considering the differential in a neonate with hypoplastic anemia. The diagnosis of DBA is suggested by anemia and reticulocytopenia appearing in the first 6 months of life. Certain unusual features of the RBCs such as macrocytosis, elevated fetal hemoglobin, and increased erythrocyte adenosine deaminase activity (eADA) may assist in diagnosis but may be difficult to interpret in the newborn period and can be confounded by RBC transfusion. eADA is elevated in 80% of patients with DBA and has high sensitivity and specificity of 84% and 95%, respectively.[198] Other disorders which can also be associated with elevated eADA include cartilage-hair hypoplasia, myelodysplastic syndromes, paroxysmal nocturnal hemoglobinuria, and acquired immunodeficiency syndrome. The role for genetic testing is increasing, with various next-generation sequencing panels identifying upwards of 70% of cases.[199]

Many patients achieve durable remissions from anemia when treated with corticosteroids. Those who do not respond to corticosteroids require chronic RBC transfusions and are at risk of transfusion hemosiderosis. Transfusion-dependent DBA can be cured by allogeneic bone marrow transplantation from an HLA-matched donor with overall survival as high as 91% in patients less than 10 years of age.[200] The risk of malignancies in patients with DBA is increased, with a cumulative incidence of 13.7% by 45 years of age and a median age of presentation of 35 years (11 to 70).[197] There is an overrepresentation specifically of myelodysplastic syndrome, acute myeloid leukemia, colon carcinoma, and osteogenic sarcoma.

Deficiency of Adenosine Deaminase 2

First described in 2014,[201,202] deficiency of adenosine deaminase 2 (DADA2) is an autoinflammatory disorder with a highly variable presentation, classically including early onset vasculitis, mild immunodeficiency, marrow failure, and systemic inflammation. It is an autosomal recessive, monogenic disease involving *ADA2* (previously *CECR1*). Given the heterogeneity and rarity of the disease, the differential often includes autoimmune lymphoproliferative syndrome (ALPs), combined variable immunodeficiency (CVID), Castleman syndrome, DBA, and polyarteritis nodosa (PAN), among others. 24% of patients are diagnosed before the age of one and 77% before the age of 10.[203] Patients as young as 2 months have been reported.[204] Hematologically, one review described 4% of patients presenting with a pure red cell aplasia while other hematologic abnormalities include anemia (13%), thrombocytopenia (6%), lymphopenia (16%), neutropenia (7%), and pancytopenia (4%). Diagnosis requires a high degree of suspicion, and patients who present with red cell aplasia may have protracted diagnostic journeys after failing initial DBA therapies.[205]

Historically, immunosuppressive agents were considered first-line therapy in DADA2, especially for patients presenting with PAN and recurrent strokes. Flares, however, were frequently encountered when tapers were attempted. Azathioprine, cyclosporine, tacrolimus, cyclophosphamide, methotrexate, and various others have also been utilized in this patient population without much success.[203] Currently, the mainstay of treatment is anti-tumor necrosis factor agents (etanercept, infliximab, adalimumab). Now with over 3000 cases reported to date,[204] patients have seemed to fall into one of three groups defined by their predominant clinical manifestation—CVID, early-onset stroke, or bone marrow failure. These groupings have seemed to predict responsiveness to various therapies. Specifically, patients with red cell aplasia and marrow failure have proven to be more refractory to TNF inhibitors.[206] Therefore, treatment for this subgroup more often involves hematopoietic stem cell transplantation with 14 patients having been cured (median follow-up of 18 months) to date.[203] In patients with documented hypogammaglobulinemia and a clinical immunodeficiency, immunoglobulin substitution and the use of prophylactic antibiotic and antivirals are standard of care antibiotic, and antiviral treatments have been used routinely.[203]

Pearson Marrow-Pancreas Syndrome

Pearson syndrome (Pearson marrow-pancreas syndrome) is a rare multisystem mitochondrial disorder characterized by a transfusion-dependent anemia with variable other cytopenias, endo- and exocrine dysfunction of the pancreas, and proximal tubular insufficiency, among others. Patients present in infancy but can also present at birth.[207,208] The phenotype evolves from one in which the hematologic manifestations dominate to one of muscle dysfunction and severe neurological symptoms. Anemia, however, was present in all patients in one report,[209] with the median age of diagnosis of 5 months. The cytopenias do not tend to respond to growth factors but spontaneously resolve over time if the patient survives. Most patients die in infancy,

however, secondary to metabolic derangements or infections. Allogeneic HSCT has been utilized in this patient population with mixed outcomes.[208,210–212]

The classic bone marrow findings include vacuolization in myeloid and erythroid progenitors, ringed sideroblasts, and global hypocellularity. An unremarkable bone marrow exam, specifically within the first month of life, however, does not rule out the diagnosis,[207] underscoring the importance of mtDNA analysis as the phenotype may vary and correlates with mtDNA gene mutation load.[213,214]

Congenital Dyserythropoietic Anemia

There are four main types of congenital dyserythropoietic anemia (CDA) with type I being the most likely one to present in the newborn period. The disease manifests with an anemia (macrocytic in roughly 70% of cases), ineffective erythropoiesis, and iron overload as well as occasional skeletal anomalies (hand, feet, frontal bossing, scoliosis).[215] There have been occasional cases where the diagnosis was made in utero.[216] A report of 45 patients with CDA diagnosed within the neonatal period described small for gestational age, hepatosplenomegaly, early jaundice, persistent pulmonary hypertension, and transient thrombocytopenia as other manifestations aside from the anemia. Within this cohort, 80% of them ($n = 36$) required blood transfusion during the first month of life.[217] The erythroid morphology on bone marrow studies is markedly abnormal with erythroid hyperplasia with megaloblastic changes, basophilic stippling, Howell-Jolly bodies, and a small proportion of multi-nucleated erythroblasts. The pathognomonic morphologic feature for this disease is internuclear chromatin bridges.[218,219] 90% of patients with type I CDA are homozygous or compound heterozygous for mutations in one of two genes: *CDAN1* on chromosome 15q15 or *C15ORF41* on chromosome 15q14.[220,221] Aside from supportive therapy (transfusion, chelation, etc.), specific treatment depends on the type of CDA. Therapies for type I include alpha-interferon[222,223] and HSCT.[224–226] Of note, splenectomy has not been shown to be of any benefit in patients specifically with type I CDA.[227]

Osteopetrosis

Infantile malignant osteopetrosis (IMO) is a rare, autosomal recessive, genetically diverse disorder of early childhood. Anemia within the first month of life can be one of the first manifestations, especially for those patients who lack a family history.[228] Ablation of normal marrow space with pathologically increased bone density (dysfunctional osteoclasts lead to failure of bone resorption) leads to hypoplastic anemia secondary to decreased hematopoietic capability. However, given the subsequent extramedullary hematopoiesis, sequestration and increased destruction of RBC have also been cited as contributing factors to anemia in these patients. Hepatosplenomegaly has been described as a prominent feature.[229–231] Other manifestations include hypocalcemia, dental anomalies, cranial nerve dysfunction (from encroachment of nerve foramina in the skull), and gross motor delays. If untreated, overall survival at 6 years of age is roughly 30%, primarily due to complications from bone marrow failure.[228] As osteoclasts are hematopoietically derived, HSCT has been used as a curative therapy.[230–232] The application of gene therapy has recently led to the first clinical trial employing lentiviral-mediated delivery of T-cell immune regulator 1 gene (*TCIRG1*), the affected gene in roughly 50% of patients with IMO.[233]

Congenital Infections

Various maternal infections have been associated with red cell aplasia in the fetus. These include, but are not limited to rubella, CMV, Epstein-Barr virus adenovirus, hepatitis A, B, and C, human immunodeficiency virus, and perhaps the most well-known, parvovirus B19. The majority of adults have been exposed and express antibodies to B19. However, for women lacking antibodies, primary infection during pregnancy leads to a 30% risk of vertical transmission.[234] In the largest prospective study of 1018 pregnant women with acute infection,[235] fetal death was reported in 6.3% of pregnancies. The rate of fetal loss decreased as infection occurred later in gestation; 13%, 9%, and 0% occurring in the first, second, and third trimester, respectively. Red cell aplasia in parvovirus B19 infection can be explained by the narrow cellular tropism. P blood group antigen is its cellular receptor and is present in high concentrations on RBCs and their precursors.[236] Productive infections therefore occur only in CD36 human erythroid progenitor cells. In the same large study,[235] hydrops occurred in 3.9% of pregnancies and similarly decreased in frequency when the maternal infection occurred later in gestation (4.4% vs. 0.8% when ≤32 weeks vs. >32 weeks, respectively). When trying to determine the etiology of non-immune hydrops, fetal infection can be confirmed either with PCR testing of amniotic fluid or detection of B19 IgM in fetal blood. For affected pregnancies, management is typically expectant. However, if anemia is suspected, MCA Doppler assessment for fetal anemia should be obtained. Supportive intrauterine transfusions should be administered if severe anemia is detected. Of note, a complete blood count should also be obtained to assess for concomitant thrombocytopenia which has been appreciated in roughly one third of parvovirus-infected fetuses with hydrops.[237,238] Thrombocytopenia may lead to complications during fetal procedures if not recognized and addressed with platelet transfusion as well.

Physiologic Anemia of Infancy and Anemia Prematurity

At birth, the mean hemoglobin of term infants (17 g/dL) is slightly greater than in premature infants (16 g/dL). The hemoglobin concentration in term infants subsequently decreases to a nadir at which it remains throughout the first year of life (Table 69.7). Termed *physiologic anemia of infancy*, this anemia characterized by low (relative to adult values) hemoglobin is a normal part of development and has no adverse clinical effects. A similar process, called *anemia of prematurity* occurs in premature infants, but the hemoglobin decreases more rapidly and reaches a lower nadir. After 1 year of age, there is little difference between the hemoglobin values of term and premature infants.

Physiologic Anemia of Infancy

With the onset of respirations at birth, considerably more oxygen is available for binding to hemoglobin, and the hemoglobin-oxygen saturation increases from approximately 50% to 95% or more. Furthermore, the normal developmental switch from fetal to adult hemoglobin synthesis actively replaces high-oxygen-affinity fetal hemoglobin with lower-oxygen-affinity adult hemoglobin, which can deliver a greater fraction of hemoglobin-bound oxygen to the tissues. Therefore, immediately after birth the increase

in blood oxygen content and tissue oxygen delivery downregulates erythropoietin production; consequently, erythropoiesis is suppressed. In the absence of erythropoiesis, hemoglobin levels decrease because there is no replacement of aged RBCs as they are normally removed from the circulation. Iron from degraded RBCs is stored for future hemoglobin synthesis. The hemoglobin concentration continues to decrease until tissue oxygen needs are greater than oxygen delivery. Normally, this point is reached between 6 and 12 weeks of age, when the hemoglobin concentration is 9.5 to 11 g/dL. This physiologic hemoglobin decrease does not represent anemia in the true sense of the term; rather, it is a normal adjustment reflecting the presence of excess capability for oxygen delivery relative to tissue oxygen requirements. It is unnecessary to administer iron during this period because it does not prevent the physiologic decrease in hemoglobin, and any iron administered is added to stores for future use. As hypoxia is detected by renal or hepatic oxygen sensors, erythropoietin production increases and erythropoiesis resumes. The iron previously stored in reticuloendothelial tissues can then be used for hemoglobin synthesis and is typically sufficient for hemoglobin synthesis, even in the absence of dietary iron intake, until approximately 20 weeks of age. In fact, the American Academy of Pediatrics recommendation that any cow milk or soy formula used to supplement breast milk be iron fortified has the goal of preventing the late iron deficiency that can occur when the shift is made to the typical cow's milk–based, iron-poor diet of later infancy and toddlerhood.[239]

Anemia of Prematurity

The anemia seen in preterm infants is more profound and occurs earlier (see Table 69.7). Because symptoms may occur, the anemia of prematurity is considered nonphysiologic. The cause of anemia is multifaceted. The lower hemoglobin may be in part a physiologic response to the lower oxygen consumption in premature infants compared with that in term infants, a consequence of their diminished metabolic oxygen needs.[240] An important component in the first few weeks of life is blood loss due to sampling for the many laboratory tests necessary to stabilize the clinical status of these infants, particularly those with cardiorespiratory problems. The erythropoietic response to anemia also is suboptimal, a significant problem because demands on erythropoiesis are heightened by the short survival of the RBCs of premature infants (approximately 40 to 60 days instead of 120 days as in adults) and the rapid expansion of the RBC mass that accompanies growth. The basis for suboptimal erythropoiesis in prematurity appears to be inadequate synthesis of erythropoietin in response to hypoxia. Fig. 69.12 illustrates the magnitude of the deficiency, which, as shown by Stockman et al.,[241] is greatest in the smallest, least mature infants. Because the liver is the predominant source of erythropoietin during fetal life, it has been proposed that relative insensitivity of the hepatic oxygen sensor to hypoxia explains the blunted erythropoietin response seen in premature infants.[242] The spontaneous resolution of the anemia that occurs by approximately 40 weeks' gestational age is in keeping with a developmental switch from the relatively insensitive hepatic oxygen sensor to the renal oxygen sensor, which is exquisitely sensitive to hypoxia, because by this time the predominant site of erythropoietin synthesis has shifted to the kidneys. The problem does not lie with altered sensitivity of erythroid progenitors to erythropoietin because this has been shown to be normal.[243]

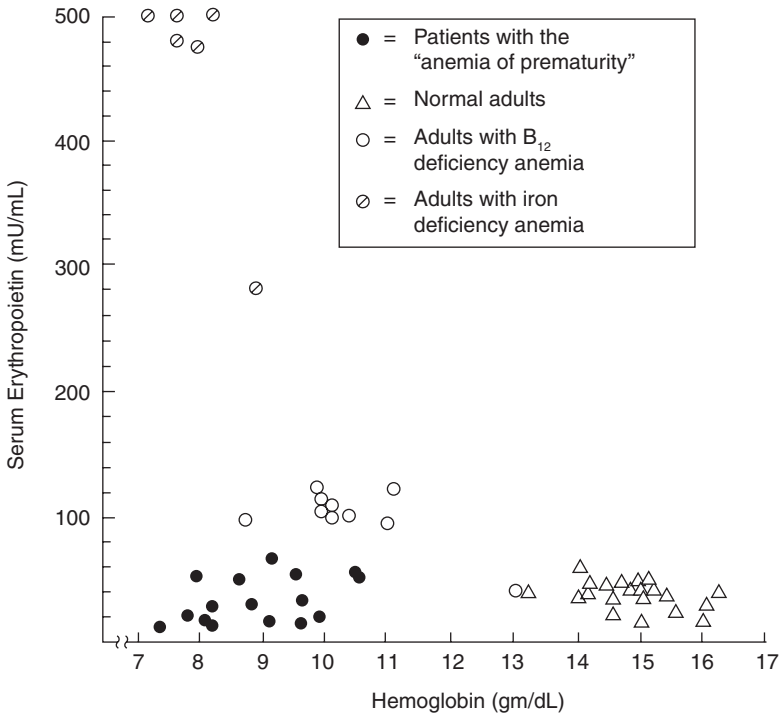
TABLE 69.7 Hemoglobin Changes During the First Year of Life

Week After Birth	Term	Premature (1.2–2.5 kg)	Premature (<1.2 kg)
0	17.0 (14.0–20.0)*	16.4 (13.5–19.0)	16.0 (13.0–18.0)
1	18.8	16.0	14.8
3	15.9	13.5	13.4
6	12.7	10.7	9.7
10	11.4	9.8	8.5
20	12.0	10.4	9.0
50	12.0	11.5	11.0
Lowest hemoglobin: mean (range)	10.3 (9.5–11.0)	9.0 (8.0–10.0)	7.1 (6.5–9.0)
Time of nadir	6–12 wk	5–10 weeks	4–8 weeks

*Hemoglobin concentration (g/dL).

The anemia of prematurity occurs even in nutritionally replete infants, but it may be heightened by deficiencies of folate, vitamin B12, or vitamin E.[244] Premature infants are endowed at birth with significantly less vitamin E than is present in term infants, and unless supplemental vitamin E is provided, this deficiency state persists for 2 to 3 months. Vitamin E is an antioxidant compound vital to the integrity of erythrocytes, and in its absence, these cells are susceptible to lipid peroxidation and membrane injury. One clinical consequence of vitamin E deficiency is that hemolytic anemia can occur in small premature infants weighing less than 1500 g at 6 to 10 weeks of age.[245,246] This hemolytic anemia, which is characterized by reduced vitamin E levels and increased RBC peroxide hemolysis, rapidly disappears following vitamin E administration. A logical conclusion is that vitamin E deficiency might contribute to the anemia of prematurity in a more general sense. In fact, premature infants given daily vitamin E (15 units/day) had higher hemoglobin levels and lower reticulocyte levels than a control group not given the vitamin.[245] However, more recent studies found no hematologic benefit for the administration of 25 or 50 international units of vitamin E daily to premature infants.[247,248] A Cochrane review of the use of vitamin E in preterm infants suggested that routine vitamin E supplementation increased the hemoglobin concentration by a significant but small amount.[249] In very low-birthweight infants, there was a decreased risk of retinopathy and blindness but increased risk of sepsis. This review concludes that the routine use of high-dose intravenous vitamin E, or to achieve tocopherol levels greater than 3.5 mg/dL, is not supported by evidence. Although it has become standard practice to administer vitamin E to all premature infants, the hemoglobin nadir in these babies is still lower than that in term newborns, indicating that anemia is largely caused by other factors such as erythropoietin deficiency.

The optimum management of anemia of prematurity requiring intervention beyond nutritional and vitamin support remains controversial.[250–252] Primary questions include the role of erythropoietin and the application of liberal versus restrictive transfusion thresholds.



• **Fig. 69.12** Hemoglobin levels and corresponding serum erythropoietin levels. Values are from infants with the anemia of prematurity, normal adults, adults with vitamin B_{12} deficiency anemia, and adults with iron deficiency anemia. (From Ross MP, Christensen RD, Rothstein G, et al. A randomized trial to develop criteria for administering erythrocyte transfusions to anemic preterm infants 1 to 3 months of age. *J Perinatol*. 1989;9:246–253.)

Treatment of Anemia of Prematurity With Recombinant Human Erythropoietin

Because a relative deficiency of erythropoietin is present in the anemia of prematurity, a number of studies have evaluated the safety and efficacy of recombinant human erythropoietin (rHuEPO) therapy in this setting. The optimal timing for initiation of rHuEPO therapy and the optimal dose have yet to be determined. Trials have used two different timing strategies: early treatment (before 8 days of age) and "late" treatment. The former has the goal of preventing anemia of prematurity, whereas the latter aims to treat anemia of prematurity and decrease transfusions after the acute stage. Although many studies have shown a modest decrease in RBC transfusion with either approach, there have not been significant differences between these two practices.[253,254] Although infants require relatively high doses of rHuEPO/kg compared to adults because of more rapid clearance and higher volume of distribution, a trial of high dose (1500 units/kg/week) as compared to low dose (750 units/kg/week) resulted in no difference in transfusion requirement.[255] Even in very small premature infants less than 1300 g birthweight with the highest likelihood of needing a transfusion, the effects are minimal, except in infants with birthweight <1000 g.[256] To optimize response to rHuEPO, infants must have adequate protein intake, receive vitamin E, and receive iron supplementation. To achieve the best results, supplemental oral iron at a dose of at least 6 to 9 mg/kg/day needs to be administered. It may be possible to use parenteral iron supplements, particularly in young very low-birthweight infants who are not able to take oral iron[257] or as a supplement to oral iron.[258]

A metanalysis of 21 prospective controlled trials of rHuEPO treatment of the anemia of prematurity was published.[259] Although there was considerable variation between studies, in general the efficacy of rHuEPO in reducing the need for red cell transfusions was modest. The authors concluded that it was premature to recommend rHuEPO for standard therapy for the anemia of prematurity. More recently, Juul et al. published data from the multicenter PENUT (Preterm Erythropoietin Neuroprotection Trial) which enrolled 941 neonates between the ages of 24 and 27 weeks' gestation, randomized to either erythropoietin treatment (1000 units/kg q 48 hours ×6 doses and then 400 units/kg three times a week as maintenance dosing through a postmenstrual age of 32 weeks) or placebo (sham injections) at the same interval. This study demonstrated a relative rate (RR) of transfusions of 0.66 (95% CI, 0.59 to 0.75) and RR of donor exposure of 0.67 (95% CI, 0.58 to 0.77) in the treatment versus the placebo arms.[260] They go further to suggest that the historical lack of demonstrable benefit from erythropoietin therapy is likely related to the following differences between studies: targeted populations, dosing, duration of treatment, and iron supplementation. Despite this elegant work, there remains wide variation in the use of rHuEPO for very low-birthweight infants in the United States,[252] and there is disagreement regarding the cost-benefit ratio of rHuEPO therapy.[261–264]

Although studies in adults have raised questions about the safety of rHuEPO and have led to reductions in its use in patients with malignancy and with renal insufficiency, there are no definitive data supporting safety concerns in premature infants. Previous questions regarding neutropenia and retinopathy of prematurity have not been borne out as significant.[265] In fact, interest in potential neuroprotective effects of rHuEPO has led to careful testing of three different high doses of rHuEPO in extremely low-birthweight infants ≤1000 g and ≤28 weeks of age.[266] Erythropoietin treatment may have a particularly important role to play in the management of infants whose parents refuse to allow blood transfusions on religious grounds.[267]

Red Blood Cell Transfusion Therapy in Premature Infants

It has been estimated that of the infants born weighing less than 1500 g in the United States each year, 80% received multiple RBC transfusions.[268,269] Most transfusions given in the first several weeks of life are to replace losses from phlebotomy required for laboratory monitoring during ventilator support and other intensive care measures. After the first few weeks of life, most transfusions are given to treat the symptoms of anemia of prematurity. The risks associated with use of allogeneic RBC transfusion in premature infants include exposure to viral infections, graft-versus-host disease, electrolyte and acid-base imbalances, exposure to plasticizers, hemolysis when T antigen activation of RBCs has occurred, and immunosuppression.[268,270] Many strategies to reduce the need for allogeneic RBC transfusion in premature infants have been developed and have resulted in a marked decrease in the number of transfusions and number of donor exposures for most premature infants. Reducing phlebotomy losses by use of noninvasive monitoring techniques has been of only limited usefulness.[268] Donor exposures can be reduced by assigning a specified bag of adult donor blood to a sick neonate for multiple transfusions,[30] particularly because it has been shown that blood stored for up to 35 days in CPDA-1[271] or AS-3[27,29] is safe for use in this setting. Defining strict criteria for RBC transfusions also can reduce the number of donor exposures in routine nursery practice.[272,273]

Lachance et al.[274] measured oxygen consumption, myocardial function, resting energy expenditure, and other physiologic variables before and after RBC transfusions. They concluded that in asymptomatic anemic premature infants, oxygenation was well maintained without RBC transfusions when the hemoglobin level was 6.5 g/dL or more. Nelle et al.[275] studied a similar group of asymptomatic anemic premature infants and found that RBC transfusion improved systemic oxygen transport as well as transport in the cerebral and gastrointestinal arteries. When clinical features of hypoxia are absent or findings are equivocal, an elevated blood lactate level may predict a need for transfusion,[276] but in the experience of Frey and Losa[277] it adds little value to the decision-making process in the individual patient. A more recent modality of measuring cerebral oxygenation by near-infrared spectroscopy (NIRS) has accumulated some promising data[278–281] but requires further clinical validations before it can be widely applied.

Traditionally, RBC transfusions have been given to replace phlebotomy losses or in the presence of symptoms thought to reflect hypoxia (e.g., tachycardia, tachypnea, dyspnea, apneic spells, poor feeding).[282,283] However, studies to validate such practices have yielded conflicting results.[241,284–288] A more recent retrospective chart review of 52 ELBW infants admitted to a NICU over the course of 1 year assessed transfusion rates as well as both short- and long-term effects of transfusion.[289] Of these neonates, 90% received at least one RBC transfusion. In this cohort, the outcomes of those transfused were worse (increased risk of bronchopulmonary dysplasia [BPD], necrotizing enterocolitis [NEC], and diuretic use) without any clinical benefit (no improvement in weight gain, heart rate, apnea, or ventilatory/oxygen needs). Although the numbers are small, this experience demonstrates the controversy and underscores the need for further study and guidelines.

Universal guidelines for the optimal approach to the transfusion of preterm infants have yet to be developed. One of the elusive outcomes which continues to drive much of the research is the effect of various transfusion strategies on neurodevelopmental outcomes (NDOs). To date, there have been four randomized control trials addressing these questions.[290–293] Perhaps laying the groundwork for these studies were the Iowa[294] and the PINT[295] studies.

The Iowa study enrolled 100 preterm infants with birth weights between 500 and 1300 g. Infants were enrolled between December 1992 and June 1997. Each infant was assigned to a transfusion algorithm with either high or low hemoglobin thresholds. The thresholds varied within each group depending on weight and level of required respiratory support. Outcomes included the number of RBC transfusions, number of donors, survival to discharge, occurrence of patent ductus arteriosus, germinal matric or intraventricular hemorrhage (IVH), periventricular leukomalacia, retinopathy of prematurity (ROP), BPD, duration of assisted ventilation, duration of supplemental oxygen therapy, number, frequency, and severity of apneic episodes, time to regain birth weight, time to double birth weight, and length of hospitalization. Only 10% to 12% of patients required transfusions. The restrictive group received fewer transfusions (3.3 ± 2.9) as compared to the liberal group (5.2 ± 4.5), but the number of donor exposures was similar (2.8 ± 2.5 for liberal group, 2.2 ± 2.0 for restrictive group). There was more apnea in the restricted transfusion group. In addition, although the study was not designed to detect a difference in CNS outcome, there was a higher rate of grade IV intraventricular hemorrhage (IVH) and periventricular leukomalacia in the restricted group.

The PINT study enrolled 451 extremely low birth weight (ELBW) infants between January 2000 and February 2003. Each infant was assigned within 48 hours of birth to a transfusion algorithm with either high or low hemoglobin thresholds. The thresholds varied within each group depending on postnatal age, blood sampling method (capillary vs. central), as well as whether the baby was receiving respiratory support. Their primary outcome was death before home discharge or survival with any of the following: severe retinopathy, BPD, or brain injury on cranial ultrasound. Secondary outcomes included hemoglobin level, number of RBC transfusion, numbers of donor exposures, rate of growth, and serum ferritin change. Although their study was not powered to assess them, other parameters recorded included NEC, apnea requiring treatment, culture proven infections, time in oxygen, time to extubation, and time to discharge. Their conclusion was that a more liberal strategy increased the number of infants receiving transfusion (89% in the restrictive group vs. 95% in the liberal) but that there was little obvious clinical benefit. Post hoc analysis, however, was suggestive of an increased risk of cognitive delay when infants were treated per the more restrictive algorithm.

These findings spurred the four subsequent studies looking at neurodevelopmental outcomes.[290–293] The findings of these randomized control studies along with other observational studies[296,297] suggest discordant effects on short- and long-term NDOs. The short-term NDOs (<2 years) appear to favor a more liberal approach whereas the longer-term NDOs (school age and older) appear to favor the restrictive approach.[298] The current theory behind this phenomenon lies in the neuroprotective role of erythropoietin.[260,265,299–303] When using a more liberal transfusion threshold, endogenous EPO production is suppressed.[287,294,304] These data from McCoy as well as the others are reassuring especially given the increased rates of periventricular leukomalacia and IVH in the restrictive group.[294]

Despite controversies, there is agreement that the optimum approach to the anemia of prematurity includes delayed clamping of the umbilical cord (at least 30 seconds);[305] limiting blood loss

by phlebotomy; use of dedicated RBC units to minimize donor exposure; optimizing nutrition including protein, iron, folate, vitamin B12, and vitamin E; and use of standardized transfusion guidelines.[306]

Polycythemia

Neonatal polycythemia is observed in 0.4% to 4% of neonates and is usually caused by one of two conditions: increased intrauterine erythropoiesis or fetal hypertransfusion (Table 69.8).[307] Other causes seen in older children, such as arterial hypoxemia (cyanotic heart disease and pulmonary disease), abnormal hemoglobins, or hypersecretion of erythropoietin by tumors, are rare, and primary polycythemia or polycythemia vera is virtually nonexistent. In normal term infants, delayed clamping of the cord leading to an increased transfer of placental blood to the infant is the most common cause of polycythemia. In the setting of acute intrapartum hypoxia, increased placental transfusion also may account for the observed increase in fetal RBC mass, according to animal studies by O et al.[308] Placental insufficiency and chronic intrauterine hypoxia, as seen typically in small-for-gestational-age infants, most commonly underlie increased intrauterine erythropoiesis.

TABLE 69.8 Etiology of Neonatal Polycythemia

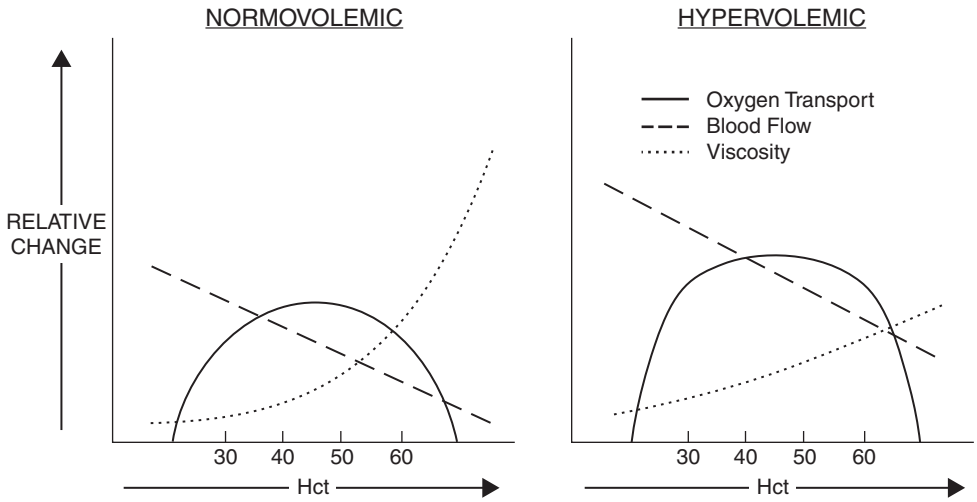
Active (Increased Intrauterine Erythropoiesis)	Passive (Secondary to Erythrocyte Transfusions)
Intrauterine hypoxia	Delayed cord clamping
Placental insufficiency	Intentional
Small-for-gestational-age infant	Unassisted delivery
Postmaturity	Maternofetal transfusion
Toxemia of pregnancy	Twin-twin transfusion
Drugs (propranolol)	
Severe maternal heart disease	
Maternal smoking	
Maternal diabetes	
Neonatal hyperthyroidism or hypothyroidism	
Congenital adrenal hyperplasia	
Chromosome abnormalities	
Trisomy 13	
Trisomy 18	
Trisomy 21 (Down syndrome)	
Hyperplastic visceromegaly (Beckwith syndrome)	
Decreased fetal erythrocyte deformability	

Data from Oski FA, Naiman JL. *Hematologic Problems in the Newborn.* 3rd ed. Philadelphia: Saunders; 1982.

As the hematocrit increases, blood viscosity increases exponentially (Fig. 69.13). Blood flow is impaired by hyperviscosity at hematocrits of 60% or more. Oxygen transport, which is determined by both hemoglobin levels (i.e., oxygen-binding capacity) and blood flow, is maximal in the normal hematocrit range. At low hematocrits, oxygen transport is limited by reduced oxygen-binding capacity, whereas at higher hematocrits, reduction in blood flow secondary to hyperviscosity may similarly limit oxygen transport. At any given hematocrit, expansion of the blood volume beyond the normal level (hypervolemia) distends the vasculature, decreases peripheral resistance, and increases blood flow and, ultimately, oxygen transport. These physiologic observations have implications for therapy of polycythemia.

Most polycythemic infants have no symptoms, particularly if the polycythemia becomes apparent only on routine neonatal screening. Symptoms, when present, usually are attributable to hyperviscosity and poor tissue perfusion or to associated metabolic abnormalities such as hypoglycemia and hypocalcemia. Common early signs and symptoms include plethora, cyanosis (resulting from peripheral stasis), lethargy, hypotonia, poor suck and feeding, and tremulousness. Serious complications include cardiorespiratory distress (with or without congestive heart failure), seizures, peripheral gangrene, NEC, renal failure (occasionally resulting from renal vein thrombosis), and priapism. Because the elevated RBC mass increases the catabolism of hemoglobin, hyperbilirubinemia is common and gallstones occasionally occur.

In the symptomatic infant, a venous hematocrit of 65% or more (or a hemoglobin greater than 22 g/dL) confirms the presence of polycythemia. In screening apparently healthy newborns for polycythemia, however, account must be taken of a number of physiologic variables that influence the hematocrit during the first 12 hours of life:



• **Fig. 69.13** Effect of hematocrit on viscosity, blood flow, and oxygen transport.

1. Time of cord clamping—immediate clamping (within 30 seconds) minimizes placental transfusion.
2. Age at sampling—values increase from birth to a peak at 2 hours, gradually decreasing to cord levels around 12 to 18 hours.[309,310]
3. Site of sampling—values from blood extracted by the heel stick method exceed those from venous blood (the difference can be minimized by prewarming the heel).
4. Method of hematocrit determination—spun values are higher than those obtained by electronic cell counter and show better correlation with blood viscosity.[311]

One way to standardize and simplify screening for polycythemia is as follows: At birth, clamp the cord at about 30 to 45 seconds; at 4 to 6 hours of age, obtain a blood sample from a warmed heel stick and perform a spun hematocrit determination. If the result is greater than 70%, repeat the test on a venous sample. A venous hematocrit of 65% or more indicates polycythemia. By this approach, 1% to 5% of newborns are polycythemic; the range largely reflects differences in altitude at which the study population resides. Because the hematocrit is lower with increasing prematurity, polycythemia is seen less frequently in preterm infants than in term babies.

Following diagnosis, an attempt should be made to determine the cause of polycythemia (see Table 69.8). The condition is particularly common in infants of diabetic mothers or those with Down syndrome[312] and may also occur in the setting of maternal hypertension[313] or, rarely, fumaric aciduria.[314] However, no apparent cause is found in most cases. Studies to determine the effects of polycythemia are dictated by the clinical findings but should usually include serum bilirubin, glucose, calcium, urea nitrogen, and creatinine levels.

Treatment by isovolumetric partial exchange transfusion (PET) is recommended to reduce the RBC mass without inducing hypovolemia. However, the precise indications for PET and the impact of the procedure on outcome remain controversial, without any clear guidelines in place. In a review of randomized or quasirandomized studies of infants with symptomatic or asymptomatic polycythemia,[315] there was no evidence of difference in either long-term neurodevelopmental outcome or short-term neurobehavioral scores. There was a trend toward an earlier improvement in symptoms, but an apparent increase in risk of NEC (RR 8.68, 95% CI 1.06 to 71.1), although several of the studies used fresh frozen plasma. The general finding that polycythemic infants have a poorer outcome than concurrent infants without polycythemia has been thought to be related to the underlying cause of the polycythemia; in one study, perinatal risk factors other than polycythemia were best correlated with outcome and thought to underlie both the polycythemia and the developmental delay.[316] A small randomized control trial compared treatment with 25 mL/kg NS over 6 to 8 hours to no treatment in a group of 55 neonates >34 weeks' gestation with hematocrits between 65% and 75%.[317] Both groups received maintenance fluids. Their primary outcome was need for PET, the rate of which did not differ between the two groups: 22.2% in the treatment group versus 28.6% in the group that did not receive extra fluids.

Many neonatal intensive care units use PET for symptomatic infants with hematocrits greater than 60% to 65%, and for asymptomatic infants with hematocrits greater than 65% to 70%. However, the Committee on the Fetus and Newborn of the American Academy of Pediatrics acknowledged in their 1993 statement that "there is no evidence that exchange transfusion affects long term outcome… Universal screening for polycythemia fails to meet the methodology and treatment criteria and also, possibly the natural history criterion." Thus, Schimmel et al.[318] made the following recommendations based on the available evidence, until such time as systematic studies can be conducted directly measuring blood viscosity to allow development of objective criteria for PET in polycythemia:

- In symptomatic patients with hematocrit >65%, PET with normal saline should be used to reduce possible ongoing injury to tissues by enhancing blood flow.
- In asymptomatic polycythemic infants with presumed normal or increased blood volume, careful monitoring (glucose, cardiorespiratory) is adequate.
- In infants with presumed reduced plasma or blood volume status, treat with early feeding or intravenous fluids.
- For asymptomatic polycythemic infants with presumed normal blood and plasma volume, consider PET only if repeated venous hematocrits are >75%.

A retrospective cross-sectional analytical study utilizing similarly restrictive management for 190 polycythemic infants revealed no increased risk of short-term complications in the following three groups:[319]

1. Hematocrit 65% to 69%: treated as healthy neonates
2. Hematocrit 70% to 75% and asymptomatic hydrated with D10 solution at 100 mL/kg/day and NPO for at least 24 hours or until hematocrit decreased to <70%.
3. Symptomatic polycythemic neonates, regardless of their degree of polycythemia and those with hematocrit >75% were administered PET using NS solution either through umbilical or peripheral vascular access.

Of note, they considered infants symptomatic if in addition to polycythemia at least one of the following was present: hypoglycemia (glucose <40 mg %), thrombocytopenia (<150 × 10^9/L), or respiratory distress requiring oxygen treatment.

Systematic reviews of several studies comparing the use of crystalloid or colloid for PET have concluded that crystalloid solutions are as effective as colloid solutions.[320,321] Thus, normal saline, as an inexpensive product that carries no risk of transfusion-associated infection or reaction to plasma proteins, should be used. Withdrawal of blood for a PET is most easily done using an umbilical artery catheter. Any vessel may be used for blood withdrawal, and all but arterial lines can be used to infuse volume. An umbilical venous catheter inserted into the right atrium also provides acceptable access, but if correct placement cannot be achieved, the catheter should be inserted just far enough into the vessel to allow blood to be withdrawn. Calculation of the total volume of blood to be exchanged for diluent uses the following formula[282]:

$$\text{Exchange volume} = \frac{\text{observed Hct} - \text{desired Hct} \times \text{BV}\left(\frac{\text{mL}}{\text{kg}}\right) \times \text{weight (kg)}}{\text{observed Hct}}$$

where blood volume (BV) usually is 100 mL/kg but in infants of diabetic mothers may be lower (80 to 85 mL/kg).

Example: A 3-kg dyspneic infant with an 80% hematocrit requires a partial exchange transfusion.

$$\text{Blood volume} = 3\,\text{kg} \times 100\frac{\text{mL}}{\text{kg}} = 300\,\text{mL}$$

$$\text{Exchange volume} = \frac{\text{observed Hct} - \text{desired Hct}}{\text{observed Hct}} = \frac{80 - 55}{80} = 0.31$$

Therefore, volume of exchange = 300 mL × 0.31 = 93 mL.

Because coexisting hypoglycemia is an important determinant of adverse neurologic outcome, careful monitoring and maintenance of adequate glucose levels and hydration are essential.

Methemoglobinemia

Methemoglobin (metHb) is an oxidized derivative of hemoglobin in which heme iron is in the ferric (Fe^{3+}) or oxidized state rather than the ferrous (Fe^{2+}) or reduced state. Methemoglobinemia adversely affects oxygen transport, shifting the oxygen dissociation curve to the left in two ways. First, ferric hemes of methemoglobin are unable to bind oxygen and second, the remaining ferrous hemes within the tetramer have increased oxygen affinity. Small amounts of methemoglobin normally are formed daily, associated with the release of oxygen from hemoglobin (auto-oxidation). MetHb that is formed rapidly is reduced through the action of the RBC enzyme cytochrome b5 reductase (Cyb5R) so that in normal persons, levels of metHb seldom exceed 1%. A second methemoglobin reductase, nicotinamide adenine dinucleotide phosphate (NADPH) methemoglobin reductase, also is present in RBCs. This enzyme has little function under normal physiologic conditions, but it is greatly activated by the presence of certain redox compounds such as methylene blue, forming the basis for the clinical treatment of methemoglobinemia.

Acquired methemoglobinemia can occur in normal individuals following exposure to chemicals that oxidize hemoglobin iron. Newborns are particularly susceptible because fetal hemoglobin is more readily oxidized to the ferric state than is hemoglobin A and because RBC Cyb5R activity is only 50% to 60% of normal adult levels during the first 4 months of life.[322] Merely marking the diapers of newborns with aniline dyes has caused methemoglobinemia. Drugs such as prilocaine, administered before birth to provide local anesthesia, can produce methemoglobinemia in both mother and infant. Although in most infants, no increase in methemoglobin levels follows the use of lidocaine-prilocaine cream (Emla cream) to provide analgesia during circumcision,[323] a few case reports of visible cyanosis due to methemoglobinemia in infants treated with this cream have appeared.[324,325] Perhaps the best-known agent that may cause methemoglobinemia is nitrite, either present de novo in ingested material or generated by administering nitric oxide to term babies in high concentrations for treatment of persistent pulmonary hypertension.[326] Nitrates can be converted to nitrite by the action of intestinal bacteria. It is for this reason that well water or foods with a high nitrate content (e.g., cabbage, spinach, beets, carrots) can produce methemoglobinemia in infants.[327] Accumulation of nitrate in the intestinal tracts of infants with diarrhea and acidosis[328,329] or symptomatic dietary protein intolerance[330] is thought to underlie the transient methemoglobinemia that occurs in these conditions.

Congenital methemoglobinemia is most commonly due to inherited disorders of hemoglobin structure or to a Cyb5R deficiency. The inherited abnormalities of hemoglobin structure that give rise to methemoglobinemia, known collectively as the hemoglobin M disorders, are rare autosomal-dominant defects caused by point mutations that alter a single amino acid in the structure of normal globin. The altered conformation that ensues favors the persistence of the ferric rather than the ferrous form of heme iron. The normal methemoglobin reductive capacity of the RBC cannot compensate for such instability of ferrous heme. There are three variants affecting the α chain (Hb M Iwate, Hb M Boston, Hb M Yantai), three variants affecting the β chain (Hb M Saskatoon, Hb M Milwaukee, Hb M Hyde Park), and six variants affecting the γ chain (Hb FM Osaka, Hb FM Fort Ripley, Hb FM Circleville, Hb F Cincinnati, Hb FM Toms River, Hb FM Viseu).[331] Only the alpha and gamma globin chain mutations are associated with neonatal methemoglobinemia, because these are the globins that form hemoglobin F. Neonatal methemoglobinemia is transient when produced by one of the six gamma chain mutations, because the normal developmental switch from fetal to adult hemoglobin eliminates all but a trace of the mutant hemoglobin.[332] Hemoglobin M heterozygotes inheriting alpha or beta globin mutations have lifelong cyanosis, but they are usually asymptomatic. No therapy is needed. The homozygous state is incompatible with life. Diagnosis of the hemoglobin M disorders is made by special tests in RBC diagnostic laboratories.

Cyb5R deficiency is an autosomal recessive disorder of which there are two phenotypes. Type I affects the soluble RBC-specific isoform and accounts for roughly 90% of Cyb5R disease. Type I defects are caused by missense mutations which give rise to enzymatically active but unstable proteins.[333] Type II affects the membrane-bound isoform of Cyb5R which plays a larger role in non-RBCs leading to methemoglobinemia along with microcephaly, developmental delay, intellectual disability, and failure to thrive. Type II defects are typically full stops or deletions leading to absent protein expression or expression of an enzymatically inactive protein.[334] Heterozygotes with type I are asymptomatic and do not have methemoglobinemia under normal circumstances. However, if challenged by drugs or chemicals that cause methemoglobinemia, they may become cyanotic and symptomatic at doses that have no effect in normal persons. Homozygotes have lifelong methemoglobin levels of 15% to 40% and are cyanotic but otherwise asymptomatic unless exposed to toxic agents. Patients with homozygous or compound heterozygous mutations for type II have methemoglobin levels ranging from 10% to 42% (mean ± SEM: 20.7% ± 3.2%).[335] Diagnosis of Cyb5R deficiency is by assay of RBC enzyme activity, a procedure available only in specialized hematology laboratories.

Cytochrome b5 deficiency is the rarest cause of congenital methemoglobinemia, having been described only in a few families.[336–340] These cases describe individuals in whom their methemoglobinemia is clinically inapparent, with only mildly elevated levels (6.1% to 8.5%). The males described in the literature presented with 46,XY disorder of sex development. This phenotype is thought to be related to Cyb5a role in facilitating CYP17A1 reduction and ultimately the production of dehydroepiandrosterone (DHEA), the principal androgen precursor.

The cardinal clinical manifestation of methemoglobinemia is cyanosis not resulting from cardiac or respiratory disease. Cyanosis present at birth suggests hereditary methemoglobinemia, whereas that appearing suddenly in an otherwise asymptomatic infant is more consistent with acquired methemoglobinemia (Box 69.3). The blood is dark and, unlike deoxygenated venous blood, does not turn red when exposed to air. Rapid screening for methemoglobinemia can be done by placing a drop of blood on filter paper and then waving the filter paper in air to allow the blood to dry. Deoxygenated normal hemoglobin turns red, whereas methemoglobin remains brown. Methemoglobin levels of 10% or more can be detected.[341] More accurate determination of methemoglobin levels is accomplished in the blood gas laboratory by co-oximetry or in the clinical laboratory using a spectrophotometer. It is important to recognize that cyanosis and symptoms resulting from methemoglobinemia do not necessarily correlate directly. Cyanosis is first clinically evident when the total amount of methemoglobin (not the percentage) reaches approximately 1.5 g/dL. This occurs

BOX 69.3 Approach to Infants with Cyanosis and Methemoglobinemia

Cyanosis With Respiratory and Cardiac Abnormalities

Blood turns red when mixed with air
Decreased arterial PO_2
Consider pulmonary, cardiac, or central nervous system disease

Cyanosis With or Without Respiratory or Cardiac Abnormalities

Blood turns red when mixed with air
Normal arterial PO_2
Consider polycythemia syndromes

Cyanosis Without Respiratory or Cardiac Abnormalities

Blood remains dark after mixing with air
Normal arterial PO_2
Consider methemoglobinemia syndromes

1. With rapid clearing of methemoglobin following methylene blue:
    a. Consider toxic methemoglobinemia (look for environmental oxidants)
    b. Consider NADH-methemoglobin reductase deficiency (perform enzyme assay)
2. With reappearance of methemoglobinemia after initial response to methylene blue:
    a. Consider NADH-methemoglobin reductase deficiency
3. With no change in methemoglobin following methylene blue:
    a. Consider hemoglobin M disorders (perform hemoglobin electrophoresis)
    b. Consider associated glucose-6-phosphate dehydrogenase deficiency (perform enzyme assay)

at methemoglobins of 10% in an individual with a total hemoglobin of 15 g/dL, where it will be delayed until the methemoglobin percentage reaches 15 in an individual with a total hemoglobin of 10 g/dL. In this way, individuals with erythrocytosis will develop cyanosis at lower methemoglobin percentages while anemia can mask cyanosis. Symptoms attributable to hypoxemia and diminished oxygen transport develop at varying levels and depend on a number of factors including the acuity of the methemoglobin accumulation as well as the underlying cause. Typically, however, symptoms do not appear until levels increase to 30% to 40% of total hemoglobin. Death occurs at levels of 70% or greater. Methemoglobinemia is not associated with anemia, hemolysis, or other hematologic abnormalities.

Treatment with intravenous methylene blue (1 mg/kg as a 1% solution in normal saline) is indicated when methemoglobin levels are greater than 15% to 20%. Doses greater than 1 mg/kg should be avoided, because they may be toxic.[342] The response to methylene blue is both therapeutic and diagnostic. Methemoglobin levels decrease rapidly, within 1 to 2 hours, if methemoglobinemia is caused by a toxic agent or by a deficiency of Cyb5R. In contrast, the hemoglobin M disorders do not respond to methylene blue. Reappearance of methemoglobinemia after an initial response to methylene blue suggests a deficiency of Cyb5R or the persistence of an occult oxidant. A poor response to methylene blue also is seen in G6PD-deficient persons because this disorder is characterized by suboptimal generation of NADPH, a required cofactor in the reduction of methemoglobin by methylene blue in deficient persons. Cases have been described in which treatment with methylene blue in patients with G6PD led to severe hemolysis.[343–345] Ascorbic acid can be used instead. In general, most infants with hereditary methemoglobinemia are asymptomatic and require no therapy.

Acknowledgment

I would like to acknowledge Dr. Dana Matthews who was the original author of this chapter in the ninth edition of *Avery's Diseases of the Newborn*. Dr. Matthews' wisdom and thoughtfulness have left an indelible mark on the world of hematology and many aspiring hematologists.

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70
Neonatal Transfusion

RAVI MANGAL PATEL AND CASSANDRA D. JOSEPHSON

KEY POINTS

- Recent multicenter randomized trials support more conservative transfusion approaches with the use of lower hemoglobin and platelet count thresholds.
- Two multicenter clinical trials did not show improvement in survival without neurodevelopmental impairment or other important outcomes with the use of higher hemoglobin thresholds for red blood cell transfusion in preterm infants. Given the lack of benefit, these data generally support the use of a lower hemoglobin threshold in most clinical circumstances for routine transfusions, although the safety of thresholds below those studied in clinical trials to date is uncertain and not generally recommended.
- One multicenter trial comparing higher (50×10^9/L) versus lower (25×10^9/L) thresholds for prophylactic platelet transfusion showed an increased risk of death or serious bleeding among infants randomized to a higher threshold. These data suggest that a lower threshold should be used in most clinical circumstances for prophylactic platelet transfusions, although they do not apply to actively bleeding infants.
- There is wide variation in the use of hemoglobin and platelet thresholds among centers in the United States, highlighting an opportunity for patient blood management.
- Prophylactic plasma transfusion is likely to have a minimal effect on prevention of intracranial bleeding in preterm infants, and newer measures such as thromboelastography may provide more precise data to guide the administration of plasma transfusion.
- Delaying clamping of the umbilical cord and minimizing phlebotomy-related blood losses are important strategies to minimize red blood cell transfusions and improve outcomes.

Overview

Blood transfusion is essential to modern neonatal intensive care and can be lifesaving, particularly for critically ill neonates or infants undergoing surgery. Blood components are necessary to carry and deliver oxygen to tissues, provide adequate preload to the heart to support cardiac output, and maintain a balance between hemostasis and coagulation to prevent both bleeding and thrombosis. In this chapter, specific blood component therapy and special circumstances in neonatal transfusion medicine will be reviewed.

Red Blood Cell Transfusion

Components of Red Blood Cell Transfusion

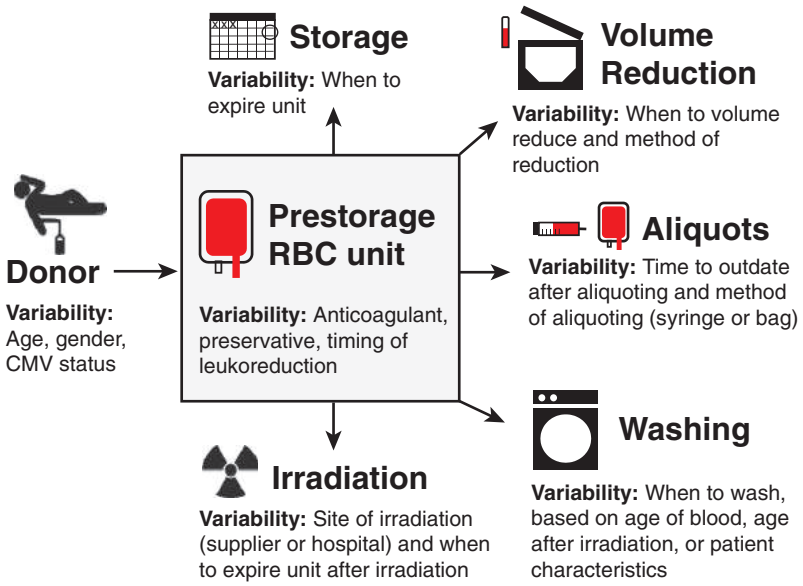
Red blood cells (RBCs) are the most commonly transfused component of whole blood. They are produced by centrifugation from whole blood or, less frequently, acquired directly from a donor by apheresis. Various storage solutions, such as citrate–phosphate–dextrose–adenine (CPDA-1) and additive–glucose–mannitol (e.g., AS-1, AS-3), containing anticoagulants and preservatives are used to maintain RBCs at 4°C during storage. AS-1 and AS-3 units contain mannitol and adenine, which are associated with diuresis and renal toxicity, respectively. Therefore, RBCs stored in AS-1 and AS-3 should not be used for large-volume (≥20 mL/kg) transfusions.

Preparation of Red Blood Cell Transfusion

Preparation of RBCs for transfusion begins with donor assessment and ends with transfusion into the neonate. The goal is to assure blood safety and maximize efficacy and response to transfusion. Accurate RBC component and recipient identification are imperative for safety. More than 300 blood group antigens, from 35 blood group systems, have been discovered on RBCs.[1] Among these, the ABO and D (also known as rhesus [Rh]) blood groups are the most important in determining the compatibility of allogenic RBC transfusion. Infants can have four ABO blood types containing the corresponding A or B antigens on the RBC surface: group A, group B, group AB, or group O (no A or B antigens). In addition, infants can be D antigen positive or D antigen negative. Testing of the plasma or serum from either the infant or the mother must include ABO and D typing of their RBCs and a screen for unexpected RBC antibodies (indirect antiglobulin test). Before non–group type O RBCs are issued, the infant's plasma or serum is tested to detect passively acquired maternal anti-A or anti-B isohemagglutinins, which usually do not develop until more than 4 months of age. Importantly, crossmatch compatibility testing and repeated ABO and D typing, as is required for all patients older than 4 months, may be omitted during any hospitalization for an infant younger than 4 months, as long as any of the following criteria are met:

1. Antibody screen is negative
2. Transfused RBCs are group O, ABO identical, or ABO compatible
3. RBCs are either D negative or the same D type as those of the patient

Testing for the isohemagglutinins must also include the antiglobulin phase of testing at 37°C (body temperature). In the presence of an immunoglobulin G (IgG) antibody, crossmatched, ABO-compatible RBCs are administered until the acquired antibody is no longer detected. Once RBC units have been properly



• **Fig. 70.1** Vein-to-Vein Processing of Red Blood Cells. Variability among centers and blood banks in processing of red blood cells, from donation to preparation before transfusion into the neonate. *CMV*, Cytomegalovirus. (From Patel RM, Meyer EK, Widness JA. Research opportunities to improve neonatal red blood cell transfusion. *Transfus Med Rev*. 2016;30:165–173.)

selected, sterile aliquots from the parent unit are produced to more accurately provide volumes of RBCs dosed based on the patient's weight. This can reduce the risk of transfusion-associated circulatory overload and also limit donor exposure–related infectious risks by repeatedly using the same RBC unit.[2] Data suggest that 20 mL/kg transfusions in very low birth weight (VLBW) infants, compared with 10 mL/kg transfusions, lead to a greater increase in hematocrit without respiratory compromise.[3] Although the optimal dose and duration of RBC administration are not known, transfusions should not run for longer than 4 hours. There is substantial variability in how blood centers and blood banks prepare RBCs (Fig. 70.1). This includes strategies to prevent cytomegalovirus (CMV) transmission,[4,5] how and when RBCs are irradiated,[6,7] when RBCs are washed,[8] and what age of RBC units are used.

The exclusive use of RBC transfusions from CMV-seronegative donors in VLBW infants has been associated with a very low risk of transfusion-transmitted CMV infection: between 0.0% and 0.3% per unit of CMV-seronegative and leukoreduced blood.[5] Historically, the risk of transfusion-transmitted CMV (TT-CMV) infection from leukoreduced transfusions from CMV-untested donors has been reported to be higher than that from approaches using both CMV-negative donors and leukoreduction.[9] In the opinion of the authors, the safest approach for transfusion of VLBW infants is to use leukoreduced RBCs from CMV-negative donors. However, given the low risks of TT-CMV infection with improvements in modern leukoreduction techniques, use of leukoreduced blood from CMV-untested donors is an acceptably safe and low-risk alternative.[10,11] Importantly, RBC transfusion should not be delayed if CMV-negative blood is unavailable, and CMV-untested blood should be used given the relatively low risk of TT-CMV infection.

Many centers limit the storage time of RBCs transfused into neonates and infants.[7,12] The maximum storage duration depends on the type of storage solution, with 35 days for CPDA-1 units and 42 days for AS-1 and AS-3 units. Recent randomized trials have provided high-level evidence regarding the safety of using stored, older RBC units. The Age of Red Blood Cells in Premature Infants (ARIPI) trial randomized VLBW infants to receive fresh blood (mean 5 days) versus standard issue blood (mean 15 days) and found no difference in a composite of mortality or morbidity between groups.[13] Similar trials in adults and children have produced concordant findings.[14–16] However, the age of RBC units may not account for other donor RBC characteristics, such as storage solutions and irradiation.[6] A recent observational study suggests that characteristics of blood donors may influence outcomes in transfused VLBW infants, with red cell transfusions from older female blood donors associated with the lowest risk of adverse short-term outcomes, although additional study is needed before selection of donors based on age and sex can be recommended.[17]

Indications for Red Blood Cell Transfusion

Common indications for RBC transfusion include anemia, bleeding, and cardiorespiratory compromise. Extremely preterm infants are among the most highly transfused populations in medicine, with 64% of extremely low birth weight infants weighing 1000 g or less at birth receiving at least one RBC transfusion during their neonatal intensive care unit (NICU) stay.[18]

Studies of RBC transfusion approaches in preterm infants suggest it is safe to practice a conservative approach. Four clinical trials enrolling at least 100 subjects each have compared the efficacy of RBC transfusion using liberal (high hemoglobin threshold) versus conservative (low hemoglobin threshold) transfusion strategies (Table 70.1).[19–22] In the Prematures in Need of Transfusion (PINT) trial, a lower hemoglobin transfusion threshold resulted in a nonsignificant increase in neurodevelopmental impairment at 18 to 21 months (odds ratio [OR] 1.74, 95% confidence interval [CI] 0.98 to 3.11), which was significantly higher in a post hoc analysis with a more inclusive measure of neurodevelopmental impairment.[23] The Transfusion of Prematures (TOP) trial enrolled 1824 infants and found that a higher hemoglobin threshold, compared to a lower threshold, did not improve survival without

TABLE 70.1 Red Blood Cell Transfusion Thresholds for Preterm Infants in Randomized Trials

Thresholds		Iowa Trial[19]	PINT Trial[20]	TOP Trial[21]	ETTNO Trial[22]
Liberal transfusion thresholds*	Upper	15.3	13.5	13.0	13.7
	Lower	10.0	8.5	10.0	9.3
Restrictive transfusion thresholds*	Upper	11.3	11.5	11.0	11.3
	Lower	7.3	7.5	7.0	7.0

*Thresholds are hemoglobin values in grams per deciliter, and differences between upper and lower thresholds within each transfusion arm reflect the range based on an infant's respiratory illness severity and postnatal age. See the text for additional information on the trials.
ETTNO, Effect of transfusion thresholds on neurocognitive outcome of extremely low birth weight infants; *PINT*, prematures in need of transfusion; *TOP*, transfusion of prematures.

neurodevelopmental impairment at 22 to 26 months (relative risk 1.00; 95% CI 0.92 to 1.10).[21] In addition, there was no difference in survival or other common morbidities. Similar findings were reported in another multicenter trial from Germany, where no difference in death or disability at 24 months was noted between more liberal (higher) versus restrictive (lower) thresholds for RBC transfusion (risk difference 1.6%; 95% CI −4.8% to 7.9%).[22] Both of these more recent trials support use of RBC thresholds within the ranges studied and favor the use of lower hemoglobin thresholds, given the lack of benefit with more liberal thresholds. However, the safety of thresholds below those evaluated in the aforementioned trials is uncertain and should be avoided.

RBC transfusions are sources of parenteral iron, and fewer RBC transfusions using lower hemoglobin transfusion thresholds without appropriate enteral iron supplementation may increase the risk of iron-deficiency anemia. Iron-deficiency anemia is associated with adverse long-term neurodevelopmental outcomes[24,25] and higher amounts of enteral iron supplementation is associated with better cognitive outcomes at 2 years of age.[26] Recent trends suggest an increasing use of conservative transfusion thresholds to decrease RBC exposure,[27,28] mirroring patient blood management approaches in adults designed to minimize blood exposure.[28] However, there is wide variation in pretransfusion thresholds among US centers.[18]

For more mature preterm and term infants, transfusion approaches are largely based on expert opinion, owing to the lack of randomized trials. In select populations of infants, such as those undergoing surgery, hematocrit values of 40% or higher may be desired.[29] By contrast, asymptomatic preterm infants may tolerate a hematocrit of 21% before needing RBC transfusion (see Table 70.1). In neonates without an ongoing source of blood loss, iron supplementation or erythropoiesis-stimulating agents may be sufficient to help restore RBC volume. However, a multicenter trial of high-dose erythropoietin did not show a reduction in death or severe neurodevelopmental impairment at 2 years of age with the use of erythropoietin (relative risk 1.03; 95% CI 0.81 to 1.32).[30] Additional studies are needed to guide appropriate thresholds in term infants, particularly among neonates undergoing surgery,[29] including cardiac surgery[31] and those receiving extracorporeal membrane oxygenation support, given the association between higher transfusion rates and increased mortality in this population.[32]

Risks of Red Blood Cell Transfusion

The risks of blood component therapy, specifically RBC transfusion, are reviewed here. However, many of the immunologic and nonimmunologic risks discussed are based on studies in adult transfusion recipients and have not been adequately studied in the neonatal population.

TABLE 70.2 Risk of Transfusion-Transmitted Infections in the United States

Infection	Estimated Risk per Unit*
HIV	1 in 2 million
Zika	None reported in United States (<1 in 3 million)
CMV	Historically estimated risk up to 1%–3% for leukoreduced RBC transfusions from CMV-untested donors, although likely substantially lower.[47] For RBC products from CMV-negative donors, estimated risk of 0% (95% CI 0.0%–0.3%)[5]
Hepatitis B	1 in 2 million
Hepatitis C	1 in 2 million
HTLV-I/II	<1 in 3 million
West Nile virus	<1 in 3 million
Malaria	<1 in 3 million
Chagas disease	<1 in 3 million

*Unless specified, estimates are from Busch et al.[33]
CI, Confidence interval; *CMV*, cytomegalovirus; *HIV*, human immunodeficiency virus; *HTLV-I/II*, human T-lymphotropic virus I/II; *RBC*, red blood cell.

Immunologic Complications

Historically, the primary risk of blood transfusion has been infection. However, current estimates of the risk of transfusion-transmitted infections suggest that modern donor selection and post-donation diagnostic testing strategies reduce the risk to below 1 in 2,000,000 for most infections (Table 70.2).[33] Acute immunologic complications of transfusion include hemolytic transfusion reactions, immune-mediated platelet destruction, febrile nonhemolytic reactions, allergic reactions, anaphylaxis, and transfusion-related acute lung injury (TRALI).[34] Formation of antibodies to ABO blood group antigens (anti-A and anti-B IgM and IgG types) typically occurs after 3 to 4 months of age.[35] Therefore, transfusion reactions related to ABO blood group incompatibility are less likely to occur in neonates. Delayed immunologic complications include delayed hemolytic

reactions, alloimmunization to white blood cells, RBCs, and platelet antigens in blood components, posttransfusion purpura, and transfusion-associated graft-versus-host disease. Transfusion-related immunomodulation (TRIM) is a potential entity described in adults that involves immunosuppressive effects of blood transfusion, which may be beneficial in solid-organ transplant but also can potentially increase the risk of infection and malignancy.[36] However, many of the studies on TRIM were done in an era before leukoreduction, and TRIM has not been investigated in neonates.

Transfusion-Related Acute Lung Injury

Although transfusion-related acute lung injury (TRALI) has not been well studied in neonates and infants, it is one of the most severe complications of blood component transfusion in other populations. TRALI is largely caused by substances in plasma and platelet components but can also occur after RBC transfusion. The causes of TRALI are grouped into immune and nonimmune types. In adults, immune TRALI is estimated to occur in 1 per 5000 transfusions, with case–fatality rates of 6% to 9% among those affected.[37] However, the incidence and risks of TRALI in neonates are unclear. Immune TRALI is suspected to be due to substances within transfused blood products that elicit an immune response, such as HLA and granulocyte-binding alloantibodies. Nonimmune TRALI is thought to occur following stored platelet and RBC transfusions. Nonimmune TRALI is more benign than immune TRALI and is thought to be mediated by biologically active lipids.

Transfusion-Related Acute Gut Injury

Transfusion-related acute gut injury (TRAGI), in which necrotizing enterocolitis (NEC) occurs within a short (e.g., 48 hours) time period after RBC transfusion, has been suggested as a possible transfusion-related adverse event.[38,39] However, the association between RBC transfusion and NEC has only been reported in some observational studies,[40] with a more recent metaanalysis reporting highly heterogeneous findings among studies with no overall association.[41] Other studies suggest that anemia may be the underlying risk factor for NEC[42] and gut injury,[43] or that both anemia and subsequent RBC transfusion are necessary to elicit gut injury.[44] However, no difference in the incidence of NEC was observed in randomized trials comparing higher versus lower transfusion thresholds (see Table 70.1). Therefore, there remains uncertainty about whether RBC transfusion causes NEC.

Nonimmunologic Complications

Infection

Viral, bacterial, parasitic, or prion infections are possible from blood transfusion. Donor screening by history questionnaire, infectious testing, and donor deferral substantially reduces the risk of these infections but does not eliminate them. Additionally, there is the potential for yet undiscovered infections to be transmitted through transfusion, as occurred with human immunodeficiency virus (HIV) among hemophilia patients in the 1980s.[45] Donor screening based on guidance from the Food and Drug Administration (FDA)[46] involves testing the donor's ABO group and Rh type, including testing for a weak D antigen. Infectious testing is based on FDA-licensed tests and involves screening patients for HIV, hepatitis B virus, hepatitis C virus, Human T-lymphotropic virus I/II, West Nile virus, syphilis, and *Trypanosoma cruzi*, the cause of Chagas disease.[33] Donors can also be screened for CMV. CMV rates are much higher in southern geographic locales as compared to temperate and cooler regions. This causes a wide variation in seroprevalence of donors of 40% to 80% in different regions of the United States. This circumstance leads to variation among blood centers in the use of a CMV donor testing strategy to prevent CMV transmission, given the low risks of transmission.[47]

Emerging pathogens such as Zika virus and Ebola virus highlight the residual risk of infections,[48] including those that are yet to be identified. This is particularly relevant given global travel and the effect of epidemics such as dengue[49] and chikungunya[50] on transfusion medicine. Pathogen reduction offers an alternative that has the potential to increase the safety of the blood supply and has been recently approved by the FDA in the United States for use with plasma and platelet transfusions.[51] Clinical trials evaluating pathogen-reduction technologies for whole blood and RBCs are ongoing.[51]

Pathogen inactivation works by combining ultraviolet irradiation with photosensitizers, such as psoralen, that damage pathogen nucleic acids, preventing replication and host infection. This can work for a wide range of viruses, bacteria, parasites, and other pathogens. In addition, this technology can be used to reduce the risk for emerging infections, particularly when donor testing strategies are not available or fully implemented. However, pathogen reduction does not inactivate all pathogens, such as hepatitis A virus and hepatitis E virus. In addition, pathogen reduction is contraindicated for infants treated with certain phototherapy devices, because of the potential reaction between the psoralen additive and ultraviolet light from phototherapy that can lead to skin erythema.[46]

Transfusion-Associated Circulatory Overload

Large-volume (>20 mL/kg) RBC transfusions may place the infant at risk of transfusion-associated circulatory overload (TACO) that can lead to congestive heart failure and pulmonary edema. Patients with severe anemia without acute bleeding may require slow correction of anemia with RBC transfusion to reduce the risk of TACO. Additionally, certain subsets of neonates, such as those with fetal anemia and hydrops fetalis, may need isovolumetric RBC transfusion using an exchange transfusion. In this procedure, RBCs without whole blood reconstitution with plasma are administered, and whole blood is removed from the patient. Whole blood exchange transfusion for hemolytic disease of the fetus and newborn is discussed later in this chapter.

Hypothermia

Large-volume RBC transfusions can increase the risk of hypothermia because RBC units are stored at 4°C. Thus, blood warmers in the setting of large-volume or massive transfusion are indicated. The possibility of hypothermia resulting from transfusion should also be monitored in operative transfusions, when patients may have acute bleeding requiring rapid large-volume transfusions, coupled with potential cold stress from the operating room environment.

Metabolite Derangements

Electrolyte abnormalities can occur following RBC transfusion. Additive solutions in donor RBCs include mannitol (AS-1), a diuretic, and citrate (CPDA-1, AS-3), a calcium chelator, which can lead to hypocalcemia. This is particularly of concern in infants receiving massive transfusion or exchange transfusion. Additional risks include changes in pH and hyperkalemia or hypokalemia, particularly among patients with impaired renal function in the

setting of massive transfusion. Washing of RBCs may reduce the risk of hyperkalemia.

Transfusion-Related Death

Although rare, fatal reactions can result from any of the complications previously discussed and should be reported to the FDA. Historically, acute hemolysis from ABO-incompatible transfusions was the leading cause of deaths from blood transfusion.[52] Such reactions are now uncommon because of improvements in blood banking practices. Fatal hemolytic reactions are especially rare in neonates due to the lack of antibodies to blood group antigens in the first few months of life.[35] Currently, TRALI is the most frequent cause of acute transfusion fatality among all blood recipients, accounting for 37% of deaths. However, there is uncertainty if this complication occurs in neonatal patients.[53] Transfusion-transmitted bacterial sepsis is the third most common cause of reported transfusion-related fatalities, with more than two-thirds of infections caused by gram-negative organisms. Although a number of organisms have been reported, *Klebsiella pneumoniae, Escherichia coli*, and *Staphylococcus epidermidis* are the three most common organisms, and all attributed to platelet transfusion.[54,55]

Platelet Transfusion

Component of Platelet Transfusion

Two types of platelet units are available in the United States, platelet concentrates (known as *random-donor* or *whole blood–derived platelets*) and apheresis platelets, known as *single-donor platelets*. Platelet concentrates are produced from whole blood drawn from a donor, whereas single-donor platelets are collected via an apheresis technique that returns the remaining whole blood components to the donor. These two methods of platelet collection yield distinctly different numbers of platelets per unit. One platelet concentrate contains approximately 7×10^{10} platelets, in contrast to one single-donor apheresis platelet concentrate with 3 to 6×10^{11} platelets per unit. Pooling of five to eight platelet concentrates from different donors is required to equal the same number of platelets in an apheresis platelet unit. Platelets have a short shelf life (limited to 5 days) and are stored at room temperature and preserved with constant, gentle agitation.

Indications for Platelet Transfusion

Platelet transfusions, typically dosed at 10 to 15 mL/kg, are nearly always administered prophylactically in the setting of thrombocytopenia, defined as a platelet count of less than 150,000/μL, or in response to severe bleeding. Thrombocytopenia is a common occurrence in neonates requiring intensive care, affecting an estimated 18% to 35% of neonates on NICU admission and close to 70% of extremely low birth weight infants.[56] However, only a fraction of these infants receive platelet transfusions. Platelet transfusions are estimated to be used in 5% to 9% of infants cared for in US NICUs. There is considerable debate as to the appropriate prophylactic platelet transfusion thresholds for neonates, with wide variation in practices.[18,57,58]

Three randomized trials in preterm infants have evaluated the use of more liberal versus conservative platelet transfusion thresholds (Table 70.3),[59–61] the largest of which reported a significant increase in the risk of death or severe bleeding with the use of a more liberal threshold for prophylactic platelet transfusions.[60] Findings from this trial were consistent across subgroups of infants with higher and lower baseline risks of bleeding or mortality.[62] Multicenter observational studies among US NICUs report wide variation in platelet thresholds for transfusion. In one study, thresholds ranged from less than 10,000/μL to more than 150,000/μL. In that study, platelet transfusion was not associated with a lower risk of intraventricular hemorrhage (IVH) after controlling for the severity of thrombocytopenia.[56] Another multicenter study reported the median pretransfusion platelet count was 71×10^9/L for neonates, with the 10th to 90th percentile ranging from 26 to 135×10^9/L. Complicating the assessment of platelet threshold and risk of bleeding are contributors to platelet dysfunction, such as nonsteroidal anti-inflammatory medications, acidosis, or concomitant coagulopathy.[63] Functional assays, such as closure time following stimulation with collagen and adenosine diphosphate, may be more important than platelet count in determining bleeding risk.[64]

TABLE 70.3 Platelet Transfusion Thresholds for Preterm Infants in Randomized Trials

Thresholds	Andrew et al.[59]	Curley et al.[60]	Kumar et al.[61]
Year published	1993	2019	2019
Liberal transfusion thresholds*	150	50	100
Restrictive transfusion thresholds*	50 or bleeding	25	20
Summary	No significant difference in new intracranial hemorrhage (28% vs. 26%, $P = .73$)	Increase in death or severe bleeding with higher threshold (OR 1.57; 95% CI 1.06–2.32)	No significant difference in closure of a patent ductus arteriosus (adjusted hazard ratio 1.4; 95% CI 0.57–3.47)

*Platelet transfusion thresholds are reported as 10^9/L.
CI, Confidence interval; *OR*, odds ratio.

Risks of Platelet Transfusion

One of the major risks of platelet transfusions is bacterial sepsis, although this is relatively uncommon, with an estimated occurrence of 1 in every 100,000 platelet transfusions.[33] Approximately 1 in every 2000 to 3000 platelet units contains clinically relevant bacteria concentrations.[33,51] Currently, in the United States, it is recommended that all blood banks employ bacterial risk control strategies. Pathogen inactivation is one approach to decrease this residual risk even further, although the effects of this approach on transfusion of platelets into immature neonates require additional study given the relatively low risks of platelet transfusion–associated sepsis and concerns about platelet refractoriness.[65]

Plasma and Cryoprecipitate Transfusion

Components of Plasma and Cryoprecipitate Transfusion

Plasma is the aqueous, acellular portion of whole blood and includes albumin, the most abundant of the plasma proteins, along with complement (predominantly C3), enzymes, transport molecules, Igs (γ-globulins), and coagulation factors. The coagulation factors in plasma include (1) fibrinogen, (2) factor XIII, (3) von Willebrand factor, (4) factor VIII, primarily bound to its carrier protein von Willebrand factor (~100 ng/mL), and (5) vitamin K–dependent coagulation factors II (prothrombin), VII, IX, and X. Plasma products, fresh frozen plasma (FFP), or F24 plasma is mainly produced from whole blood and less frequently from plasmapheresis collections. FFP is frozen within 6 to 8 hours of collection, while F24 plasma is frozen within 24 hours of collection.

Cryoprecipitate is an insoluble precipitate that is formed by the thawing of FFP and then refreezing it in 10 to 15 mL of plasma within 1 hour. This produces a product with high concentrations of factor VIII (80 to 150 U/unit), von Willebrand factor (100 to 150 U/unit), fibrinogen (~250 mg/unit), factor XIII (150 to 250 U/unit), and fibronectin (~2 mg/mL). Cryoprecipitate can be stored at temperatures of −18°C or lower and can be maintained for up to 1 year.

Indications for Plasma and Cryoprecipitate Transfusion

Plasma transfusions are used in NICU patients for a variety of indications, most often to treat or prevent bleeding in the setting of coagulopathy or disseminated intravascular coagulation.[66] Additionally, plasma transfusion may be used as a colloid to increase intravascular volume, including situations in which low plasma oncotic pressure is suspected or patients have not responded to crystalloid therapy. The incidence of plasma transfusion varies widely based on the degree of prematurity, need for surgery, and presence of a comorbid condition.[18] The wide variation in plasma transfusion may be related to variation in the assessment of coagulation and treatment of abnormal coagulation function studies.[67] Evidence suggests that increasing coagulation testing is associated with more frequent use of plasma transfusion.[68]

Plasma transfusion is often used in preterm infants to treat abnormal coagulation tests. However, there is no evidence to support an association between abnormal coagulation values in the first week of birth and serious hemorrhage, including IVH.[69] No contemporary clinical trials exist to guide the use of plasma transfusion in neonates, particularly as it relates to the prevention of bleeding. The most common INR associated with plasma transfusion in neonates is 1.7, with the 10th and 90th percentiles 1.2 and 2.8, respectively.[18] One randomized trial compared the prophylactic use of plasma compared with dextrose infusion or gelatin plasma (Gelofusine) and found no beneficial effect of early plasma transfusion with regard to death or cranial ultrasound scan abnormality.[70]

Cryoprecipitate is used to treat or prevent bleeding in infants with acquired hypofibrinogenemia (fibrinogen level <100 or 150 mg/dL) or as part of a massive transfusion protocol (discussed later in this chapter). A single unit of cryoprecipitate usually contains 15 to 20 mL, although there is a paucity of data to guide optimal dosing, with infants typically transfused 5 to 10 mL/kg (or corresponding unit dose such as 0.5 units depending on weight). Of note, a full unit of cryoprecipitate may be too high a dose for most infants.

Risks of Plasma and Cryoprecipitate Transfusion

The risks of plasma transfusions include TRALI, as mentioned previously, and infection. In addition, two studies have reported an increased risk of thrombosis among neonates[71] and children[72] who received plasma transfusion. In the study by Maruyama et al.,[71] a total dose of FFP of more than 50 mL/kg in the first 5 days after birth was associated with an increased risk of venous thrombosis (adjusted OR of 5.9, 95% CI 1.1 to 41.8). Potentially, the use of thromboelastography, which measures multiple aspects of clot formation, strength, and fibrinolysis, may provide a more precise measure of coagulation to better guide the use of plasma transfusion in neonates.[73]

Special Circumstances

Massive Transfusion

Massive transfusion may be required in neonates with large-volume blood loss. On the basis of evidence from adults and children, the use of massive transfusion protocols with balanced ratios of blood products (RBCs, plasma, platelets, cryoprecipitate) may decrease the adverse effects of massive transfusion, particularly on the ability of the neonate to maintain hemostasis.[74] In addition, the risks of transfusion previously discussed, such as metabolic complications or TACO, are increased in massive transfusion and require close monitoring. The appropriate ratio of blood products is uncertain, but studies[74,75] have suggested use of a 1:1 ratio of RBCs and FFP, with additional alternating use of platelet and cryoprecipitate transfusions. In adults, the use of whole blood among trauma patients requiring massive transfusion has been evaluated and may be associated with better outcomes,[76] but this has not been sufficiently evaluated in preterm infants. The use of fresh whole blood for cardiopulmonary bypass priming has no advantage over the use of a combination of products during surgery for congenital heart disease, with whole blood priming associated with an increased length of stay and more fluid overload.[77]

Exchange Transfusion

The indications for exchange transfusion include severe hyperbilirubinemia, often in the setting of hemolytic disease of the fetus and newborn with ABO or Rh incompatibility or other minor RBC antigen–antibody incompatibilities with the mother, as well as anemia with fluid overload (e.g., hydrops fetalis) or hemochromatosis. Exchange transfusion is a relatively uncommon treatment following the introduction of Rh_0(D) immune globulin prenatally administered to the mother and intensive phototherapy for the infant. In one series, only 5 of 111,009 infants in a cohort from California NICUs received exchange transfusion for severe hyperbilirubinemia (bilirubin level >30 mg/dL),[78] and more recent data estimate this may be as low as 1.9 per 100,000 live births.[79]

For infants with severe hyperbilirubinemia, exchange transfusion is the most effective method of removing bilirubin rapidly, while simultaneously removing and diluting the offending antibody and RBC antigen target for the antibody. Typically, a double-volume exchange transfusion is recommended.[80] This involves removing twice the estimated amount of circulating blood volume (70 to 80 mL/kg for term infants, 90 to 100 mL/kg for preterm infants) and replacing it with

RBCs reconstituted with plasma over 1 to 3 hours, to a target hematocrit of the infant. The RBCs should be leukoreduced, and, if they are going to a fetus for intrauterine transfusion, they should be irradiated. Small volumes, 5 mL/kg or less, with absolute aliquot volumes ranging from 5 to 20 mL, are exchanged serially, with each exchange occurring in several minutes. This procedure replaces approximately 85% of native circulating RBCs.

The most common risks following exchange transfusion are hypocalcemia (estimated to occur in 29% to 38% of infants), thrombocytopenia (38% to 44%), and infection.[81] Less common risks include catheter-related complications, thrombosis, bleeding, hypothermia, necrotizing enterocolitis, and cardiac arrhythmia. The historical risk of death from exchange is reported to be 3 per 1000 procedures (0.3%), although the current modern-era risk is difficult to estimate given the infrequent use of this therapy and differences in patient populations of infants undergoing exchange transfusion.

In addition to double-volume exchange transfusions, partial exchange transfusions involve removing whole blood from a patient and replacing it with an equal volume of colloid or crystalloid. This is often done in response to polycythemia (hematocrit ≥65% to 70%), although the benefit of partial exchange transfusions in reducing complications from polycythemia has not been well established.[82]

Intravenous Immune Globulin

Intravenous immune globulin (IVIG) therapy is a therapy using pooled Ig from multiple donors, often several hundred. In neonates, the most common indication is treatment of hemolytic disease of the fetus and newborn that is refractory to intensive phototherapy.[83] IVIG works by blocking Fc receptors in the splenic reticuloendothelial system from destroying RBCs or platelets that are bound to maternal circulating antibodies, although the exact mechanisms are not clear. Treatment with IVIG decreases the risk of exchange transfusion and, among those receiving exchange transfusion, decreases the risk of subsequent exchange transfusion.[84] However, the effect of IVIG in studies with a low risk of bias shows no clear benefit.[85] Overall, IVIG is well tolerated and has been extensively studied in the treatment of sepsis.[86] However, hypotension is a common risk, and the risk can be reduced by decreasing the infusion rate.[87] In addition, rare risks, including viral infection[88] and necrotizing enterocolitis,[89] have been reported, although in a systematic review and meta-analysis, no serious adverse effects of IVIG were identified.[86]

Neonatal Alloimmune Thrombocytopenia

Neonatal alloimmune thrombocytopenia (NAIT) is a rare disorder that can lead to severe bleeding, including intracranial hemorrhage, in the fetus or neonate. Thrombocytopenia results from maternal alloimmunization against paternally derived platelet antigens, most commonly human platelet antigen (HPA)-1a,[90] although more than 27 different HPAs have been associated with NAIT. NAIT is estimated to occur in approximately 1 per 1000 live births.[91] Typically, the mother is negative for HPA-1a and often, but not always, positive for anti-HPA-1a antibodies.[92] The fetus is HPA-1a positive, inheriting the gene from the father. Pregnant women become sensitized to the platelet antigen, resulting in transplacental passage of antiplatelet antibodies that cause immune-mediated destruction of platelets and thrombocytopenia.

Severe hemorrhage is estimated to occur in 10 per 100,000 neonates effected with NAIT, and hemorrhage commonly occurs before birth.[93] Therefore, prenatal IVIG treatment has been investigated and has been found to increase fetal platelet counts,[94] although the beneficial effect on reducing intracranial hemorrhage is unclear. If NAIT is diagnosed in an infant, future pregnancies typically undergo close monitoring, with testing of the father and, if indicated, the fetus. After birth, treatment involves transfusion using platelets obtained from the mother that are negative for the offending paternal antigen or from a random donor. There is no clear benefit of IVIG use postnatally,[95] but it may be used if platelets are not immediately available[96] given antibody–mediated platelet clearance. For most infants with NAIT, thrombocytopenia resolves over time as the passive antibody degrades.[97] Additional information about NAIT is given in Chapter 68 and readers may want to review the International Collaboration for Transfusion Medicine Guidelines (ICTMG) for treatment recommendations.[96]

Decreasing the Need for Red Blood Cell Transfusion

Anemia is nearly universal in extremely preterm infants,[98] largely owing to phlebotomy-related blood losses.[99] The effect of erythropoiesis-stimulating agents, such as erythropoietin and darbepoetin, on reducing RBC transfusion and donor exposure has been modest,[100,101] although some studies show promising beneficial effects of erythropoiesis-stimulating agents on long-term neurocognitive outcome in extremely preterm infants[102,103]; other studies have shown no long-term neurodevelopmental benefit.[30] Strategies to reduce phlebotomy-related blood losses[104] are important aspects of patient blood management.

Placental transfusion from delayed cord clamping is an important strategy to decrease the need for RBC transfusion[105] and reduce in-hospital mortality.[106] Delayed cord clamping should be considered as part of an institutional transfusion protocol. Cord milking should be avoided in extremely preterm infants, particularly those <28 weeks' gestation, given the potential for an increased risk of severe IVH.[107]

Suggested Readings

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71

Neonatal Leukocyte Physiology and Disorders

JOHN T. BENJAMIN, BENJAMIN A. TORRES, AND AKHIL MAHESHWARI

KEY POINTS

- The neonate and the young infant depend primarily on the innate immune system for host defense. With limited prior exposure to infectious and environmental antigens, the adaptive immune arm is still in a phase of structural and functional development.
- Neutropenia is frequently encountered during the neonatal period.
- Neonatal neutrophils show a wide range of functional deficiencies in movement, phagocytosis, and microbial killing.
- With the exception of a few deficiencies, neonatal monocytes and macrophages are functionally comparable to their counterparts in adults.
- Neonatal T-cell and B-cell populations are still developing. Several adaptive mechanisms, such as the presence of B1 cells that can function without assistance from T cells and the production of immunoglobulins with polyspecific antigen binding, are unique to the neonate and partially mitigate the deficiencies in adaptive immunity.
- The innate lymphoid cells are a recently described exciting new subset in innate immunity that are likely to play a major role during the neonatal period and early infancy.

This chapter presents an overview of neonatal leukocyte physiology and quantitative and qualitative disorders of leukocytes. Topics include the normal physiology and defects associated with neonatal hematopoiesis, neutrophils, monocytes, lymphocytes, dendritic cells (DCs), and innate lymphoid cells (ILCs) (Fig. 71.1). Novel therapeutic approaches are also discussed.

Neutrophil Physiology and Function

Ontogeny

Neutrophils are an important line of defense in the cellular innate response (see Fig. 71.1). The life cycle of a neutrophil can conceptually be divided into three phases, representing time spent in (1) marrow, (2) blood, and (3) tissues. The earliest neutrophilic precursors, myeloblasts, promyelocytes, and myelocytes, are capable of cell division and thus are referred to as the *neutrophil proliferative pool* (NPP). In later stages of maturation, neutrophils lose their ability for cell division. These metamyelocytes, bands, and segmented neutrophils continue to differentiate in situ and constitute the neutrophil storage pool (NSP). In adults, the NSP is a sizeable reservoir of neutrophils that can be rapidly mobilized into the bloodstream when needed. However, the NSP is relatively much smaller in the midgestation fetus and preterm infant and can be readily exhausted during sepsis. The NSP contains about 6×10^9 cells per kilogram in adult rats. In contrast, the rat NSP at 19 days' gestation contains only about 0.9×10^9 cells per kilogram, which expands marginally to 1.2×10^9 cells per kilogram at term (21 days). Unlike in fetal and newborn rodents, in whom the liver and spleen house a significant fraction of the NPP and NSP,[1] neutrophil production and storage in human neonates occur primarily in the bone marrow.[2]

Circulating and Marginated Blood Neutrophil Pools

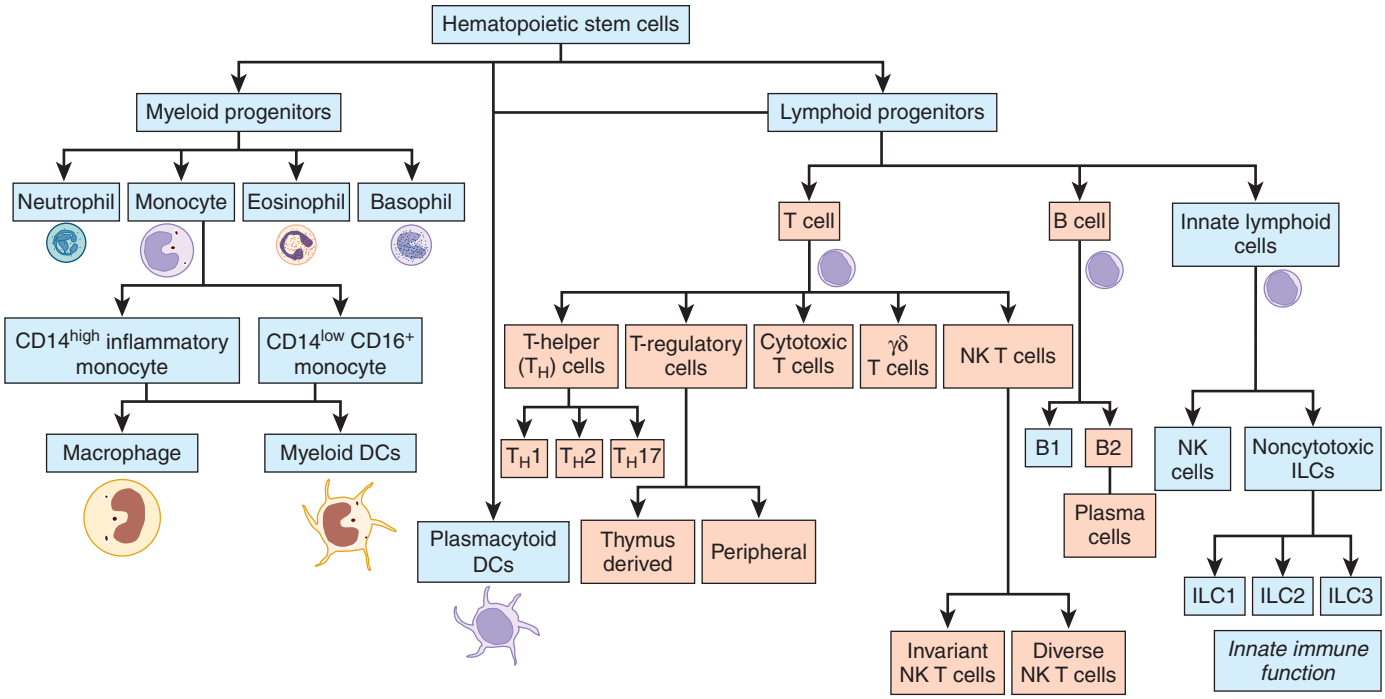
Circulating neutrophils are distributed into two compartments of approximately equal size, the circulating and marginated pools. As the name suggests, neutrophils in the circulating compartment freely circulate in the bloodstream, whereas those in the marginated compartment are transiently attached to the endothelium. In healthy adults, the circulating and marginated pools both contain approximately 0.4×10^7 cells per kilogram.[3] The marginated cells can move into the circulation for short periods of 30 to 45 minutes after strenuous crying or exercise or following administration of epinephrine or corticosteroids.[4,5]

Neutrophils remain in circulation for a few hours (half-life of about 6.3 hours) and then migrate into the tissues. During infection, neutrophil trafficking into the tissues is increased. In fetal sheep, intra-amniotic exposure to endotoxin caused an initial drop in circulating neutrophil counts due to tissue emigration, which was followed by a gradual increase over the next 6 days. The length of time that neutrophils spend in the tissues and their subsequent fate is not well understood.

Neutrophil Heterogeneity

There is now evidence for the existence of different neutrophil subsets with distinct molecular markers. Details of differences in function are still emerging. Three molecular markers have been evaluated most frequently:

- Olfactomedin 4 (OLFM4) is a glycoprotein that has been suggested to act as a tumor suppressor and has recently been identified in specific granules of approximately 25% of circulating



• **Fig. 71.1** Leukocyte Populations in the Neonate and the Young Infant. Hematopoietic stem cells differentiate along the myeloid and lymphoid lineages to ultimately give rise to leukocyte populations that participate in the innate *(blue background)* or adaptive *(orange background)* immune responses. *DC*, Dendritic cell; *ILC*, innate lymphoid cell; *NK*, natural killer.

human neutrophils. The expression of OLFM4 could negatively regulate the efficiency of bacterial killing in a subset of neutrophils.[6]

- The surface glycoprotein CD177 (NB1) is a 55-kDa glycosylphosphatidylinositol-anchored receptor that is expressed at various levels on circulating neutrophils. Several distinct functions have recently been attributed to CD177, including high-affinity binding to platelet–endothelial cell adhesion molecule–1 and the ability to associate with the serine protease PR3. During infection, CD177+ neutrophil subsets may show increased tissue infiltration as aided by the associated cell surface PR3.[7]
- Intercellular adhesion molecule (ICAM)-1–positive neutrophils are believed to undergo reverse transendothelial migration from the tissues to enter the bloodstream and may be involved in the systemic dissemination of inflammation.[8]

Recent studies demonstrate additional heterogeneity in neutrophil phenotypes depending on age, microenvironment, priming by microbiota, and exposure to inflammation. These distinct phenotypes result in differences in cytokine production, phagocytosis, and neutrophil extracellular traps (NETs) production.[9] For example, while neutrophils in the spleen are $CD26L^{low}$, $CD11b^{hi}$, $ICAM\text{-}1^{hi}$ and produce NETs,[10] neutrophils in lymph nodes express CCR7, LFA-1, and CXCR4 and interact with T lymphocytes and mediate T cell activation.[11] Similarly, exposure to antibiotic-resistant *Staphylococcus aureus* results in two distinct subsets of murine neutrophils based on TLR expression, cytokine production, and macrophage activation potential. PMN-1 neutrophils express TLR2/4/5/8, produce IL-12, and classically activate macrophages; PMN-2 neutrophils express TLR2/4/7/9, produce IL-10, and alternatively activate macrophages.[12] Thus, neutrophils are complex inflammatory cells that can mediate distinct immune and non-immune functions both during homeostasis and in various disease states.

Neonatal Neutropenia

Statistically, neutropenia is defined as an absolute neutrophil count (ANC) less than two standard deviations below the mean value, or below the fifth percentile, for postnatal age. Manroe et al.[13] established reference values for ANCs in term and preterm infants during the first 28 days of life for both healthy infants and those with perinatal complications. Mouzinho et al.[14] studied serial white blood cell counts in healthy preterm very low-birthweight (VLBW) infants to investigate whether this patient cohort had neutrophil counts different from those found in previous studies in which cohorts consisted mostly of term infants. They detected a wider range of the ANC, mostly resulting from a downward shift of the lower boundary, especially during the first 60 hours of life. However, there was no difference in absolute total immature neutrophil counts or in the ratio of immature neutrophil counts to total neutrophil counts. Schmutz et al.[15] showed that the ANC peaked at 6 to 8 hours for neonates born at 28 weeks' gestation or later but at 24 hours for those born before 28 weeks' gestation. Table 71.1 provides the 5th and 95th percentiles for ANCs at 72 to 240 hours among neonates in that study. In that study, ANCs were higher in neonates born after a prolonged period of labor than in those born by elective cesarean delivery. Female infants also had higher ANCs, averaging about 2000/μL more than their male counterparts.

In neutropenic infants, the pathophysiologic mechanisms include exhaustion of myeloid progenitors, inadequate response of the progenitor cells to proliferative or maturational signals, and increased usage and destruction. Fig. 71.2 highlights the causes of

neutropenia in the newborn period. Some of the more frequently encountered causes of neonatal neutropenia are discussed in the following sections.

Sepsis-Induced Neutropenia

Neonates with overwhelming sepsis often develop neutropenia, which illustrates some of the differences between adult and neonatal neutrophils. Neonates have fewer neutrophil progenitors and a diminished precursor storage pool, so neutrophils are easily depleted in stress conditions.[16] Following experimental sepsis with group B streptococci, adult rats respond with a transient decrease in circulating neutrophil counts, followed by significant neutrophilia associated with a twofold to threefold increase in the progenitor pool (colony-forming unit–megakaryocyte) and an increase in the proliferative rate to 75% of the maximal capacity.[17,18] In contrast, neonatal rats under the same conditions had a decrease of 50% of their progenitor pool and failed to increase their myeloid proliferative rate, which, as discussed previously, was already at near maximal levels. Most important, during experimental sepsis, neonatal rats had further depletion of their already reduced NSP reserves by almost 80%, compared with a decline of 33% in adult rats.

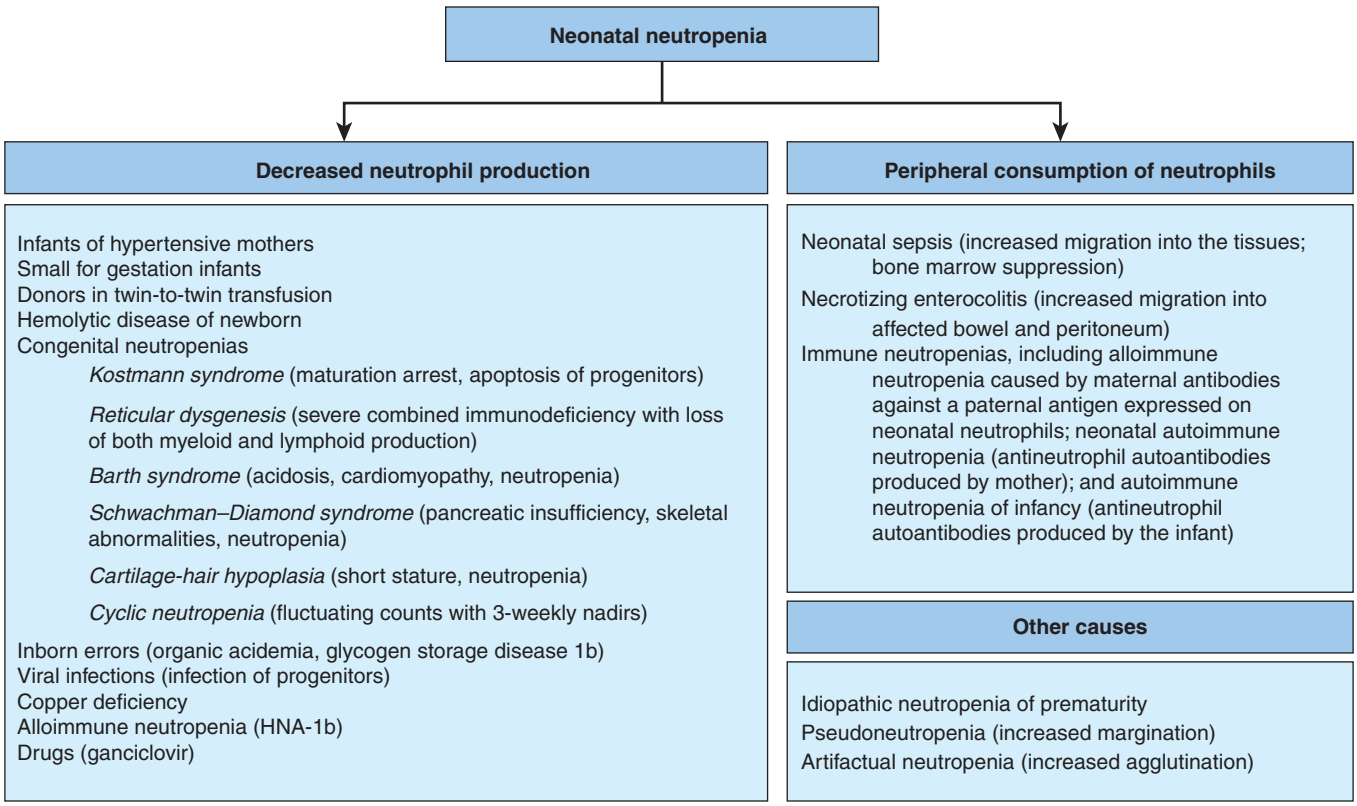
TABLE 71.1 Absolute Neutrophil Count in Neonates at 72–240 Hours of Age

Gestational Age	5th Percentile	95th Percentile
>36 weeks	2700/μL	13,000/μL
28–36 weeks	1000/μL	12,500/μL
<28 weeks	1300/μL	15,300/μL

From Schmutz N, Henry E, Jopling J, Christensen RD. Expected ranges for blood neutrophil concentrations of neonates: the Manroe and Mouzinho charts revisited. *J Perinatol.* 2008;28(4):275–281.

Immune-Mediated Neonatal Neutropenia

Immune-mediated neutropenia is important to consider as a diagnostic possibility in infants with persistent neutropenia. Alloimmune neonatal neutropenia occurs as a result of maternal sensitization to neutrophil antigens present on the infant's neutrophils (paternally acquired) that are not present on the maternal neutrophils, with subsequent production of immunoglobulin G (IgG). Neutrophil-specific antibodies are found in the maternal and infant sera, but the mother has a normal neutrophil count. Alloimmune neonatal neutropenia is estimated to occur at a frequency of 3% of live births.[19] The antigens most commonly involved in the United States are human neutrophil antigen (HNA)-1a, HNA-1b, and HNA-2a. Because the antibodies are IgG, which crosses the placenta, peripheral blood counts show profound neutropenia. The condition is self-limiting and typically resolves within 6 to 7 weeks, during which time the neonate is susceptible to infections, mostly cutaneous in nature. Most infections are mild, although infants with profound neutropenia are at risk of life-threatening infections and should be monitored closely.[20] Neonatal autoimmune neutropenia occurs when mothers have antineutrophil antibodies that cross transplacentally and bind fetal neutrophils. This form of neutropenia is generally milder in severity than alloimmune neutropenia.[21]



• **Fig. 71.2** Causes of Neonatal Neutropenia. *HNA*, Human neutrophil antigen.

Maternal Hypertension–Associated Neutropenia

One of the most common and well-described causes of transient neonatal neutropenia is maternal hypertension. Neonatal neutropenia is inversely related to birthweight and gestational age and directly related to the severity of hypertension. Infants of hypertensive mothers seem to have decreased production of neutrophils, but the cause is uncertain. Several studies have demonstrated a decrease in the numbers of neutrophil progenitor cells, decreased cycling of these cells, a relatively normal NPP and NSP, and the absence of a "left shift."[22] Studies show conflicting evidence for the risk of infection in these infants,[14,23,24] although the risk is probably low because neutropenia resolves in most cases within 72 hours, and almost always in 5 to 7 days.[25,26]

Idiopathic Neutropenia of Prematurity

This is generally a benign form of neutropenia that presents after the early neonatal period (weeks 4 to 10). Neutropenia is usually transient and recovers spontaneously in a majority of patients. Differential leukocyte counts show few immature neutrophils (in the presence of neutropenia), suggesting a transient suppression of neutrophil regeneration in these patients. In some premature infants, idiopathic neutropenia can be severe (sometimes ANC <500/μL) and prolonged, but even in these infants neutropenia resolves spontaneously.[27]

Treatment of Neonatal Neutropenia

Recombinant granulocyte colony-stimulating factor (G-CSF) and granulocyte-macrophage colony-stimulating factor (GM-CSF) are often used to treat neonatal neutropenia. G-CSF stimulates neutrophil production, maturation, and release from the marrow and also reduces neutrophil apoptosis. [28] GM-CSF generates both granulocyte and macrophage colonies from precursor cells.[29] G-CSF is the primary systemic regulator of the circulating neutrophil concentrations.[28] GM-CSF may not play a major role in the steady state but is induced in inflamed tissues.[30]

Recombinant G-CSF or GM-CSF has been used in neonatal sepsis with conflicting results.[31–33] In a meta-analysis of five studies, G-CSF was shown to reduce mortality (odds ratio 0.17, 95% confidence interval [CI] 0.03 to 0.70).[34] However, this protective effect was no longer significant when nonrandomized studies were excluded. The role of GM-CSF in the prevention or treatment of neonatal sepsis is also unclear.[35–37] Carr et al.[38] reviewed the efficacy and safety of G-CSF/GM-CSF in the treatment or prophylaxis of neonatal sepsis. In preterm infants with suspected sepsis, G-CSF or GM-CSF did not reduce mortality at 14 days after the start of therapy (relative risk [RR] of death 0.71, 95% CI 0.38 to 1.33). However, in a subgroup of 97 infants with sepsis and neutropenia (ANC <1700/μL), there was a significant reduction in mortality (RR 0.34, 95% CI 0.12 to 0.92; number needed to treat 6, 95% CI 3 to 33).

Recombinant G-CSF is highly effective in correcting immune-mediated neutropenia. Along with its effects on neutrophil production, G-CSF may decrease antigen expression on neutrophils, rendering them less vulnerable to circulating antibodies,[39] and also improve neutrophil function.[40] Similarly, G-CSF is also effective in many patients with congenital neutropenias.[41,42] Kostmann syndrome presents in early infancy with low ANCs, often less than 200/μL, and recurrent bacterial infections. These patients have mutations in the neutrophil elastase gene *ELANE*.[43] G-CSF can reduce the need for antibiotics and hospitalization[42] and can improve survival in these patients. Cyclic neutropenia is another rare hematologic disorder that may be modified by the use of G-CSF. Cyclic neutropenia is characterized by regular drops in ANCs to levels as low as 250/μL at 3-week intervals.[44] Although the marrow may look normal during periods of higher ANCs, it shows a characteristic "maturational arrest" of myeloid cells during or just before the onset of severe neutropenia. G-CSF treatment can be used to raise neutrophil counts during periods of severe neutropenia.[45] These patients may not respond to GM-CSF.[46] While neutropenia resolves spontaneously in most patients with idiopathic neutropenia of prematurity, in one study, G-CSF treatment induced a rapid rise in ANC, indicating an adequate marrow neutrophil reserve in these patients.[47]

G-CSF is administered intravenously or subcutaneously at a dosage of 5 to 10 μg/kg/day. The response to G-CSF therapy and a rise in ANC usually occurs within 24 to 48 hours. In an occasional patient, G-CSF therapy will not raise blood neutrophil counts. In such a patient, G-CSF doses can be increased in increments of 10 μg/kg at 7- to 14-day intervals if the ANC remains below 1000/μL.[48,49] Doses can be reduced or withheld once the ANC exceeds 5000/μL. G-CSF treatment is usually tolerated in neonates without adverse effects. Long-term G-CSF therapy in congenital neutropenias has been associated with splenomegaly, thrombocytopenia, osteoporosis, myelodysplastic syndrome/leukemia, and the development of anti–G-CSF antibodies.[50,51]

Intravenous immunoglobulin (IVIG) is effective in about 50% of infants with alloimmune and autoimmune neutropenia.[52–54] IVIG can mobilize neutrophils from the NSP, although repeated doses may be needed for a sustained effect.[55,56] While steroids are generally not effective in alloimmune neutropenia,[57,58] may raise the ANC for short periods in autoimmune neutropenia of infancy. Exchange transfusions are generally not effective in immune-mediated neutropenia.[59]

Monocyte Physiology and Dysfunction

Ontogeny

Embryonic macrophages are found among hematopoietic cells in the yolk sac at 3 to 6 weeks' gestation. The fetal liver becomes the primary site of hematopoiesis from 6 weeks until midgestation, and the bone marrow then becomes the lifelong center of blood cell production.[60] Monocytes are present in high proportions in the early hematopoietic tissues, with approximately 70% of hematopoietic cells at 4.5 weeks' gestation morphologically identifiable as monocytes. This proportion falls from 1% to 2% during the next 6 weeks as erythroid cells become predominant. The precursors of monocytes, monoblasts, and promonocytes continue to be present in the fetal liver; however, intravascular monocytes are not observed until the fifth month of gestation. Circulating monocytes do not appear with regularity until hematopoiesis is first established in the bone marrow after the 10th week of gestation.

During ontogeny, macrophages in the fetal liver express CD11b as early as 12 weeks' gestation. The classic monocyte marker, CD14, does not appear until about 15 to 21 weeks' gestation. CD14 expression on circulating mononuclear cells is equivalent in cord blood and adult peripheral blood. CD11a, CD11b, and CD11c are expressed in lower densities on cord blood monocytes than on adult cells. There is also a lower expression of class II major histocompatibility complex (MHC) antigens HLA-DR, HLA-DP, and HLA-DQ on neonatal monocytes compared with adult monocytes. The density of these class II MHC antigens has been correlated with the antigen-presenting capacity of monocytes

in vitro, although the effect of this deficiency on neonatal host defense is not clear. Other important monocyte markers are the receptors for the Fc moiety of IgG (FcγR) and the Toll-like receptors (TLRs). FcγR receptors are important in the process of monocyte and macrophage phagocytosis of microbes and antibody-dependent cytotoxicity. Monocytes constitutively express the high-affinity receptor FcγRI (CD64) and FcγRII (CD32). TLRs play a critical role in recognition of microbial pathogens. Term neonatal monocytes express normal basal levels of TLR2 and TLR4 but show reduced tumor necrosis factor (TNF) release in response to stimulation with a range of TLR agonists.[61–63] Cord blood monocytes of preterm neonates, however, have lower TLR4 expression than adult peripheral blood monocytes.[64]

Macrophage colony-stimulating factor (M-CSF) is a hematopoietic growth factor that regulates the proliferation, differentiation, and functional activation of monocytes. Normally detected in human serum, M-CSF plays an important role in enhancing the effector functions of monocytes and macrophages. Serum M-CSF levels are increased in cord blood and rise further during the neonatal period.

Circulating Monocytes

Term infants show a relative monocytosis that persists through the neonatal period. Although there is some disagreement about normal blood monocyte counts in neonates, we have described normal ranges of absolute monocyte counts (AMCs) using data from more than 62,000 blood counts.[65] In this cohort, blood monocyte concentrations increased almost linearly between 22 and 42 weeks' gestation. Monocyte concentrations also increased during the first 2 weeks postnatally. These data are consistent with previous kinetic studies in human fetuses that show a similar maturational increase in the concentrations of monocyte precursors.[66,67] In neonates, monocytosis has been associated with prematurity, blood transfusions, albumin infusions, and theophylline therapy. Monocytosis has also been described in infants with congenital infections such as candidiasis and syphilis.[68,69] and in association with immune-mediated neutropenia.[70] In contrast, monocytopenia is not seen frequently in neonates, except in growth-restricted preterm infants who may have low monocyte counts as part of an overall suppression of all leukocyte lineages.[71] Recently, we showed that a fall in AMCs can be a useful diagnostic marker of necrotizing enterocolitis (NEC) in VLBW infants.[72] In this study, we compared blood counts obtained at the onset of feeding intolerance with the last available counts obtained before the onset of symptoms. In an infant with feeding intolerance, a drop in AMC of more than 20% indicated NEC with a sensitivity of 0.70 (95% CI 0.57 to 0.81) and specificity of 0.71 (95% CI 0.64 to 0.77). The negative predictive value was 88%, indicating that the test may be valuable for exclusion of the diagnosis of NEC in infants with feeding intolerance due to other causes. Despite modest diagnostic accuracy, AMC is a convenient tool because the information is already available at no extra cost in complete blood counts from automated hematology analyzers.

Monocyte Subsets

Increasing evidence indicates that peripheral blood monocytes are a heterogeneous population comprised of two major subpopulations. The predominant subtype is "classic" $CD14^+CD16^-$ monocytes, which express C-C chemokine receptor (CCR)2, CD64, and CD62L and represent nearly 80% to 90% of all blood monocytes. The remaining fraction comprises the "nonclassic" $CD14^{low}CD16^+$ monocytes that lack CCR2.[73] Both subsets express the receptor for fractalkine, CX3C chemokine receptor (CX_3CR)1, but $CD14^{low}CD16^+$ monocytes characteristically express higher levels. $CD16^+$ monocytes are composed of at least two populations with distinct functions. Monocytes that express CD16 and CD14 ($CD14^+CD16^+$) also express the Fcγ receptors CD64 and CD32, have phagocytic activity and produce TNF and interleukin (IL)-1β in response to lipopolysaccharide (LPS). In contrast, monocytes that express CD16 but very low levels of CD14 ($CD14^{dim}CD16^+$) lack the expression of other Fc receptors, are poorly phagocytic and do not produce TNF or IL-1β in response to LPS.[74] This subset of "resident" monocytes patrol blood vessels in the steady state and extravasate during infection with *Listeria monocytogenes* or during tissue healing.[75]

Monocyte Function

Monocytes are capable of directed movement (chemotaxis) in response to chemoattractants produced by bacteria or by host cells at the site of injury or invasion. The chemotactic capabilities of neonatal and adult peripheral blood monocytes have been compared, and chemotaxis was found to be less pronounced in neonates than in adults (Table 71.2).

During an acute infection, circulating monocytes become activated and migrate into the tissues. During this process, monocytes adhere to the endothelium through the interaction of the integrins (CD11a, CD11b, CD11c, and CD18) expressed on the monocyte cell membrane, with ICAM-1 or ICAM-2 on the endothelial surface. Finally, the activated monocyte moves through the endothelium to the site of inflammation or infection. Preliminary studies demonstrate that the levels of monocyte adhesion molecule expression are comparable in neonate and adult peripheral blood.[76] The CD11b–CD18 complex (macrophage one antigen/complement receptor 3) promotes monocyte trafficking to the sites of infection by binding ICAM-1 and is also involved in the recognition of opsonized microbial pathogens.

TABLE 71.2 Function and Phenotype of Adult Versus Neonatal Mononuclear Cells

Cells	Function/Phenotype	Adult	Neonate
Monocytes	Chemotaxis	↑	↓
	Phagocytosis	↑	↓
	Adhesion	≈	≈
	Respiratory burst	≈	≈
Dendritic cells	Expression of CD83, CD86	↑	↓
	Mixed lymphocyte reaction	↑	↓
	IL-12 (p40) production	↑	↓
	IL-12 production	↑	↓
	IL-10 production	↓	↑
Natural killer cells	Expression of CD8, CD57	↑	↓
	Expression of ICAM-1, CD161	↑	↓
	Cytolytic activity	↑	↓

ICAM-1, Intercellular adhesion molecule1; *IL*, interleukin.

Antimicrobial activity of monocytes includes oxygen-dependent mechanisms such as the respiratory burst, which through a complex series of reactions forms highly reactive hydroxyl radicals that damage host and microbial membranes. The ability of fetal and neonatal monocytes to kill pathogens (*Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli*, and *Candida albicans*) is generally comparable to that of the monocytes in adult peripheral blood (see Table 71.2), although isolated preterm monocytes may show weaker superoxide production and degranulation in vitro than monocytes from term neonates.

On exposure to microbial antigens, monocytes and macrophages become activated by collaborative actions of soluble recognition proteins, including CD14 and TLRs, and produce several cytokines and chemokines contributing to the inflammatory process. IL-1β, interferon (IFN)-α, and TNF are synthesized at similar levels in adults and neonates. Kaufman et al.[77] detected lower TNF production in LPS-stimulated adherent monocytes from preterm infants compared with monocytes from term infants; however, no difference was seen in the production of IL-1β or IL-6. They also found lower expression of CD11b and CD18 adhesion receptor subunits in preterm monocytes. Although TLR expression of term neonatal monocytes is similar to that of adult monocytes, there are important functional differences. Levy et al.[78] reported that cord blood monocytes are less sensitive to TLR ligands than adult monocytes for TNF induction. The innate immune responses of neonatal monocytes to TLR agonists may be biased toward high IL-6 levels but low TNF levels in vitro because of distinct neonatal cellular (monocyte) and humoral (serum) factors, and such a pattern was also evident in vivo.[79]

Developmental Defects in the Phagocytic Immune System in Neonates

The immaturity of the phagocytic immune system predisposes neonates to increased morbidity and mortality during bacterial sepsis. This impairment is attributed to developmental deficiencies in both the innate immune system and the adaptive immune systems (see Fig. 71.1). In the following section, we use the term *phagocyte* to refer mainly to neutrophils and the monocyte/macrophage lineage. Although immature DCs also show phagocytic activity, there is limited information about these cells in the neonate. The phagocyte system depends on the presence of adequate numbers of phagocytes in circulation to function efficiently, the ability to respond to signals from the sites of inflammation, the ability of phagocytes to migrate to these sites, and the capability of phagocytes to ingest and kill invading microorganisms.

Several studies show important differences in TLR expression on neonatal versus adult leukocytes. The basal expression of TLR2 and TLR4 in neutrophils and monocytes from healthy adults and term neonates is similar.[16] During sepsis, neonates showed a sustained upregulation of TLR2 on monocytes but not on neutrophils, and there was no change in TLR4 expression on monocytes or neutrophils.[62] In VLBW infants, monocytes express TLR4 at much lower levels than in mature infants and adults and show significantly lower LPS-stimulated IL-1β, IL-6, and TNF release in vitro.[64] Further, CD14 and MD-2, co-receptors for gram-negative and gram-positive cell wall constituents alongside TLR2 and TLR4, respectively, are also expressed at lower levels on preterm infant monocytes.[80] Stimulation of newborn monocytes with LPS produced a significant decrease in the expression of MyD88, supporting the premise of impaired TLR4-mediated signaling[81] Belderbos et al.[82] compared TLR-induced cytokine responses of whole blood leukocytes from cord blood from healthy term neonates, neonatal venous blood at the age of 1 month, and adult venous blood. On TLR4 activation, neonatal leukocytes (both at birth and at 1 month of age) showed a skewed pattern of cytokine expression, with low levels of T-helper (T_h) type 1–polarizing cytokines such as IL12p70 and IFN-α and more of the anti-inflammatory cytokine IL-10. In contrast, cytokine responses to TLR3, TLR7, and TLR9 matured by 1 month of age.

Recent studies indicate that epigenetic changes during development may underlie the observed maturation in monocyte function with advancing gestational age. Histone modifications H3K4me3 and H3K4me1 affect promoter sites of various immune genes (IL1B, IL6, TNF) in monocytes. Increased H3K4me3 activation at these promoter sites (compared to H3K4me1 activity leading to increased H3K4me3/H3K4me1 ratio) is observed in monocytes with advancing gestational age and thought to be important in modifying the epigenetic landscape of monocytes to a more mature and functional state with age.[80]

The immaturity of neonatal host defense is also characterized by profound deficiencies in quantitative and qualitative phagocytic effector cell function, particularly during stress or bacterial sepsis. The initial step in mounting a host defense response is directed migration or chemotaxis (*chemo* refers to a chemical substance; *taxis* refers to rearrangement) of activated phagocytes toward the site of microbial invasion. Such movement occurs along concentration gradients of chemoattractants, which may include bacterial products such as *N*-formyl-L-methionyl-L-leucylphenylalanine (f-MLP), leukotriene B_4, complement products such as C5a, or chemokines. At the site of inflammation, the phagocytes ingest and kill the pathogens by oxygen-dependent and oxygen-independent mechanisms. Neutrophils from both preterm and term neonates show numerous qualitative abnormalities, including decreased deformability and impaired functions, including chemotaxis, phagocytosis, adherence, bacterial killing, aggregation, and oxidative metabolism. In contrast to neutrophils, neonatal monocytes are more comparable in function to the monocytes of adults.

GM-CSF increases adult neutrophil oxidative metabolism by augmenting superoxide anion production. Additionally, GM-CSF increases chemotaxis, promotes phagocytosis of *Staphylococcus aureus* and augments neutrophil aggregation by the increased expression of surface adhesion molecules.[83,84] GM-CSF is not a direct stimulant of neonatal neutrophil function, but cord blood leukocytes show increased superoxide production if primed with GM-CSF before exposure to f-MLP or opsonized zymosan particles.[85]

The diminished inflammatory response of neonatal neutrophils results in a high incidence of microbial invasion. Significant defects in the upregulation of surface-active glycoprotein receptors (C3bi) and reduced aggregation also predispose the neonate to impaired response to bacterial infection. C3bi expression has been compared in adult and neonatal neutrophils and was found to be significantly less in neonatal neutrophils when stimulated by f-MLP– or zymosan-activated serum. However, cord blood neutrophils incubated with GM-CSF demonstrated a significant induction of C3bi expression. Also, a significant increase in C3bi expression was seen when cord blood neutrophils were pretreated with GM-CSF and subsequently stimulated with the calcium ionophore A23187. The upregulation of the C3bi receptor by GM-CSF also appears to correlate with an enhancement of neutrophil aggregation. GM-CSF also primes neutrophils

for increased neutrophil aggregation following agonist (f-MLP) stimulation.

G-CSF and TNF have also been reported to modulate the function of adult and neonatal neutrophils in a manner similar to that of GM-CSF. Priming cord blood neutrophils with G-CSF or TNF and subsequent stimulation with f-MLP induces the expression of C3bi receptors and enhances the bacterial and phagocytic activity and superoxide generation.

Lymphocyte Contributing to Acquired Immunity

Lymphocytes form critical components of the acquired immune system and constitute 20% of blood leukocytes. There are two broad categories: (1) T lymphocytes, which mature in the thymus and subsequently seed peripheral lymphatic organs, including the spleen and lymph nodes; and (2) B lymphocytes, which are produced in the bone marrow, mature in secondary lymphoid organs, and subsequently differentiate into antibody-secreting plasma cells. The following sections outline the development and function of T and B lymphocytes in the neonate.

T-Lymphocyte Physiology and Function

Ontogeny

T-cell precursors undergo initial differentiation and maturation in the thymus, an organ that arises from the third branchial arch at approximately 6 weeks' gestation.[86] Hematopoietic stem cells destined to become T-cell precursors move to the thymus by 9 weeks' gestation, initially entering the subcapsular cortical areas, and then undergo a period of differentiation as they move from the cortex to the medulla of the thymus. Lymphoid progenitors entering the cortical area do not express the T-cell receptor (TCR) or the coreceptors CD4 and CD8 and are therefore referred to as *double-negative thymocytes*. These cells genetically rearrange α and β genes to generate a TCR with unique antigen specificity. In the next stage of differentiation, this newly rearranged TCR is expressed on the cell surface along with *both* CD4 and CD8, and the cells are termed *double positive*. T cells with TCRs that recognize MHC bound to microbial peptides are "positively selected." Following the double-positive stage, T cells lose expression of either CD4 or CD8 to become "single positive," and during this stage, T cells that recognize "self-peptides" are negatively selected to undergo apoptosis. At the end of this differentiation process, mature T cells that enter the medulla are either $CD4^+$ or $CD8^+$ and can bind host MHC but only when it is not bound to self-antigens. Approximately 98% of thymocytes are eliminated during this process.

T-Cell Receptor Repertoire

The TCR is composed of two different protein chains that are associated with CD3, a protein involved in signal transduction. Most T cells carry a TCR composed of α and β chains, but in about 5% of all T cells, the TCR comprises γ and δ chains instead. Each TCR protein chain contains an immunoglobulin-like extracellular region with a variable (V) domain at its N-terminal and a constant (C) domain at the C-terminal end. The variable domains contain hypervariable, complementary-determining regions (CDRs). The third CDR (CDR3) imparts affinity for a specific processed antigen.[87]

TCR diversity arises from genetic recombination of DNA encoding for a variable (V), diversity (D), and joining (J) gene segments in individual T cells using recombination activating gene (RAG)1 and RAG2 recombinases or gene conversion using cytidine deaminases. These recombinations create a diverse pool of T cells with a wide repertoire of antigen specificity.

T-Cell Subtypes

T cells are broadly categorized as $CD4^+$, $CD8^+$, or T cells that are neither $CD4^+$ nor $CD8^+$. $CD4^+$ T cells recognize antigens bound to class II MHC molecules and are further classified into T_h cells and T-regulatory cells, commonly referred to as T_{regs}.

T-Helper Cells

Naïve $CD4^+$ cells differentiate into three distinct effectors T_h subsets: T_h1, T_h2, and T_h17.[88] T_h1 cells are inflammatory cells that interact with mononuclear phagocytes and provide cellular immunity against intracellular pathogens and virus-infected cells. T_h1 cells produce IL-2, IFN-γTNF, IL-13, and GM-CSF. T_h2 cells associate with B cells and defend against helminths and play a role in allergic reactions through regulation of antibody isotype switching. T_h2 cells produce IL-4, IL-5, IL-9, IL-10, IL-12, and IL-13.[89] T_h17 cells protect against infections, where they take on a T_h1-like effector function to promote pathogen clearance by enhancing neutrophil recruitment to sites of infection, and by activating macrophages. These cells can also play a pathogenic role in allergic, autoimmune, and other chronic inflammatory diseases.[90–92] T_h17 cells produce cytokines such as IL-17A, IL-17F, IL-21, IL-22, and IL-26.

Regulatory T Cells

T_{regs} are derived from naïve T cells.[93] The transcriptional regulator forkhead box P3 (FoxP3) plays a critical role in the development of T_{regs} and is often used as a biomarker for these cells. T_{regs} downregulate the immune response by expressing inhibitory cytokines (IL-10, transforming growth factor β), cytotoxic molecules, modulators of cyclic AMP, and cytokine competition to limit effector and/or antigen-presenting cell function.[94]

Two populations of T_{regs} have been identified: thymus-derived T_{regs} (tT_{regs}) and peripherally derived T_{regs} (pT_{regs}). tT_{regs} develop in the thymus, whereas pT_{regs} develop in the periphery from naïve T cells.[95] $CD4^+CD25^+FoxP3^+$ T_{regs} are important contributors to the control of the immune response in the fetus/neonate. In the fetus, $CD4^+$ T cells have a predisposition to differentiate into tolerogenic T_{regs} that promote self-tolerance. During midgestation, $CD4^+CD25^+FoxP3^+$ T_{regs} may constitute 15% to 20% of all $CD4^+$ cells in the lymphoid tissues.[96,97] T_{regs} represent less than 5% of all $CD4^+$ cells in cord blood from term infants and in the adult peripheral blood.[97] Reductions in $CD4^+CD25^+$ T_{regs} numbers and/or functional impairment of these cells is thought to be important in the pathogenesis of necrotizing enterocolitis.[98,99]

Cytotoxic T Lymphocytes

Following antigen presentation and activation, $CD8^+$ T cells can differentiate into cytotoxic T lymphocytes (CTLs). These cells protect against intracellular pathogens and play a role in graft rejection and tumor surveillance.[100,101] CTLs cause cytotoxicity by releasing pore-forming mediators (perforin/granzyme system) or by activating the Fas/Fas ligand-dependent apoptotic pathway.[102,103]

Gamma-Delta (γδ) T Cells

γδ T cells are detectable in the fetal thymus and liver at 6 to 8 weeks' gestation. At 16 weeks' gestation, γδ T cells constitute 10%

of circulating T cells, but the proportion gradually declines to about 3% at term.[104,105] Postnatally, these cells are present mainly on skin and mucosal surfaces. A unique feature of the γδ T-cell sub-population is the characteristic T-cell receptor composed of a γ-chain and a δ-chain, genes that rearrange during γδ T-cell maturation in the thymus.[106] Although the role of γδ T cells remain unclear, these cells respond to nonpeptide microbial metabolites, show cytotoxicity, and produce various cytokines, including IFN-γ and TNF.[107,108] Recent studies indicate that IL-17A-producing γδ T cells are important to mediate protective immune responses to influenza infection during the neonatal period.[109] The cytotoxicity of neonatal γδ T cells is significantly less than that of their counterparts in adults,[110] although recent studies indicate an adult-like functional maturation in these cells by 2 years of age.[111]

Natural Killer T Cells

Natural killer (NK) T cells are a subset of T cells that express TCR αβ chains, as well as a variety of NK cell markers.[112,113] These cells recognize both exogenous and endogenous lipid antigens in the context of the MHC-like molecule CD1d. CD1d-restricted NK T cells are classified into two main subsets: type I or invariant NK T cells and type II or diverse NK T cells. Type I NK T cells express a conserved αβ TCR and constitute less than 1% of T cells in cord blood.[114,115] These cells have been identified in the human fetal small intestine.[116] Type II NK T cells are more abundant than type I NK T cells in humans and express relatively diverse TCR α and β chains.

Circulating T Cells

Term neonates have higher counts of $CD4^+$ T cells in peripheral blood than adults. The CD4/CD8 ratio is high (up to 4.9:1) in neonates and declines to adult values (2:1) by 4 years of age.[117,118] In cord blood, 85% of the T cells express CD38 (compared with less than 5% in adults) but lack other markers of activation.[119] Nearly 80% of all T cells in cord blood display a naïve CD45RA phenotype, compared with less than half of the circulating T cells in adults.[120,121] The proportion of memory CD45RO T cells reaches adult levels in the second decade.[120]

Neonatal T-Lymphocyte Function

T cells form a critical component of the adaptive immune defense system in the neonate. Although term infants have higher T-cell counts in peripheral blood than adults,[122] preterm neonates tend to have lower $CD4^+$ and $CD8^+$ lymphocyte counts than their term counterparts. This deficiency could, at least in part, place the preterm infant at risk of infection. In contrast to the $CD4^+$ effector T cells, preterm infants have more T_{regs} in cord blood than do term infants or adults in peripheral blood.[123]

Some studies suggest an altered function of $CD4^+$ T cells in neonates compared with adults. T_h1 cell function is decreased in human neonates. Neonatal $CD4^+$ T cells secrete less IL-12 and IFN-γ and express less CD154 (CD40 ligand) on their surface than their counterparts in adults.[124] Cord blood lymphocytes also show a limited capacity to produce IL-17.[125] Similarly, both preterm and term neonatal T_{regs} appear less functional than their adult counterparts. T_{regs} from neonates limits contact between DCs and conventional T cells, downmodulating the expression of costimulatory molecules by DCs. This results in decreased DC immunogenicity and impaired T-cell activation.[126] CTL function is also less efficient than in the neonate compared with the adult.[127] Neonatal CTLs express less perforin than their counterparts in adults. Circulating inhibitors, such as alpha-fetoprotein and prostaglandins, have been implicated as the cause of impaired CTL activity in neonates.[128]

In contrast, other studies indicate that neonatal T cells may not be truly deficient or immature versions of adult T cells but rather represent distinct populations of cells that are, in fact, well suited for adaptations during the perinatal period. These studies suggest a layered immune system model for CD4+ and CD8+ T cells where T cells that arise in the fetal and neonatal period have distinct characteristics and transcriptomic profiles from their adult counterparts. These cells continue to persist into adulthood (alongside the T cells originating in adulthood) and continue to maintain their cell-intrinsic behavior.[129] Critical features that may distinguish neonatal T cells from adult T cells include their origin from fetal hematopoietic stem cells, rapid capacity for proliferation and differentiation, low TCR diversity, increased expression of TLRs, and enhanced ability to respond to inflammation and danger signals and an impaired ability to form memory T cells.[129]

T-cell signals from antigen presentation and T-cell–derived cytokines such as IL-2, IL-4, IL-5, IL-6, IL-10, IL-13, and IFN-γ are required for B-cell proliferation, differentiation, and survival.[130] T-cell and B-cell cross-talk also involves receptor-ligand pairs such as CD40 (on B cell)–CD40 ligand (on T cell), which is critical during B-cell immunoglobulin isotype switch, and B7–CD28, CD11a (lymphocyte function-associated antigen–1)–ICAM-1, and CD58–CD2.[130] Neonatal T cells show lower CD40 ligand expression than adults and are less efficient at providing humoral and CD40-dependent activation signals to B cells.[131,132]

B-Lymphocyte Physiology and Function

Ontogeny

B-cell progenitors are derived from pluripotent hematopoietic cells in the bone marrow.[133] The first recognizable B-cell progenitor is the large pre-B cell, which is characterized by the presence of cytoplasmic μ heavy chains. Pre-B cells can be identified in the fetal liver as early as 7 weeks' gestation and in the marrow by 12 weeks. Immature B cells undergo a selection process analogous to T cells to eliminate self-identifying clones.[134,135] Once B cells begin to express surface IgM (sIgM), they are ready to leave the bone marrow to enter the peripheral circulation[136]; $sIgM^+$ B cells can be seen in the fetal liver by 9 weeks and in the bone marrow, peripheral blood, and spleen by 12 weeks. B cells with surface IgA, surface IgG, and surface IgD (sIgD) isotypes appear between 10 and 12 weeks.[137] IgM/IgD^+ B cells populate the lymph nodes at 16 weeks. Plasma cells are first seen around 20 weeks' gestation. By 22 weeks, the proportion of B cells in the spleen, peripheral blood, and marrow is similar to that in adults.[137,138] The bone marrow becomes the exclusive site for B-cell maturation by 30 weeks' gestation.

B-Cell Subtypes

In the fetus and neonate, most B cells express CD5, a T-cell antigen, on the cell surface. These $CD5^+$ B cells have been referred to as B1 cells to differentiate them from the conventional B2 cells seen in adults. These B1 cells show a greater capacity for bone marrow–independent self-renewal, display an unusual $CD11b^+$ $sIgM^{high}$ $sIgD^{low}$ phenotype, and show constitutive expression of the transcription factor signal transducer and activator of transcription 3.[139,140] These cells are the predominant B-cell type during fetal life and show a distinctive anatomic localization in the spleen and the peritoneal cavity.[141,142] In adults, CD5 expression can be found on about 25% to 35% of all B cells and on less than

5% of mononuclear cells in peripheral blood. In contrast, $CD5^+$ B cells represent 90% of all B cells in cord blood. The proportion falls to 75% to 80% during infancy and approaches adult levels only by late adolescence.[141,142]

The role of B1 cells in the fetus is still unclear. Their unique localization, their broad polyspecific specificities, and the restricted immunoglobulin repertoire suggest a role in innate immunity rather than in adaptive immunity.[139] Unlike follicular B2 cells, which respond to protein antigens and show immunoglobulin heavy chain class switching and affinity maturation, B1 cells respond mainly to T-cell–independent carbohydrate antigens.[143,144]

Regulatory B (Breg) cells are a rare B-cell subpopulation that is $CD19^+CD24^{hi}CD38^{hi}$ and known to promote tolerance and prevent unwanted immune responses. A greater number of Bregs (expressed as a proportion of all B cells) are present in cord blood compared to adult peripheral blood. Cord blood Bregs are also functionally more immunotolerant compared to their adult counterparts and produce higher levels of IL-10 and inhibit IFN-γ and IL-4 production by cocultured T cells.[145] These cells are thought to be important in the fetus to promote immune tolerance to the haploidentical mother, allowing for the normal continuation of pregnancy.

Circulating B Cells

Cord blood contains more B cells than adult peripheral blood, although the ratio of B cells to circulating T cells is similar.[146] B-cell counts peak at 3 to 4 months and then decline gradually to adult levels by 6 years of age[147]). Preterm infants have B-cell counts comparable to those of term infants, although the number may be smaller in growth-restricted infants.[148] Unlike adults, most B cells in cord blood express activation markers such as CD25, CD23, and transferrin receptors.[149]

Neonatal B-Lymphocyte Function

Immunoglobulin Production

The fetus and the neonate can mount an antibody response to antigenic challenges, although they may not be able to respond to all the antigens in a vaccine or may show other deficiencies, such as a delayed isotype switch.[150] Overall, postnatal age seems to be a better predictor of antibody response than gestational age. Both preterm and term infants immunized with diphtheria toxoid in the first 10 days after birth showed weaker antibody responses than similarly immunized adults, but the response was more robust when the vaccine was administered at 1 to 2 months of age.[150] Similarly, premature infants respond poorly to the hepatitis B vaccine in early infancy but show antibody responses comparable with those of their term counterparts in later infancy.[151]

Immunoglobulin Repertoire

Immunoglobulin diversity is generated by DNA recombination involving various V, D, or J gene segments, giving rise to a large number of V(D)J permutations.[152] Additional diversity is generated by imprecise gene segment joins, the addition of extra nucleotides to the splice junction of the VDJ joins, and somatic mutations.[153,154]

In the fetus and neonate, the immunoglobulin repertoire is relatively limited. During early gestation and midgestation, the heavy chain V (V_H) gene segments located closest to the J segments are utilized preferentially, resulting in shorter CDR3 regions than in adults. This altered architecture of the antigen-binding site has been postulated to allow greater polyspecificity of antigen binding, although at the cost of lower antibody affinity.[155,156] The utilization of V_H segments spreads out more evenly with increasing gestation but does not reach adult levels.[157] In general, the antibody response in neonates is of low affinity and restricted to the IgM isotype. Somatic mutation of the heavy and light immunoglobulin variable region genes and the selection of higher affinity antibody-producing B cells are also limited at birth and increase slowly after 10 days of age.[158]

Serum Immunoglobulin Levels

At birth, most of the Igs present in serum are derived from the transplacental transfer of maternal IgG (particularly IgG1 and IgG3) during the third trimester.[159,160] Serum IgG levels in term neonates (up to 1000 mg/dL) may be similar to or higher than those in maternal serum, although preterm infants, who did not receive the maternal antibody, have lower IgG levels.[161,162] During early infancy, Ig levels fall because of normal turnover to reach a nadir of 300 to 500 mg/dL at 3 to 5 months of age, when the infant starts producing increasing amounts of his or her own. This nadir is reached earlier and is typically lower in preterm infants.[163]

Dendritic Cell Physiology and Function

DCs are specialized mononuclear cells with highly developed antigen-presenting capabilities that play a pivotal role in humoral and cellular immunity by initiating T-cell responses. DCs have a distinct morphology and are noted for their irregular shape with veil-like ruffled cell membranes (lamellipodia), many pseudopods, and numerous membrane processes. Microscopic examination of DCs reveals prominent mitochondria, endosomes, and lysosomes within the cytoplasm, which are necessary for the processing of antigens. The morphology of DCs in cord blood and adult peripheral blood is identical, although cord blood contains a lower proportion of DCs (0.5%) than adult blood (1%).

Ontogeny

Most DCs are derived from pluripotent hematopoietic stem cells differentiating along myeloid or lymphoid lineages. An exception may be the *follicular DCs*, a nonmigratory population of DCs located in the paracortical areas of the spleen and lymph nodes, which may be of mesenchymal origin.[164]

Dendritic Cell Subtypes

In humans, DCs are usually classified into the following four subsets[165]:

1. *Myeloid DCs (mDCs) or conventional DCs (cDCs)* express typical myeloid antigens CD11c, CD13, CD33, and CD11b and are homologous to mouse $CD11c^+$ "classic" or "conventional" DCs. In humans, mDCs may be split into $CD1c^+$ and $CD141^+$ fractions.[166,167] $CD1c^+$ mDCs are the major population of human mDCs in the blood (1% of peripheral blood mononuclear cells), tissues, and lymphoid organs. In lymph nodes, $CD1c^+$ DCs are found as "interdigitating cells" of T-cell areas. The expression of CD141 (thrombomodulin) is seen in a smaller proportion of mDCs (0.03% of peripheral blood mononuclear cells). These cells show enhanced ability to take up necrotic cells, sense viral nucleic acids with TLR3 and TLR8, and cross-present antigen to $CD8^+$ T cells.[166]

2. *Plasmacytoid DCs (pDCs)* typically lack myeloid antigens and are distinguished by the expression of CD123 (IL-3 receptor), CD303 (C-type lectin domain family 4, member C), and CD304 (neuropilin). These cells are not related to plasma cells; the name reflects subtle lymphoid features and abundant secretory properties. Two populations of pDC are thought to exist based on their expression of CD2. While both subsets express CD123 and CD303, $CD2^+$ DCs lack the high IRF7 and IFN-I producing capabilities of $CD2^-$ DCs.[168] In quiescent tissues, there are few pDCs, but they may constitute up to 20% of MHC class II–positive cells in lymph nodes and are rapidly recruited to both sites during inflammation. Further, pDCs express high levels of TLR7 and TLR9 and release type 1 IFN in response to viruses.[169]
3. *$CD14^+$ DCs* are a third subset of $CD11c^+$ DCs found in tissues and lymph nodes. Originally described as "interstitial DCs," these cells are more monocyte-like than the other mDCs and may arise from classic $CD14^+$ monocytes.[170]
4. *Langerhans cells and microglia* are two specialized self-renewing DC populations found in stratified squamous epithelium and the brain parenchyma, respectively.

DC populations in the blood include pDCs and $CD1c^+$, and $CD141^+$ mDCs; these cells are immature forms of those found in tissues and lymph nodes. Most epithelial tissues contain "migratory" DC populations that acquire antigens and migrate via the afferent lymphatics to lymph nodes, where they present antigens to T cells. In the steady state, tissues contain $CD1c^+$ mDCs, $CD141^+$ mDCs, and $CD14^+$ DCs but few pDCs. Lymphoid tissue contains blood-derived nonmigratory "lymphoid" or "resident" DCs and some migratory DCs from the tissues.[171]

Mature DCs express high levels of CD80 and CD83 on the cell membrane. Several agents have been shown to increase CD80 and CD83 expression on DCs, including TNF, bacterial endotoxin, and monocyte-derived inflammatory signals. Interactions with other cells also can induce maturation of DCs, as demonstrated by T-cell–mediated cross-linking of CD40.

Morphology

DCs express numerous surface molecules commonly found on mononuclear cells, MHC molecules, CD4, CD45 isoforms, adhesion molecules (ICAM-1, ICAM-2, ICAM-3, and leukocyte function-associated antigen 1), and Fc receptors (FcγRI [CD64] and FcγRII [CD32]). Maturation of DCs is commonly associated with high expression of CD80 and CD83. On stimulation, DCs upregulate the costimulatory molecules CD40, CD80, and CD86. Cord blood DCs express lower levels of ICAM-1 (CD54), MHC class I (HLA-ABC), and MHC class II (HLA-DR), CD80, and CD40 antigens than DCs in adult peripheral blood.[172,173] Liu et al.[174] compared monocytes from cord blood and adult peripheral blood for the capacity to generate DCs; cord blood monocytes generated fewer DCs, which also showed lower MHC class II expression. The endocytotic ability and ability to stimulate CD3+ T cells were also reduced in cord blood DCs.

Function

The activation of TLR pathways has been shown to mediate DC maturation. Krutzik et al.[175] demonstrated that TLR activation of monocytes induces rapid differentiation to DCs that could be expanded by TLR-mediated upregulation of GM-CSF. Cord blood monocyte-derived DCs showed lower expression of the maturation markers CD83 and CD86 and produced fewer IL-12 (p40, p70) than adult DCs.[82,172,176] In mixed leukocyte culture with adult CD4+ T cells, neonatal DCs induced significantly lower levels of IFN-γ but higher levels of IL-10 than did adult DCs (see Table 71.2). Furthermore, neonatal DCs also performed poorly as accessory cells for T-cell mitogenic responses.[173]

Innate Lymphoid Cell Physiology and Function

ILCs include the classic cytotoxic NK cells and the recently described noncytotoxic ILC populations.[177] These cells are characterized by a classic lymphoid cell morphology but, unlike the adaptive T and B cells, do not show antigen specificity and function as a part of the innate immune system.

Natural Killer Cell Physiology and Function

NK cells have the ability to lyse target cells and secrete immunomodulatory cytokines.[178] These cells make up 10% of peripheral blood lymphocytes and are characterized by the lack of the lymphocyte marker CD3 but an expression of CD56.[179]

Ontogeny

NK cells develop from the committed lymphoid progenitors in the aortogonadomesonephron and later within the fetal liver.[180] Lineage-specific NK-cell progenitors are first identified in the fetal thymus and later in the bone marrow.[181,182] Human NK-cell development requires early growth factors such as FMS-like tyrosine kinase (Flt)-3 ligand or Kit ligand and later IL-15. Based on the expression profile of cell surface markers and functional maturity, distinct stages of NK-cell maturity have been recently described.[183]

Natural Killer Cell Subtypes

NK cells can be classified on the basis of the surface density of CD56 into $CD56^{bright}$ NK cells and $CD56^{dim}$ NK cells. Early NK cells are $CD56^{bright}$, which then undergo further differentiation into $CD56^{dim}$ NK cells. The $CD56^{dim}$ NK cells are the more cytotoxic of the two categories and represent 90% of the NK-cell population. These cells are also the predominant NK cell population in peripheral blood and express CD16 (FcγRIII), and show antibody-dependent cellular cytotoxicity.[183,184]

Function

NK cells mediate their effector immune function by cytotoxicity and/or expression of a plethora of pro-inflammatory cytokines.[183] NK cells express a repertoire of classes of receptors that bind to MHC class I or MHC class I–like molecules that regulate whether NK cells will be activated or inhibited.[179,185] When NK cells fail to interact with the MHC class I molecules and the activating receptor is activated, NK-cell–mediated cell lysis will ensue, as occurs in the case of infection.[186] After activation, lysosome-like vesicles containing perforin, serine esterases, and sulfated proteoglycans are secreted toward the target cell. Perforin forms pores in the target cell, leading to osmotic lysis. The serine esterases, including granzymes, induce apoptosis.[178,186,187] After stimulation with cytokines, $CD56^{bright}$ NK cells produce

positive feedback loops by secreting IFN-γ, TNF, and GM-CSF. In addition, these cells also produce chemokines that recruit macrophages and other antigen-presenting cells for more efficient control of the infection.[188,189]

Neonatal NK cells show functional immaturity, which leads to an impaired immune system. Cord blood NK cells show lower expression of CD8 and CD57, two markers associated with NK cell maturation[190] (see Table 71.2), but show higher levels of the immaturity marker NKG2A.[191] Cord blood NK cells express less ICAM-1 and CD161, which are involved in endothelial adhesion and activation, respectively, than adult NK cells.[190] Cord blood NK cells are also functionally deficient when compared to their adult counterparts. When challenged with MHC class I deficient cells, cord blood NK cells degranulate less and produce less IFN γ and TNFα compared to adult cells.[191] Neonatal NK cells also show less cytolytic activity, which results in impaired clearance of intracellular pathogens.[190,192]

Noncytotoxic Innate Lymphoid Cell Physiology and Function

Ontogeny

Similar to NK cells, noncytotoxic ILCs are also derived from the common lymphoid progenitor that differentiates into precursors committed to the various subsets. Recent studies have confirmed that NK cells and noncytotoxic helper ILCs belong to distinct cell lineages.[193,194]

Innate Lymphoid Cell Subtypes

Noncytotoxic ILCs are characterized by classic lymphoid cell morphology and express IL-2 receptor α and IL-7 receptor α, but unlike the adaptive T and B cells, ILCs do not exhibit antigen specificity. Noncytotoxic ILCs are largely tissue-resident cells and are divided into three main groups: group 1 ILCs (ILC1s), group 2 ILCs (ILC2s), and group 3 ILCs (ILC3s), including lymphoid tissue inducer (LTi) cells that are necessary for the formation of lymph nodes and intestinal Peyer patches during embryonic development.[195] These subsets are defined on the basis of differential requirements for transcription factors during development, expression of effector cytokines, and the acquisition of other distinct effector functions.[196] In addition to their direct role in mediating innate immune responses, ILCs interact closely with non-hematopoietic cells in the tissue microenvironment and also participate in metabolic homeostasis and tissue repair and remodeling.[197]

All three ILC subsets function in the absence of antigen specificity but exhibit remarkable functional similarity with the T_h cell subsets, T_h1, T_h2, and T_h17, respectively, in cytokine expression and effector function and are therefore thought to be innate counterparts to T lymphocytes.[177] ILC1s express the T-bet transcription factor and produce T_h1-associated cytokines such as IFN-γ and TNF to protect against intracellular bacteria and parasites. ILC2s express the transcription factor GATA-binding protein three and produce T_h2-associated cytokines (including IL-4, IL-5, IL-9, and IL-13) and/or the epidermal growth factor receptor ligand amphiregulin. ILC2s become activated in response to epithelial-cell derived cytokines IL-33, IL-25, and TSLP and promote type 2 inflammation seen during tissue repair, allergic disorders, and antihelminth immunity. Recent findings also implicate ILC2s in the development of inflammatory lung diseases such as asthma.[195]

ILC3s express the transcription factor RAR-related orphan receptor γT, have a cytokine signature similar to that of T_h17 cells, and produce IL-17A, IL-17F, IL-22, GM-CSF, and TNF, to promote antibacterial immunity, chronic inflammation, or tissue repair. ILC3s are a heterogenous group of cells that include the $CCR6^+$ ILCs and two other subsets of cells distinguished by their presence of the NK cell receptor (NCR).[195] $CCR6^+$ ILC3s encompass $CD4^+$ and $CD4^-$ LTi cells. Recent findings indicate that depending on specific pathogenic signals, ILC subsets retain the capacity to change phenotype and function and therefore exhibit some degree of plasticity.[197]

Function

There is limited information on ILCs in the fetus and neonate. ILC2 cells are detectable in cord blood and may be present in higher proportions in male neonates than in female neonates.[198] ILC2s influence adipocyte progenitor cells to differentiate into white or beige fat and can therefore affect thermal homeostasis.[199] In a murine model of gastroschisis, organs exposed to amniotic fluid were shown to contain high numbers of ILC2 and ILC3 and eosinophilia, which was reversed by the administration of an anti-IL5 antibody.[200] In another study, ILC3s were implicated as a source of increased IL-17 levels seen in patients with pre-eclampsia, and gestational/chronic diabetes was associated with ILC3s.[201]

ILC3s are abundant on mucosal surfaces, particularly in the gastrointestinal tract. There is evidence that the development and function of some subsets of ILC3s are induced in response to microbial colonization and are involved in enhancing B-cell IgA production.[202] IL-22 production by ILCs plays an important role in containing and shaping the composition of the intestinal microbiota.[203] Further, ILC3s also promote the proliferation of intestinal stem cells in certain conditions.[199] Yet, NCR^- ILC3s may also have a pathogenic role in colitis.[204] A fine balance of ILC3 function may therefore be important for intestinal homeostasis.

Summary

Immaturity of neonatal neutrophils, monocytes, lymphocytes, DCs, and ILCs predisposes newborns to an increased incidence and/or severity of infectious complications. An increased understanding of the genetic mechanisms responsible for these defects will provide insight regarding future treatment strategies to prevent and/or treat serious or overwhelming infection in the newborn.

Acknowledgments

The authors thank Evan B. Shereck, Carmella van de Ven, and Mitchell S. Cairo for their expertise in this area and their contributions to the previous version of this chapter.

Suggested Readings

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Neonatal Hyperbilirubinemia and Kernicterus

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KEY POINTS

- Early clinical jaundice or rapidly developing hyperbilirubinemia is often a sign of hemolysis, the differential diagnosis of which commonly includes immune-mediated disorders, red cell enzyme deficiencies, and red cell membrane defects.
- Knowledge of the maternal blood type and antibody screen is critical in identifying non-ABO alloantibodies in the maternal serum that may pose a risk for severe hemolytic disease of the newborn.
- Knowledge of the hour-specific predischarge bilirubin measurement, the infant's gestational age in weeks, and hyperbilirubinemia neurotoxicity risk factors are critical to determining appropriate timely postbirth hospitalization follow-up and evaluation.
- Hyperbilirubinemia in late-preterm neonates ($34^{0/7}$ to $36^{6/7}$ weeks' gestation) is more prevalent, more pronounced, and more protracted than in their term counterparts, and these immature neonates are more vulnerable to bilirubin-induced brain injury.
- Glucose-6-phosphate dehydrogenase (G6PD) deficiency is a noteworthy cause of bilirubin encephalopathy worldwide. Clinicians everywhere must have a high index of suspicion for G6PD deficiency in neonates whose genetic heritage derives from Africa, the Middle East, the Mediterranean region, and Asia.

Hyperbilirubinemia

Hyperbilirubinemia is the most common clinical condition requiring evaluation and treatment in the newborn, and a frequent reason for hospital readmission during the first week of life. Although generally a benign, postnatal, transitional phenomenon, some neonates develop marked, potentially hazardous bilirubin levels that can cause serious brain injury.[1] Acute bilirubin encephalopathy (ABE) may ensue and evolve into kernicterus (chronic bilirubin encephalopathy), a permanent disabling neurologic condition classically characterized by (1) the movement disorders of dystonia and/or choreoathetosis, (2) hearing loss caused by auditory neuropathy spectrum disorders, and (3) oculomotor paresis.[1]

Total serum bilirubin (TSB) is the measure of albumin-bound bilirubin, whereas the small circulating fraction not bound to albumin or other serum proteins is indexed by the unbound or "free" (Bf) bilirubin level. There is a keen interest in circulating Bf, its measurement, and its ability to predict bilirubin-induced neurologic injury. Indeed, Bf is the vehicle of bilirubin's biologic effects in the brain. However, bilirubin-induced neurotoxicity depends on a complex interaction between the level and duration of the central nervous system (CNS), Bf exposure, and the innate cellular characteristics of the developing CNS that may predispose or protect against bilirubin-induced neuronal injury. At present, the measurement of circulating Bf is not generally available in clinical laboratories.[1] As a result, clinicians must rely on the TSB and the bilirubin/albumin (B/A) ratio, an imperfect surrogate of circulating Bf, to index the risk for ABE and drive treatment decisions.

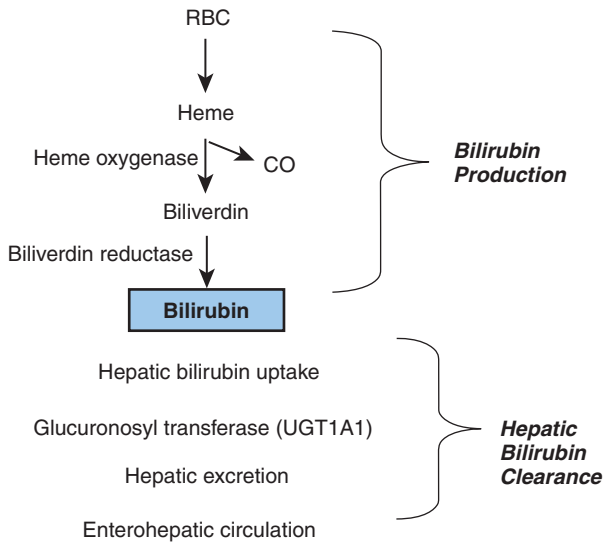
The genesis of neonatal hyperbilirubinemia reflects the interplay of developmental red blood cell (RBC), hepatic, and gastrointestinal immaturities that result in an imbalance favoring bilirubin production over hepatic enteric bilirubin clearance (Fig. 72.1). The equation below summarizes the interactions among the rates of bilirubin production (a), the enterohepatic circulation of bilirubin (b), and bilirubin elimination (c), in determining the TSB at any postnatal time point t, where TSB_0 is the cord blood TSB.[2]

$$TSB_t = TSB_0 + \sum [a(t) + b(t) - c(t)]^{\Delta t}$$

A variety of clinical conditions can increase the bilirubin load or decrease bilirubin clearance and thereby contribute to neonatal hyperbilirubinemia in any given infant (Box 72.1). In a small fraction of neonates, a constellation of conditions may lead to hazardous levels of hyperbilirubinemia that pose a neurotoxic risk. Accelerated RBC turnover (hemolysis) plays a pivotal role in increasing the risk for subsequent severe hyperbilirubinemia and in potentiating the risk of bilirubin neurotoxicity. Treatment interventions are therefore recommended at a lower bilirubin level whenever hemolysis is present.

Increased Hepatic Bilirubin Load: Hemolytic Disease

The causes of hemolysis in the neonatal period can be broadly grouped into five major categories: (1) abnormalities in red cell membrane structure, (2) red cell enzyme defects, (3) hemoglobinopathies, (4) acquired causes of hemolysis, and (5) immune-mediated mechanisms (see Box 72.1).



• **Fig. 72.1** Schematic of Bilirubin Production and Hepatic Bilirubin Clearance in Neonates. Heme, produced largely by the breakdown of red blood cells (RBCs), is catabolized by heme oxygenase to produce an equimolar amount of carbon monoxide (CO) and biliverdin; the latter is reduced to unconjugated bilirubin by biliverdin reductase. Unconjugated bilirubin is taken up by the hepatocyte via facilitated diffusion, bound to glutathione S-transferase (ligandin), and conjugated with glucuronic acid by hepatic uridine diphosphate-glucuronosyltransferase 1A1 (UGT1A1). Conjugated bilirubin is excreted into bile via multidrug resistance protein-2, a portion of which may be deconjugated by intestinal β-glucuronidase and reabsorbed into the portal circulation, enhancing the hepatic bilirubin load (enterohepatic circulation).

Red Cell Membrane Defects

Of the many red cell membrane defects that lead to hemolysis, only hereditary spherocytosis, elliptocytosis, stomatocytosis, and infantile pyknocytosis manifest themselves in neonates.[3–5] Establishing a diagnosis of these disorders is often difficult because newborns normally exhibit a marked variation in red cell membrane size and shape.[3,6] Spherocytes, however, are not often seen on RBC smears of hematologically normal newborns, and when prominent, suggest a diagnosis of hereditary spherocytosis, as does an elevated mean corpuscular hemoglobin concentration (MCHC >36.5 to 37 g/dL) or MCHC to mean corpuscular volume (MCV) ratio (MCHC/MCV >0.36).[7] Given the likelihood of autosomal dominant inheritance, a positive family history can often be elicited. The diagnosis of hereditary spherocytosis can be confirmed using the incubated osmotic fragility test coupled with fetal red cell controls or eosin-5-maleimide flow cytometry.[7] Symptomatic ABO hemolytic disease must be excluded by performing a direct Coombs test, as affected infants can manifest prominent microspherocytosis. Moreover, hereditary spherocytosis and symptomatic ABO hemolytic disease can occur in the same infant and result in anemia and severe hyperbilirubinemia.[8]

Hereditary elliptocytosis and stomatocytosis are rare but reported causes of hemolysis in the newborn period.[3] Infantile pyknocytosis is a transient red cell membrane abnormality manifesting itself during the first few months of life. The pyknocyte, an irregularly contracted red cell with multiple spines, can normally be observed in premature infants, whereas many as 5% of red cells may manifest this morphologic variant.[5] In newborns affected with infantile pyknocytosis, however, up to 50% of red cells may exhibit the morphologic abnormality with anemia that may necessitate transfusion, and hyperbilirubinemia that can be severe enough to require control by exchange transfusion.[5] Whatever the mechanism underlying infantile pyknocytosis, the disorder tends to resolve after several months of life. Pyknocytes may also occur in other conditions, including glucose-6-phosphate dehydrogenase (G6PD) deficiency and hereditary elliptocytosis, and these must be excluded before a diagnosis of infantile pyknocytosis is made.

• BOX 72.1 Causes of Indirect Hyperbilirubinemia in Neonates

A. Increased Hepatic Bilirubin Load

1. Hemolytic disease—immune mediated (positive direct Coombs test)
   a. Rhesus isoimmunization
   b. ABO incompatibility
   c. Minor blood group incompatibility
2. Hemolytic disease—red blood cell enzyme abnormalities
   a. Glucose-6-phosphate dehydrogenase deficiency
   b. Pyruvate kinase deficiency
3. Hemolytic disease—red blood cell membrane defects
   a. Hereditary spherocytosis
   b. Elliptocytosis
   c. Stomatocytosis
   d. Pyknocytosis
4. Hemolytic disease—hemoglobinopathies
   a. Alpha-thalassemia
   b. Gamma-thalassemia
5. Extravascular blood (e.g., cephalohematoma)
6. Polycythemia
7. Enhanced enterohepatic bilirubin circulation
   a. Intestinal obstruction, pyloric stenosis
   b. Ileus, meconium plugging, cystic fibrosis
   c. Breast-milk feeding

B. Decreased Hepatic Bilirubin Clearance

1. Prematurity including late-preterm gestation
2. Hormonal deficiency
   a. Hypothyroidism
   b. Hypopituitarism
3. Impaired hepatic bilirubin uptake
   a. Patent ductus venosus
   b. *SLCO1B1* gene polymorphisms
4. Disorders of bilirubin conjugation—*UGT1A1* gene variants
   a. Crigler-Najjar syndrome type I
   b. Crigler-Najjar syndrome type II (Arias disease)
   c. Gilbert syndrome
5. Enhanced enterohepatic circulation
   a. Intestinal obstruction, pyloric stenosis
   b. Ileus, meconium plugging, cystic fibrosis
   c. Breast-milk feeding

SLCO1B1, Solute carrier organic anion transporter 1B1; *UGT1A1*, uridine diphosphate glucuronosyltransferase 1A1.

Red Cell Enzyme Deficiencies

The two most common red cell enzyme defects that can lead to hyperbilirubinemia in the neonatal period are G6PD deficiency[9–14] and pyruvate kinase deficiency.[15,16] Of these, pyruvate kinase (PK) deficiency is far less frequent. PK deficiency is an autosomal recessive disorder largely confined to populations in which consanguinity is prevalent, including newborns of Amish descent and

other isolated communities.[16–18] Pyruvate kinase deficiency often presents with anemia, reticulocytosis, and severe hyperbilirubinemia.[15] A full third of affected infants require exchange transfusion to control their hyperbilirubinemia,[19] and kernicterus is a real risk.[16,18] The diagnosis of pyruvate kinase deficiency is often difficult, as the enzymatic abnormality is frequently not simply a quantitative defect but may involve abnormal enzyme kinetics or an unstable enzyme that decreases in activity as the red cell ages. The diagnosis of pyruvate kinase deficiency should be considered whenever marked hyperbilirubinemia and a picture of nonspherocytic, Coombs negative hemolytic anemia is observed.

Glucose-6-Phosphate Dehydrogenase Deficiency

G6PD deficiency is an X-linked enzymopathy affecting hemizygous males, homozygous females, and a subset of heterozygous females (via X chromosome inactivation). G6PD deficiency is an important cause of hazardous hyperbilirubinemia and kernicterus worldwide, including the United States. Although most prevalent in Africa, the Middle East, East Asia, and the Mediterranean, G6PD deficiency has evolved into a global neonatal problem as a result of past and present migration patterns, the transatlantic slave trade, and intermarriage.[9,10,12–14,20] The condition is a noteworthy contributor to endemic rates of bilirubin encephalopathy in several developing countries[21] and accounts for a substantial and disproportionate number of neonates with kernicterus in the United States Pilot Kernicterus registry (20.8% of all reported cases).[22,23] The majority of these kernicterus cases have been in African-American neonates,[24,25] an at-risk population—given that the G6PD deficiency prevalence rates in the United States are 12.2% for African-American males and 4.1% for African-American females.[26] Other subgroups at risk for G6PD deficiency include newborns of East Asian, Greek, Italian (especially Sardinia and Sicily), and Middle Eastern descent.[13,20]

G6PD is remarkable for its genetic diversity (230 variants have been described),[14] and the mutations seen in the United States include among numerous others (1) the African A variants, a group of double-site mutations, all of which share a mutation in codon 126 (376A>G) known as *G6PD A* (which expressed alone is a nondeficient variant) coupled most commonly with the 202G>A mutation (202G>A;376A>G, known as *G6PD A−*) but on occasion with the 968T>C variant (968T>C;376A>G; also known as *G6PD Betica*), or the 680G>T mutation (680G>T; A376G); (2) the *Mediterranean* (563C>T) mutation; (3) the *Canton* (1376G>T) mutation; and (4) the *Kaiping* (1388G>A) variant.[9,14,27]

G6PD is critical to the redox metabolism of red blood cells. G6PD deficiency can result in acute severe neonatal hemolysis following exposure to oxidative stress, possibly even the stress accompanying perinatal transition to the extrauterine environment.[28] Reported hemolytic triggers in G6PD deficiency are outlined in Box 72.2. A sudden, often rapid exponential rise in TSB to potentially hazardous levels may occur and result in kernicterus that may not always be preventable.[10,12,13,24,29] Severe jaundice rather than anemia may predominate in the clinical presentation.[10,30] In some neonates, G6PD deficiency and the hepatic uridine diphosphate-glucuronosyltransferase 1A1 gene (*UGT1A1*) polymorphisms of Gilbert syndrome (that limit hepatic bilirubin conjugation) combine to significantly increase the risk of hyperbilirubinemia. Kaplan et al.[31] have demonstrated a dose-dependent genetic interaction between the *UGT1A1*28* promoter variant and G6PD deficiency that substantially increases neonatal hyperbilirubinemia risk. Details regarding this icterogenic genetic interaction and other aspects of *UGT1A1* gene variants in neonates are described later under Hepatic Bilirubin Conjugation. Coexistent nongenetic factors may also impact hyperbilirubinemia risk in G6PD-deficient neonates, as shown in those who are also late-preterm and breastfed.[32]

Caretakers must have a high index of suspicion for G6PD deficiency in populations at increased risk (Mediterranean region, Africa, the Middle East, southern and southeastern Asia) who demonstrate significant hyperbilirubinemia.[24,25] Although there has been discussion on the potential utility of screening for G6PD deficiency in the United States, no consensus has emerged on whether or how best to screen, and point-of-care testing during birth hospitalization is not routinely practiced.[33] Models of point-of-care testing have demonstrated the feasibility

• BOX 72.2 Agents Reported to Produce Hemolysis in Patients With Glucose-6-Phosphate Dehydrogenase Deficiency

Antimalarials
- Pamaquine
- Pentaquine
- Plasmoquine
- Primaquine
- Quinacrine
- Quinine
- Quinocide
  - Tafenoquine

Sulfonamides
- Sulfacetamide
  - Sulfadiazine
- Sulfamethoxazole/cotrimoxazole
- Sulfanilamide
- Sulfamethoxypyridazine
- Sulfapyridine
  - Sulfasalazine
- Sulfisoxazole
- Trisulfapyrimidine

Sulfones
- Nitrofurans
- Furaltadone
- Furazolidone
- Nitrofurantoin
- Nitrofurazone
- Thiazolesulfone

Antipyretics and Analgesics
- Acetophenetidin
- Acetylsalicylic acid
- Aminopyrine
- Antipyrone
- *p*-Aminosalicylic acid

Others
- Ascorbic acid
- Chloramphenicol
- Chloroquine
  - Ciprofloxacin
- Aniline dyes
- Dimercaprol
  - Dimercaptosuccinic acid
  - Dapsone-containing combinations
- Fava beans
  - Glibenclamide (glyburide)
  - Henna (cosmetic use)
- Methylene blue
  - Moxifloxacin
  - Nalidixic acid
- Naphthalene (used in mothballs)
- Naphthoquinones (used in mothballs)
  - Niridazole
  - Norfloxacin
  - Ofloxacin
- Paradichlorobenzenes (moth repellent, car freshener, bathroom deodorizer)
- Phenylhydrazine
  - Phenazopyridine
- Probenecid
- Quinidine
  - Rasburicase and pegloticase
  - Sulfonylureas
  - Tolbutamide
  - Tolonium chloride (toluidine blue)
- Vitamin K analogs
- Menadiol sodium phosphate

Infection
- Sepsis
- Urosepsis
- Necrotizing enterocolitis

Adapted from Oski FA, Naiman JL. *Hematologic Problems in the Newborn*, 2nd ed. Philadelphia, PA: WB Saunders; 1972 and updated from Valaes F. Severe neonatal jaundice associated with glucose-6-phosphate dehydrogenase deficiency: pathogenesis and global epidemiology. *Acta Paediatr Suppl.* 1994;394:58–76, and Luzzatto L, Ally M, Notaro R. Glucose-6-phosphate dehydrogenase deficiency. *Blood.* 2020;136(11):1225–40.

and utility of identifying G6PD-deficient newborns.[34,35] Data from other countries (e.g., Israel, Singapore, and Taiwan) show that point-of-care G6PD screening strategies are associated with reductions in the prevalence of severe hyperbilirubinemia and kernicterus.[36,37]

Hemoglobinopathies

Defects in hemoglobin structure or synthesis are rare disorders that infrequently manifest themselves in the neonatal period. Of these, the α-thalassemia syndromes are the most likely to be clinically apparent in newborns. Each human diploid cell contains four copies of the α-globin gene, and thus four α-thalassemia syndromes have been described reflecting the presence of defects in 1, 2, 3, or 4 α-globin genes. Silent carriers have one abnormal α-globin chain and are asymptomatic. Common in populations of African descent, the α-thalassemia trait (two α-thalassemia mutations) is not associated with hemolysis in newborns, but presents with an MCV of less than 95 μm^3 (normal 100 to 120 μm^3).[38] Hemoglobin H disease results from the presence of three α-thalassemia mutations and can cause hemolysis and anemia in neonates.[39] Homozygous α-thalassemia (total absence of normal α-chain synthesis resulting in hemoglobin Barts hydrops fetalis syndrome) is characterized by profound hemolysis, anemia, *hydrops fetalis*, and, without fetal transfusions, almost always stillbirth or death in the immediate neonatal period. Those few reported survivors have required lifelong transfusion and frequently evidence comorbidities including neurodevelopmental delays and associated congenital abnormalities.[40]

The pure β-thalassemias do not manifest themselves in the newborn period. In addition, γ-thalassemias are (1) incompatible with life in the homozygous form, (2) associated with transient mild-to-moderate neonatal anemia if one or two genes are involved that resolves when β-chain synthesis begins, or (3) in combination with impaired β-chain synthesis, and are associated with severe hemolytic anemia and marked hyperbilirubinemia.[41]

Acquired Causes of Hemolysis

Acquired causes of hemolysis comprise a miscellaneous group of disorders, which include among others the (1) microangiopathic hemolysis associated with disseminated intravascular coagulation or hemangiomas and (2) infection (bacterial sepsis or congenital infections). The mechanisms underlying the hemolytic process in the latter are not fully understood but may also serve as a hemolytic trigger in G6PD deficiency.

Immune-Mediated Hemolytic Disease

Immune-mediated disorders are the most common cause of hemolysis in neonates, and should be suspected when there is (1) a heterospecific mother–infant pair where the infant expresses a red cell antigen(s) foreign to the mother, (2) the presence of a maternal antibody directed to the infant RBC antigen, and (3) a positive direct Coombs test in the neonate, indicating maternal antibody bound to the infant RBC.

Non-ABO Alloantibodies

A priority in evaluating every newborn is knowledge of the maternal blood type and the maternal antibody screen (routinely performed at maternal registration upon pregnancy diagnosis) to identify non-ABO alloantibodies in the maternal serum that may pose a risk for hemolytic disease in the newborn.[42] In addition, Rhesus (Rh)-D negative women who have a negative antibody screen at registration will have a repeat screen at 24 to 28 weeks' gestation before Rh(D) immune globulin (RhIG) is administered, and a screen at delivery along with a blood type and direct Coombs test on the infant to determine the need for postpartum RhIG. If these maternal data are not known, the infant should have a direct antiglobulin test and blood type determined as soon as possible (from either umbilical cord or peripheral blood) to identify risk for hyperbilirubinemia due to hemolysis.[43]

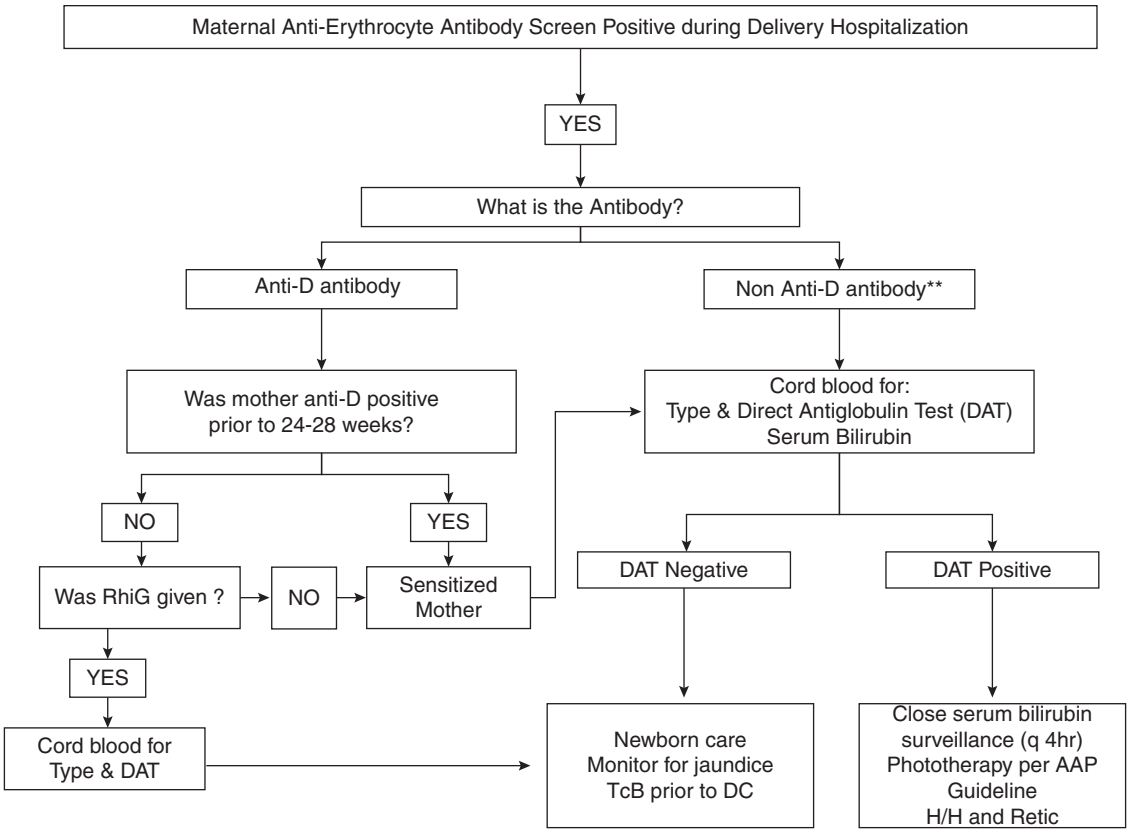
In addition to the classic Rhesus hemolytic disease of the fetus and newborn secondary to Rh-D isoimmunization, alloantibodies directed to non-D Rhesus antigens and a broad range of non-Rhesus blood group (minor) antigens are seen. Increasingly, the latter two categories comprise a clinically relevant proportion of hemolytic disease. The incidence of clinically significant sensitization to the minor blood group antigens (as determined by meta-analysis) approximates 1:330 pregnancies.[44] Only a review of the maternal antibody screen and the direct Coombs test on the infant will uncover such cases. Fig. 72.2 outlines an approach to the evaluation and management of the neonate born to a mother with a positive antibody screen.[42] A blood type and direct Coombs test are indicated at delivery (cord or infant blood) for all infants born to women with a positive antibody screen.[42]

Interpreting the results of the maternal antibody screen by neonatal caregivers is critical in identifying mothers who carry a non-ABO alloantibody, several of which can cause moderate-to-severe hemolytic disease. Table 72.1 outlines several clinical scenarios in which the maternal antibody screen is positive, accompanied by the likely clinical explanation for the positive screen.[45] The only scenario shown that does not indicate maternal sensitization is that which is secondary to RhIG administration. The latter positive anti-D maternal antibody screen must be distinguished from the occurrence of late Rh-D sensitization by confirming that the mother was anti-D antibody negative before RhIG administration and that she received RhIG. At times, the infant will also have a positive direct Coombs test secondary to maternal RhIG administration.[46–49] This finding is generally not thought to indicate a hemolytic risk.[46–50]

It is also important to note that Rh-D positive infants delivered to Rh-D negative women during the first isoimmunized pregnancy (conversion from negative to positive maternal antibody titer in that pregnancy) are at an approximately 20% risk of developing hemolytic disease requiring treatment, including the possibility of an exchange transfusion.[51] An infant born of a pregnancy during which maternal antibody conversion occurs will by definition carry the foreign antigen and may have a positive direct Coombs test. Such infants are at risk of hemolytic disease and should be monitored closely for severe hyperbilirubinemia with serial TSB measurements and not discharged early from the birth hospital.

ABO Hemolytic Disease

Hemolytic disease related to ABO incompatibility is generally limited to mothers who are blood group O and infants of blood group A or B.[52] Although this association exists in approximately 15% of pregnancies, only a fraction of infants born in this context will develop significant hyperbilirubinemia.[52,53] The diagnosis should be considered in infants who develop marked jaundice in the context of ABO incompatibility accompanied by a positive direct Coombs test, and on occasion microspherocytosis on red cell smear. The hyperbilirubinemia seen with symptomatic ABO hemolytic disease is often detected within the first 12 to 24 hours



**, determine if antibody is reported to cause hemolytic disease of the newborn

• **Fig. 72.2** An Approach to the Evaluation and Management of the Neonate Who Is Born to a Mother With a Positive Antibody Screen. Care must be taken to (1) distinguish passive anti-D as a result of Rh immune globulin administration from anti-D secondary to sensitization when maternal antibody screen is positive for anti-D at time of delivery and (2) identify all non-D alloantibodies. *AAP*, American Academy of Pediatrics; *DC*, discharge; *H/H*, hemoglobin/hematocrit, *TcB*, transcutaneous bilirubin. See also text and Table 72.1. (From Vats K, Watchko JF. Coordinating care across the perinatal continuum in hemolytic disease of the fetus and newborn: the timely handoff of a positive maternal anti-erythrocyte antibody screen. *J Pediatr*. 2019;214:212–216.)

TABLE 72.1 Interpreting Maternal Antibody Status in Rhesus-D Negative Women at Delivery

Maternal Antibody Status at Beginning of Pregnancy	Maternal Antibody Status at 24–28 Weeks Prior to Rhogam	Was Rhogam Administered?	Maternal Antibody Status at Delivery	Maternal Antibody	Diagnosis	Infant at Risk for Hemolytic Disease of the Newborn
Negative	Negative	Yes	Positive	Anti-D	Passive anti-D; Rhogam effect	Unlikely*
Negative	Negative	No	Positive	Anti-D	Late sensitization to Rh-D	Yes
Negative	Positive	No	Positive	Anti-D	Early sensitization to Rh-D	Yes
Positive	Positive	No	Positive	Anti-D	Sensitized pregnancy to Rh-D	Yes
Negative	Negative	Yes	Positive	Non-D antibody	Late sensitization to non-D antigen	Yes

*At times, the infant will also have a positive direct Coombs test secondary to maternal Rhogam administration.[45–47] This finding is generally not thought to indicate a hemolytic risk.[45–47]
From Watchko JF. Common hematologic problems in the newborn nursery. *Pediatr Clin North Am*. 2015;62:509–524.

of life ("icterus neonatorum praecox").[54] Although usually controlled with intensive phototherapy alone, a few affected infants develop hyperbilirubinemia to levels requiring exchange transfusion.[45,55] Routine screening of all ABO-incompatible cord blood is common practice in many nurseries.[56] The literature,[52,56–59] however, suggests that such routine cord blood screening is not warranted given the low yield and cost, consistent with the position of the American Association of Blood Banks.[60] This recommendation assumes appropriate surveillance for early jaundice, universal birth hospitalization bilirubin screening, and hyperbilirubinemia risk assessment before discharge and in follow-up.[43,53,59,61,62] A blood type and Coombs test are indicated in the evaluation of any newborn with early and/or clinically significant jaundice.

Infants born of ABO-incompatible mother-infant pairs who have a negative direct Coombs test appear to be at no greater risk for developing hyperbilirubinemia than their ABO-compatible counterparts[52] and the development of significant hyperbilirubinemia in such neonates should prompt an evaluation for a cause other than isoimmunization.[63] Similarly, group A or B infants born to incompatible group B or A mothers are not likely to manifest symptomatic ABO hemolytic disease, and less than 1% will have a positive direct Coombs test.[52]

Decreased Hepatic Bilirubin Clearance

Hepatic Bilirubin Uptake

During intrauterine life, fetal bilirubin is removed by the placenta and metabolized and excreted by the maternal liver. Blood flow through the newborn's hepatic arteries develops only in the first week of extrauterine life, and the ductus venosus may remain partially patent for several days, allowing blood to bypass the liver. Unconjugated bilirubin may also be a substrate for the solute carrier organic anion transporter 1B1 (SLCO1B1), a sinusoidal transporter that facilitates the hepatic uptake of a broad range of substrates in an adenosine triphosphate-independent fashion.[64] It follows that delayed developmental expression of *SLCO1B1* and nonsynonymous *SLCO1B1* gene variants may adversely impact hepatic bilirubin uptake and clearance in neonates.[65]

Hepatic Bilirubin Conjugation

The bilirubin-conjugating capacity of infants is dependent on the activity of hepatic uridine diphosphate-glucuronosyltransferase 1A1 (UGT1A1), which is developmentally expressed at 0.1% of adult levels at 17 to 30 weeks' gestation, increasing to 1% of adult values between 30 and 40 weeks' gestation, and reaching adult levels only at 14 weeks of postnatal life.[66,67] A graded upregulation of hepatic UGT1A1 activity over the first few days of life is induced by TSB itself independent of the newborn's gestational age at birth. Phenobarbital induces *UGT1A1* via the phenobarbital responsive enhancer module (PBREM) in the *UGT1A1* gene promoter element. A nonsynonymous polymorphism of *UGT1A1* PBREM (*UGT1A1*60*) is associated with an increased risk of hyperbilirubinemia.[68]

In addition to the developmentally modulated postnatal transition in hepatic bilirubin UGT1A1 activity, there are congenital inborn errors of *UGT1A1* expression, commonly referred to as the indirect hyperbilirubinemia syndromes.[69] These include the Crigler-Najjar type I and II (Arias) syndromes and Gilbert syndrome (Table 72.2).[69] Infants with Crigler-Najjar type I have complete absence of bilirubin UGT1A1 activity and are at

TABLE 72.2 Congenital Nonhemolytic Unconjugated Hyperbilirubinemia: Clinical Syndromes

	SEVERITY		
Characteristic	**Marked Crigler-Najjar Type I**	**Moderate Crigler-Najjar Type II**	**Mild Gilbert Syndrome**
Steady-state serum total bilirubin	>20 mg/dL	<20 mg/dL	<5 mg/dL
Range of bilirubin values	14–50 mg/dL	5.3–37.6 mg/dL	0.8–10 mg/dL
Total bilirubin in bile	<10 mg/dL (increased with phototherapy)	50–100 mg/dL	Normal
Conjugated bilirubin in bile	Absent	Present (only monoglucuronide)	Present (50% monoglucuronide)
Bilirubin clearance	Extremely decreased	Markedly decreased	20%–30% of normal
Hepatic bilirubin uptake	Normal	Normal	Reduced
Bilirubin UGT1A1 activity	None detected	None detected	Decreased
Genetics	Autosomal recessive	Heterogeneity of defect distinctly possible	Genetic polymorphisms: 1. Thymine-adenine $(TA)_7$ and $(TA)_8$ repeats in the *UGT1A1* promoter region. 2. G211A (Gly71Arg) *UGT1A1* coding sequence mutation identified in the Asian populations. 3. Linkage disequilibrium between $(TA)_7/(TA)_7$ and T-3279G PBREM *UGT1A1* promoter polymorphisms.

PBREM, Phenobarbital responsive enhancer module; *UGT1A1*, uridine diphosphate glucuronosyltransferase 1A1 isoenzyme.
Adapted, updated, and modified from Valaes T. Bilirubin metabolism. Review and discussion of inborn errors. *Clin Perinatol.* 1976;3:177.

significant risk for bilirubin encephalopathy and kernicterus.[70–72] Inherited in an autosomal recessive pattern, at least 85 different genetic mutations have been identified in Crigler-Najjar type I syndrome, typically nonsense or "stop" mutations in nature.[73]

Phototherapy is the mainstay of treatment for infants and children with Crigler-Najjar type I syndrome, although neonates may develop hazardous hyperbilirubinemia necessitating exchange transfusion. Liver transplantation is the only current definitive therapeutic intervention for this disorder.[71,72] Human hepatocyte transplantation to enhance hepatic UGT1A1 activity has shown limited success in these patients.[74] Stem cell and gene therapy strategies hold promise for treatment of this condition.[75,76]

In contrast, the Crigler–Najjar type II syndrome, typified by more moderate levels of indirect hyperbilirubinemia as well as low but detectable hepatic bilirubin UGT1A1 activity, appears in most cases to be mediated by missense mutations in the *UGT1A1* gene.[73] Phenobarbital can be trialed to induce residual UGT1A1 activity. These rare but important clinical syndromes must be included in the differential diagnosis of prolonged marked indirect hyperbilirubinemia.

Gilbert Syndrome

Gilbert syndrome is far more common and characterized by mild, chronic, or recurrent unconjugated hyperbilirubinemia in the absence of liver disease or overt hemolysis.[77] Hepatic *UGT1A1* activity is reduced at least 50% in affected subjects, and more than 95% of their total serum bilirubin is unconjugated.[77] Gilbert syndrome affects approximately 9% of the population, and its genetic basis in Caucasians and African Americans is an abnormal, two base pair addition to the *UGT1A1* TATAAA box promoter element giving rise to 7 ($A[TA]_7TAA$) rather than the more usual 6 ($A[TA]_6TAA$) repeats—the *UGT1A1*28* variant allele.[78]

Pediatricians have long speculated that Gilbert syndrome might contribute to indirect hyperbilirubinemia in the newborn period, and reports now show that neonates homozygous for the *UGT1A1*28* allele have accelerated jaundice, decreased fecal excretion of bilirubin monoglucuronides and diglucuronides,[79] and modestly elevated postnatal TSB levels.[80] Others have failed to demonstrate a clinically significant effect of *UGT1A1*28* alone on peak TSB. However, the coupling of *UGT1A1*28* with icterogenic conditions, e.g., G6PD deficiency and hereditary spherocytosis, markedly increases a newborn's risk for severe hyperbilirubinemia.[31,81] Several studies clearly demonstrate that the $A(TA)_7TAA$ promoter variant is prevalent in breastfed infants who develop prolonged neonatal indirect hyperbilirubinemia.[80,82] In Asian populations the nucleotide 211 guanine to adenine mutation in the coding sequence of *UGT1A1 (UGT1A1*6)* underlies a Gilbert syndrome phenotype and contributes to neonatal hyperbilirubinemia risk, including that associated with breast milk jaundice.[65,83,84]

Enhanced Enterohepatic Circulation of Bilirubin

Intestinal reabsorption of bilirubin excreted into the intestine is enhanced in neonates, adding to their hyperbilirubinemia risk. Conjugated bilirubin, as either the monoglucuronide or diglucuronide, is unstable and can be spontaneously or enzymatically hydrolyzed back to unconjugated bilirubin via intestinal β-glucuronidase and readily reabsorbed through the mucosa. Two other factors accelerating the deconjugation of bilirubin glucuronides in the newborn intestine are the mildly alkaline pH of the proximal intestine, which facilitates nonenzymatic hydrolysis, and the predominance of monoglucuronides as the main excretion form of bilirubin in the first few days of life. Neonates also lack normal intestinal flora, which metabolize conjugated bilirubin to the water-soluble and readily excretable breakdown products urobilin and stercobilin.

Other Clinically Relevant Icterogenic Conditions

Certain demographic, environmental, and genetic/inherited risk factors, among a myriad of icterogenic contributors, merit special clinical attention in addition to hemolytic conditions (Box 72.3).[43] Although each holds the potential to be a singularly important contributor to an infant's hyperbilirubinemia, more often multiple risk factors are observed in combination in marked hyperbilirubinemia.[65,85] Indeed, in infants with peak TSB levels greater than or equal to 25 mg/dL (428 µmol/L), 88% had a least two and 43% had three or more identified risk factors in one report.[85] Several merit additional comment.[43,85–87]

Late-Preterm Gestation

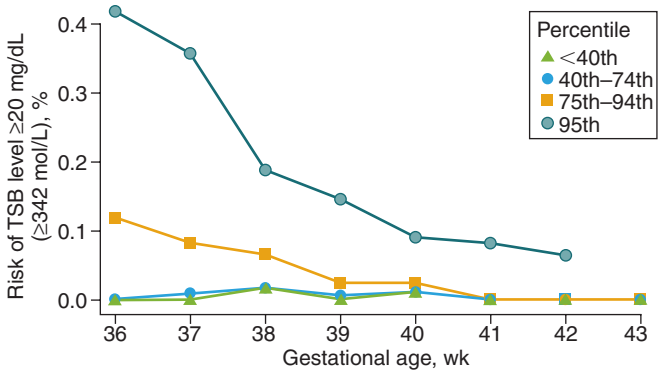
Late-preterm ($34^{0/7}$ to $36^{6/7}$ weeks' gestation) infants evidence lower UGT1A1 enzyme activity and a slower increase in UGT1A1 enzyme activity than their term counterparts during the first week of life.[66,88] This exaggerated hepatic immaturity contributes to the greater prevalence, severity, and duration of neonatal jaundice in late-preterm infants.[89] Indeed, there is an approximately eightfold increased risk of developing a TSB of greater than or equal to 20 mg/dL (342 µmol/L) in infants born at 36 weeks' gestational age (5.2%) as compared with those born at 41 weeks' gestation (0.7%).[90] This gestational age effect is even more evident when examined as a function of hour-specific TSB risk zones using the Bhutani nomogram,[91] as shown in Fig. 72.3.[86] The reported difficulty in visually assessing jaundice in late-preterm newborns[92]

• BOX 72.3 Risk Factors for Development of Significant Hyperbilirubinemia in Infants of Greater Than 35 Weeks' Gestation

- Lower gestational age (i.e., risk increases with each additional week less than 40 weeks)
- Jaundice in the first 24 h after birth
- Predischarge TcB or TSB concentration close to the phototherapy threshold
- Hemolysis from any cause, if known or suspected based on a rapid rise of increase in the TSB or TcB of greater than 0.3 mg/dL/h (in the first 24 h) or greater than 0.2 mg/dL/h thereafter
- Phototherapy before discharge
- Parent or sibling requiring phototherapy or exchange transfusion
- Family history or genetic ancestry suggestive of inherited red blood cell disorders, including G6PD deficiency
- Exclusive breastfeeding with suboptimal intake
- Scalp hematoma or significant bruising
- Down syndrome
- Macrosomic infant of a diabetic mother

G6PD, Glucose-6-phosphate dehydrogenase; *TcB*, transcutaneous bilirubin; *TSB*, total serum bilirubin.

Adapted from Kemper AR, Newman TB, Slaughter JL, et al. Clinical practice guideline revision: management of hyperbilirubinemia in the newborn infant 35 or more weeks of gestation. *Pediatrics*. 2022;150(3):e2022058859.



• **Fig. 72.3** Risk of developing a total serum bilirubin (TSB) greater than or equal to 20 mg/dL (342 µmol/L) as a function of gestational age (weeks) and risk percentile-based TSB measured at less than 48 hours using the Bhutani nomogram. (Modified from Newman TB, Liljestrand P, Escobar GJ. Combining clinical risk factors with serum bilirubin levels to predict hyperbilirubinemia in newborns. *Arch Pediatr Adolesc Med*. 2005;159(2):117.)

underscores the importance of routine birth hospitalization TSB or transcutaneous bilirubin (TcB) screening.[61]

Late-preterm neonates, because of their immaturity, often demonstrate less effective sucking and swallowing and may have difficulties achieving consistent nutritive breastfeeding, predisposing to varying degrees of lactation failure.[89,93] Suboptimal feeding was the leading reason for discharge delay during birth hospitalization in late-preterm neonates.[89,93] Late-preterm, breastfed infants merit timely post birth-hospitalization discharge follow-up and lactation support, given these risks.[61,89,94] A shortened hospital stay (<48 hours after delivery), although permitted for selected healthy term neonates, is not recommended for late-preterm neonates.[95]

Late-preterm infants are disproportionately overrepresented in the United States Pilot Kernicterus Registry, a database of voluntarily reported cases of kernicterus.[22,23,96] Moreover, the registry demonstrates that late-preterm neonates show signs of bilirubin neurotoxicity at an earlier postnatal age than term newborns, indirectly suggesting a greater vulnerability to bilirubin-induced brain injury.[96] Clinical hyperbilirubinemia management guidelines for late-preterm infants, therefore, recommend treatment at lower TSB thresholds than term newborns, an important distinction in the American Academy of Pediatrics' (AAP) practice guideline on neonatal jaundice.[43] It is also important to note that the management of late-preterm newborns born between $34^{0/7}$ and $34^{6/7}$ weeks' gestation is not addressed by the AAP guideline but set forth in an approach to management of preterm neonates.[97]

Breast-Milk Feeding

Not coincidentally, almost every reported case of kernicterus over the past three decades has been in a breastfed infant.[22] As such, exclusive breast-milk feeding, particularly in the setting of ineffective nursing and excessive weight loss, is listed as a hyperbilirubinemia risk factor by the AAP.[43,61] What does the association between exclusive breast-milk feeding and kernicterus imply with respect to the etiopathogenesis of marked neonatal jaundice? Numerous studies have reported an association between breastfeeding and an increased incidence and severity of hyperbilirubinemia, both in the genesis of early and prolonged neonatal jaundice.[65,98–101] A pooled analysis of 12 studies comprising over 8000 neonates showed a threefold greater incidence in TSB of greater than or equal to 12.0 mg/dL (205 µmol/L) and a sixfold greater incidence in levels of greater than or equal to 15 mg/dL (257 µmol/L) in breastfed infants as compared with their formula-fed counterparts.[101] Others, however, report that if adequate breastfeeding is established and sufficient lactation support is in place, breastfed infants should be at no greater risk for hyperbilirubinemia than their formula-fed counterparts.[102,103] The later studies suggest that many breastfed infants who develop marked neonatal jaundice do so in the context of a delay in lactation or varying degrees of lactation failure. Indeed, an appreciable percentage of the breastfed infants who develop kernicterus have been noted to have inadequate intake and variable but substantial degrees of dehydration and weight loss.[22,23,104] Inadequate breast-milk intake, in addition to contributing to dehydration, can enhance hyperbilirubinemia by increasing the enterohepatic circulation of bilirubin and resultant hepatic bilirubin load.[100,105] Breastfeeding-associated jaundice, however, is not associated with increased bilirubin production.[106]

Lactation failure, however, is not uniformly present in affected infants, suggesting that other mechanism(s) may be operative in breastfeeding-associated jaundice. Breast-milk feeding may act as an environmental modifier for selected genotypes and thereby potentially predispose to the development of marked neonatal jaundice.[107] In one report, the risk of developing a TSB greater than or equal to 20 mg/dL (342 µmol/L) associated with breast-milk feeding was enhanced 22-fold when combined with expression of *UGT1A1*6* or *SLCO1B1*1b* and increased 88-fold when breast-milk feedings were combined with *both UGT1A1* and *SLCO1B1* variants.[65] Several others have reported an association between prolonged (>14 days) breast-milk jaundice and expression of Gilbert syndrome variants *UGT1A1*28* and *UGT1A1*6*.[82–84] Indeed, breast-milk jaundice is now recognized as a prevalent Gilbert syndrome phenotype.[108] While recognizing the relationship between breast-milk feeding and jaundice, the benefits of breast-milk feeds far outweigh the related risk of hyperbilirubinemia. Cases of severe neonatal hyperbilirubinemia with suboptimal breast-milk feedings underscore the need for effective lactation support and timely follow-up examinations.

East Asian Ethnicity

Neonates of East Asian ethnicity, encompassing the populations of mainland China, Hong Kong, Japan, Macau, Korea, and Taiwan demonstrate a higher incidence of hyperbilirubinemia than others[107–109] and an overall increased risk for a TSB of greater than or equal to 20 mg/dL (342 µmol/L) (odds ratio [OR] 3.1, confidence interval [CI] 1.5 to 6.3).[85] Investigators have speculated as to the nature of this phenomenon, invoking potential population differences in the incidence of ABO hemolytic disease and G6PD deficiency, as well as environmental exposures to Chinese herbal medicines, among others.[110] There is little doubt that G6PD deficiency is an important contributor to hyperbilirubinemia risk in East Asian newborns.

Innate ethnic variation in hepatic bilirubin clearance also contributes to the hyperbilirubinemia risk in Asian newborns, as revealed by genetic analysis of enzymatic variants that modulate bilirubin metabolism.[110] Four different *UGT1A1* coding sequence variants: 211(G>A) (*UGT1A1*6*), 1456 (T>G) (*UGT1A1*7*), 686(C>A) (*UGT1A1*27*), and 1091(C>T) (*UGT1A1*73*) have been

described in East Asian populations, each associated with a Gilbert syndrome phenotype.[65,111] Of these, the *UGT1A1*6* variant is predominant, with an allele frequency of 11% to 13% in East Asians[111] (as high as 30% in neonates with hyperbilirubinemia ≥15 mg/dL [257 µmol/L])[112] and an associated significant decrease in UGT1A1 enzyme activity[113] greater than that seen in *UGT1A1*28*.[108] Indeed, studies of *UGT1A1*6* and **28* variants suggest a spectrum of neonatal hyperbilirubinemia risk across Gilbert syndrome genotypes.[108]

Hepatic *SLCO1B1* gene variants are also prevalent in East Asian populations,[65,114] and the *SLCO1B1*1b* variant was demonstrated to enhance neonatal hyperbilirubinemia risk.[65] Coupling *UGT1A1* and *SLCO1B1* variants together enhances hyperbilirubinemia risk, one that is further increased when that infant is also exclusively breastfed.[65]

Jaundice Observed in the First 24 Hours of Life

Jaundice appearing in the first 24 hours of life is an abnormal clinical finding[43] and an indication for immediate serum bilirubin measurement. Approximately 2.8% of newborns will evidence jaundice within 18 hours and 6.7% within 24 hours of life.[115] As contrasted with nonjaundiced newborns on the first day of life, newborns with overt jaundice in the first 24 hours of life are more likely to receive phototherapy (18.9% vs. 1.7%; relative risk [RR] 10.1, 95% CI 4.2 to 24.4) and to develop a TSB greater than or equal to 25 mg/dL (428 µmol/L) (OR 2.9, 95% CI 1.6 to 5.2).[115] Hemolytic disease, immune-mediated and otherwise, should be considered in any infant with early clinical jaundice.

Hemorrhage or Significant Bruising

Internal hemorrhage, ecchymosis, and other extravascular blood collections will enhance bilirubin production and the bilirubin load on the liver. Extravascular red cells have a markedly shortened life span, and their heme fraction is quickly catabolized to bilirubin by tissue macrophages that contain heme oxygenase and biliverdin reductase.[116] Thus cephalohematoma, subdural hemorrhage, subgaleal hemorrhage, massive adrenal hemorrhage, and marked bruising can be associated with increased serum bilirubin levels that typically manifest 48 to 72 hours following the extravasation of blood.[116] This temporal pattern is consistent with the evolution of ecchymosis and bilirubin formation in situ and also accounts for why extravascular blood can cause prolonged indirect hyperbilirubinemia.[116] An unusual but dramatic example of how extravascular blood can contribute to the genesis of hyperbilirubinemia is found in reports of marked jaundice associated with the delayed absorption of intraperitoneal blood in infants who received fetal intraperitoneal red cell transfusions.[117,118] In one such case, 13 exchange transfusions were necessary to control the hyperbilirubinemia that resolved only when approximately 87 cc of packed red cells were evacuated from the intraperitoneal cavity.[117] In this instance, the intraperitoneal blood hematocrit of 60% had the potential to contribute up to approximately 600 mg of bilirubin to the infant's bilirubin load over time.

Previous Sibling Treated With Phototherapy

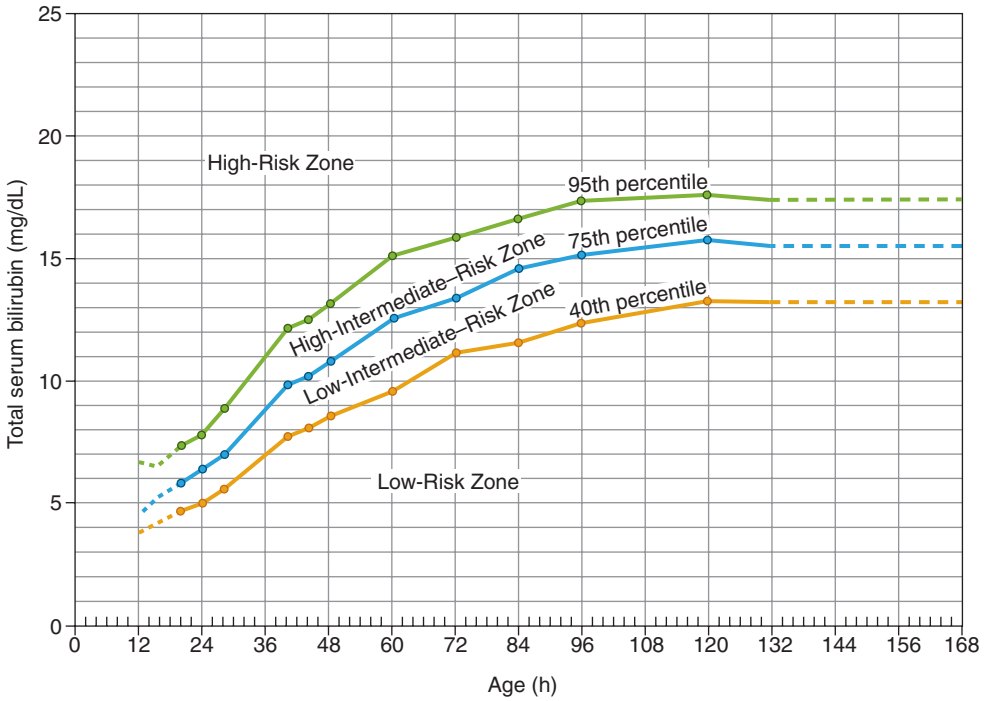
A history of a previous sibling treated with phototherapy is an identified risk factor for hyperbilirubinemia,[85,119] including concentrations greater than 15 mg/dL (257 µmol/L).[120] This relationship may reflect recurrent ABO or Rh hemolytic disease or exposure to a common environmental factor in addition to a shared genetic background.[119,121] The recurrence rate of ABO hemolytic disease is high: 88% in those infants of the same blood type as their index sibling, with almost two-thirds of the affected infants requiring treatment.[122] An excess risk in siblings independent of hyperbilirubinemia risk factors expected to recur in siblingships including breastfeeding, lower gestational age, and hemolytic disease suggests that genetic effects play an important role.[119,120] Consistent with this hypothesis, there is a higher concordance level in TSB between monozygotic (identical) than dizygotic (fraternal) twins when controlled for confounders that modulate neonatal bilirubinemia.[123]

African Ethnicity

African-American neonates demonstrate a lower overall incidence of significant hyperbilirubinemia, a lower risk for TSB greater than 20 mg/dL, comprise approximately 12% of the US population, and yet account for more than 25% of kernicterus cases in the United States.[24,25] A study using a large neonatal cohort reported that African-American newborns, despite having a reduced risk of TSB greater than 20 mg/dL, actually have a fourfold increased risk for a TSB greater than or equal to 30 mg/dL than other groups.[124] African ancestry also has been demonstrated as a risk factor for hazardous hyperbilirubinemia in infants born outside the United States.[125] G6PD deficiency is a plausible contributor to this pattern, but no effect modification by sex expected in an X-linked disorder was observed.[124] Regardless, African ancestry can no longer be considered a factor associated with decreased hyperbilirubinemia risk (as noted in the 2004 AAP Clinical Practice Guidelines for management of neonatal hyperbilirubinemia),[126] and African-American infants must be monitored for hyperbilirubinemia at least as closely as other neonates.[24,25,124] Some would caution that African-American neonates with jaundice merit special attention and follow-up.[24,25,127] Notably, Kaplan et al. report that 48.4% of G6PD-deficient African-American neonates developed a TSB greater than 75%, and 21.9% developed a TSB greater than 95% (high-intermediate and high risk zones on the Bhutani nomogram, respectively).[128] In contrast, Keren et al. report that neither the presence of a G6PD mutation or overt clinical jaundice was associated with the development of significant hyperbilirubinemia in African-American infants or African-American male infant cohorts.[87]

Combining Clinical Risk Factor Assessment With Predischarge Bilirubin Measurement

Several clinical studies show that combining clinical risk factor analysis with a birth hospitalization predischarge measurement of TSB or TcB significantly improves the prediction of subsequent hyperbilirubinemia risk.[60,85–87] An hour-specific prebirth hospitalization discharge TSB or TcB level in the high-risk zone (>95%) using the Bhutani nomogram (Fig. 72.4)[91] or close to the phototherapy threshold are risk factors for severe hyperbilirubinemia,[43,84,91,129] and the clinical factors most predictive of hyperbilirubinemia risk when combined with the risk zone characterization are lower gestational age and exclusive breastfeeding.[60,85–87] Coupling predischarge TSB or TcB measurement with gestational age and the presence of hyperbilirubinemia neurotoxicity risk factors (Box 72.4) is central to the recommendations for management and follow-up outlined in the current AAP



• **Fig. 72.4** Nomogram for Designation of Hyperbilirubinemia Risk Based on Hour-Specific Bilirubin Value. (Adapted from Bhutani V, Johnson L, Sivieri EM, et al. Predictive ability of a predischarge hour-specific serum bilirubin for subsequent significant hyperbilirubinemia in healthy term and near-term newborns. *Pediatrics*. 1999;103:6–14.)

• BOX 72.4 Risk Factors for Hyperbilirubinemia Neurotoxicity

- Gestational age less than 38 weeks, and this risk increases with the degree of prematurity
- Albumin less than 3.0 g/dL
- Isoimmune hemolytic disease (i.e., positive direct antiglobulin test), G6PD deficiency, or other hemolytic conditions
- Sepsis
- Significant clinical instability in the previous 24 h

G6PD, Glucose-6-phosphate dehydrogenase

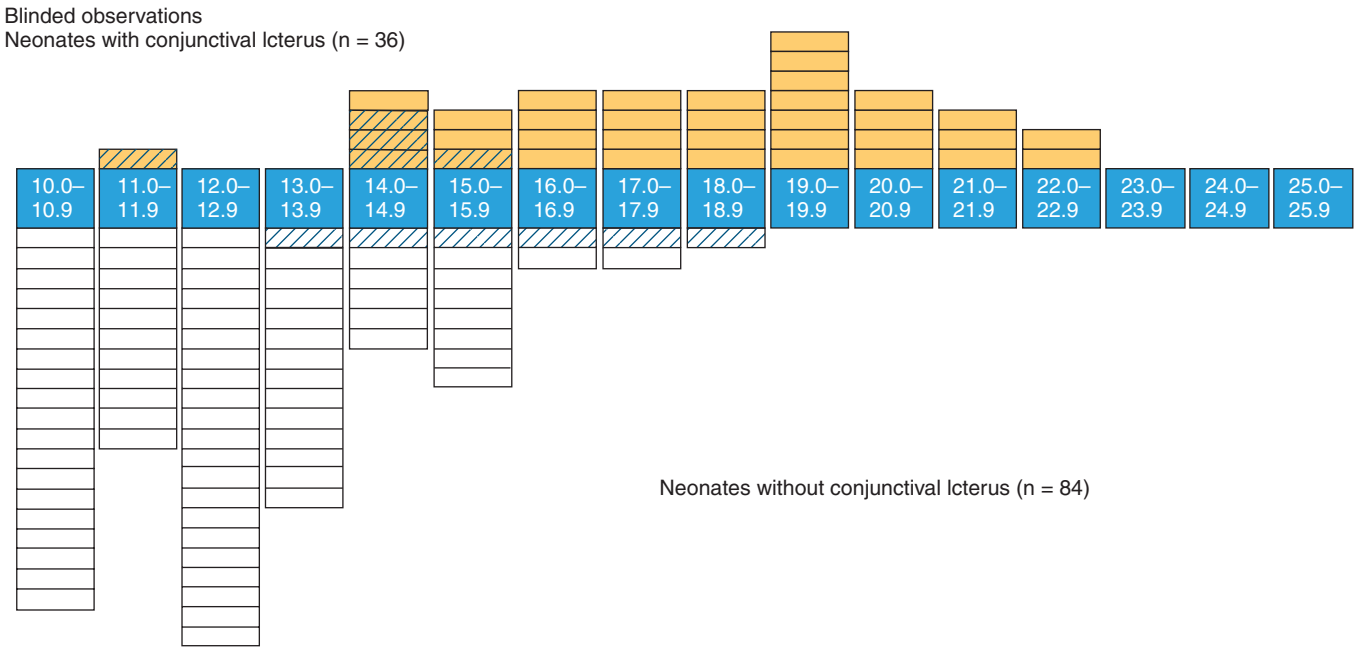
Adapted from Kemper AR, Newman TB, Slaughter JL, et al. Clinical practice guideline revision: management of hyperbilirubinemia in the newborn infant 35 or more weeks of gestation. *Pediatrics*. 2022;150(3):e2022058859.

clinical practice guideline on the management of hyperbilirubinemia in the newborn infant ≥35 weeks' gestation (see Clinical Efforts at Kernicterus Prevention later).[43] Published data show that predischarge bilirubin screening is associated with a significant reduction in the incidence of TSB greater than or equal to 25 mg/dL (428 µmol/L)[130–134] possibly by increasing the use of phototherapy.[131,134]

Clinical Evaluation of Jaundice

Jaundice is the visible manifestation in the skin of elevated serum concentrations of bilirubin. Although most adults are jaundiced when TSB levels exceed 2.0 mg/dL (34 µmol/L), neonates characteristically do not appear jaundiced until the TSB exceeds 5.0 to 7.0 mg/dL (86 to 120 µmol/L). Some degree of jaundice develops in approximately 85% of neonates,[92] and chemical hyperbilirubinemia, defined as a TSB greater than or equal to 2.0 mg/dL (34 µmol/L), is virtually universal in newborns during the first week of life. Jaundice in neonates becomes evident first on the face and progresses in a cephalocaudal fashion, with increasing hyperbilirubinemia, as classically characterized by Kramer.[135] The pattern and intensity of jaundice during the birth hospitalization, however, may not be as reliable an indicator of hyperbilirubinemia degree as previously thought.[92,136] Therefore, the AAP cautions that "*visual estimation of bilirubin levels can lead to errors*."[43] A transcutaneous bilirubin (TcB) or TSB is recommended as a complement to the clinical jaundice assessment in *every* neonate during the birth hospitalization to assist in hyperbilirubinemia detection and management.[60]

The absence of jaundice has excellent negative predictive value (99%) for developing a TSB that merits phototherapy[92]; jaundice limited to the face and upper chest likely predicts a TSB of less than 12.0 mg/dL (205 µmol/L).[136] More recent observations suggest that the presence of conjunctival icterus may be a sign of significant hyperbilirubinemia, often associated with elevations in TSB greater than 14.9 mg/dL (255 µmol/L) and greater than 95% on the Bhutani nomogram (Fig. 72.5).[137,138] Others, while confirming a strong correlation with elevated bilirubin levels, noted conjunctival icterus in neonates at lower bilirubin levels.[139] The AAP recommends that parents who detect conjunctival icterus should call their physician, who in turn should evaluate the infant, including a bilirubin measurement.



• **Fig. 72.5** Plot of individual neonates (each block = one subject) as a function of total serum bilirubin (mg/dL) and presence *(top/yellow)* or absence *(bottom/white)* of conjunctival icterus. Blinded observations of two clinicians; concordant examinations in open bars; the 11 discordant examinations are indicated by hatched bars. (Adapted from Azzuqa A, Watchko JF. Bilirubin concentrations in jaundiced neonates with conjunctival icterus. *J Pediatr*. 2015;167:840–844.)

Kernicterus Spectrum Disorders

Kernicterus spectrum disorders (KSDs) define the permanent clinical sequelae of bilirubin toxicity that become evident in the first years of life.[140] These include the extrapyramidal movement disorders (dystonia and/or choreoathetosis), hearing loss caused by auditory neuropathy, and paresis of vertical gaze.[140–142] The CNS sequelae reflect the regional topography of bilirubin-induced neuronal damage distinguished by remarkably selective involvement of the globus pallidus, subthalamic nucleus, hippocampus, red nuclei, the dentate nuclei and Purkinje cells of the cerebellum, and select brainstem nuclei.[143–145] Infants with KSD often demonstrate abnormal bilateral, symmetric, high-signal intensity on T2-weighted magnetic resonance imaging (MRI) of the globus pallidus and subthalamic nucleus, consistent with the neuropathology of kernicterus (Fig. 72.6).[142,146]

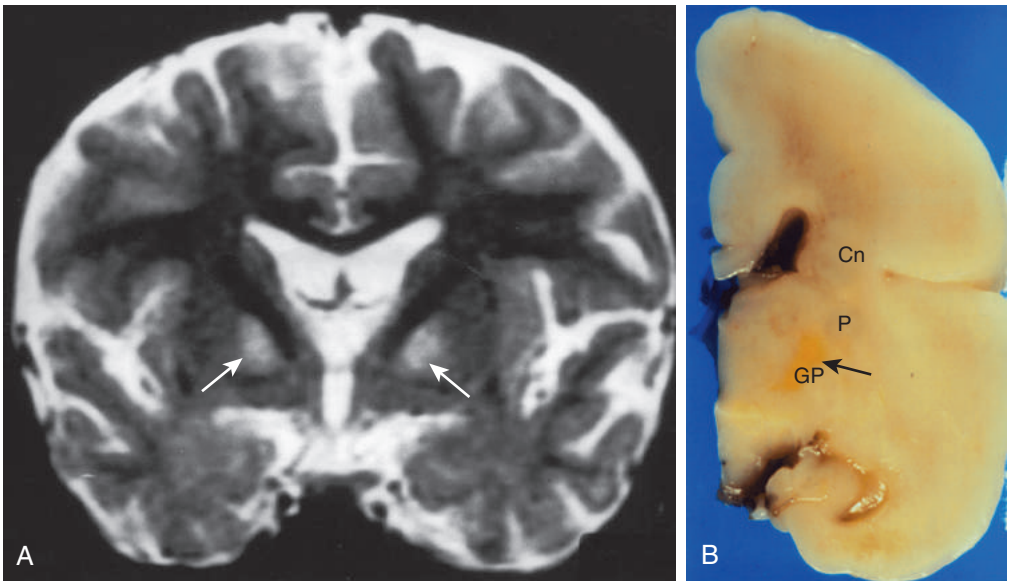
Originally described in the context of severe hyperbilirubinemia secondary to Rh hemolytic disease, kernicterus has also been reported in other hemolytic conditions (e.g., hereditary spherocytosis and pyruvate kinase deficiency), G6PD deficiency, premature neonates, and in otherwise healthy term and late-preterm gestation breastfed infants without evident hemolysis.[22,23,104] Population-based kernicterus incidence estimates for term neonates in developed countries range from approximately 0.4 to 2.7 per 100,000[147]; higher rates have been reported for (1) preterm newborns and (2) infants born in low- and middle income countries where kernicterus is a serious *endemic* problem, e.g., Nigeria, where approximately 3% of neonatal hospital admissions evidence bilirubin encephalopathy.[21,148]

Bilirubin toxicity usually does not become clinically evident until high TSB levels have been established for several hours. Acute bilirubin encephalopathy (ABE) defines an encephalopathic state characterized by a constellation of abnormal clinical signs typically progressive in their severity. In term and late-preterm gestation infants, stupor (lethargy), hypotonia, and poor sucking are seen in the initial phase.[142,149] These signs are nonspecific but in a hyperbilirubinemic infant should raise the possibility of early ABE. Clinical signs of intermediate to advanced stages of ABE are increasingly more specific to bilirubin-induced neurotoxicity and herald a marked increased risk for permanent injury.[142,149] These include hypertonia often manifested by retrocollis and opisthotonos, fever, and high-pitched cry. Inability to feed and apnea may ensue.[23] A bilirubin-induced neurologic dysfunction (BIND) score quantifies this progression and is strongly correlated with the risk of kernicterus.[150,151] Infants less than 34 weeks' gestation less frequently show these abnormal neuromotor signs. Recurrent apnea and desaturations may be the only clinical manifestations of ABE in preterm infants during the neonatal period, if any appear at all.[152,153]

Although a preponderance of the literature suggests that evidence of advanced ABE in an infant portends permanent CNS damage,[151,154,155] more recent reports suggest that at least some such infants, if treated aggressively, may escape unscathed.[156,157] The latter support the AAP recommendation for urgent exchange transfusion in any infant who is jaundiced and manifests the signs of intermediate to advanced stages of ABE (hypertonia, arching, retrocollis, opisthotonos, fever) even if the TSB is falling.[43]

Molecular Pathogenesis

Although no one disputes the neurotoxic potential of bilirubin, the cellular and molecular events that cause bilirubin-induced neurotoxicity only have been partially characterized, and there is little agreement as to which, from a mechanistic standpoint, may be the most clinically relevant. Fig. 72.7 highlights the multiple reported



• **Fig. 72.6** (A) Coronal T2-weighted MRI at the level of the basal ganglia, demonstrating bilateral, symmetric high-intensity globus pallidus (*GP*) signals *(arrows)*. (B) Deep orange-yellow staining of the *GP* of the coronal section at postmortem in another neonate. Note unstained putamen (*P*) and caudate nucleus (*Cn*). These findings illustrate the selective vulnerability and regional topography of kernicterus and concordance of neuroimaging and neuropathology in this disorder. (A from Blackwell Publishing Asia; from Shah Z, Chawla A, Patkar D, et al. MRI in kernicterus. *Austr Radiol*. 2003;47:55–57; B from *Monographs in Clinical Pediatrics*. 2000;11:78. Available at www.tandf.co.uk.)

effects of bilirubin on neurons and glial cells.[1] Bilirubin-induced neuronal cell injury probably reflects the adverse effects of hazardous unbound ("free") unconjugated bilirubin concentrations on plasma and mitochondrial and/or endoplasmic reticulum membranes (Fig. 72.8).[1,149,158,159] These membrane perturbations, in turn, precipitate untoward cellular events that culminate in increased intracellular calcium concentrations ([iCa^{2+}]). Downstream events triggered by increased [iCa^{2+}] may include the activation of proteolytic enzymes, apoptosis, necrosis, as well as abnormalities of cell cycle progression, including cell cycle arrest. Bilirubin-induced excitotoxicity, neuroinflammation, and oxidative stress have all been hypothesized to play important roles in potentiating bilirubin-induced neuronal injury. Innate cellular characteristics of the CNS may predispose or protect against bilirubin-induced neuronal injury.[1] Prematurity, hemolysis, and sepsis play key roles in enhancing bilirubin neurotoxicity risk.[43,151,160,161]

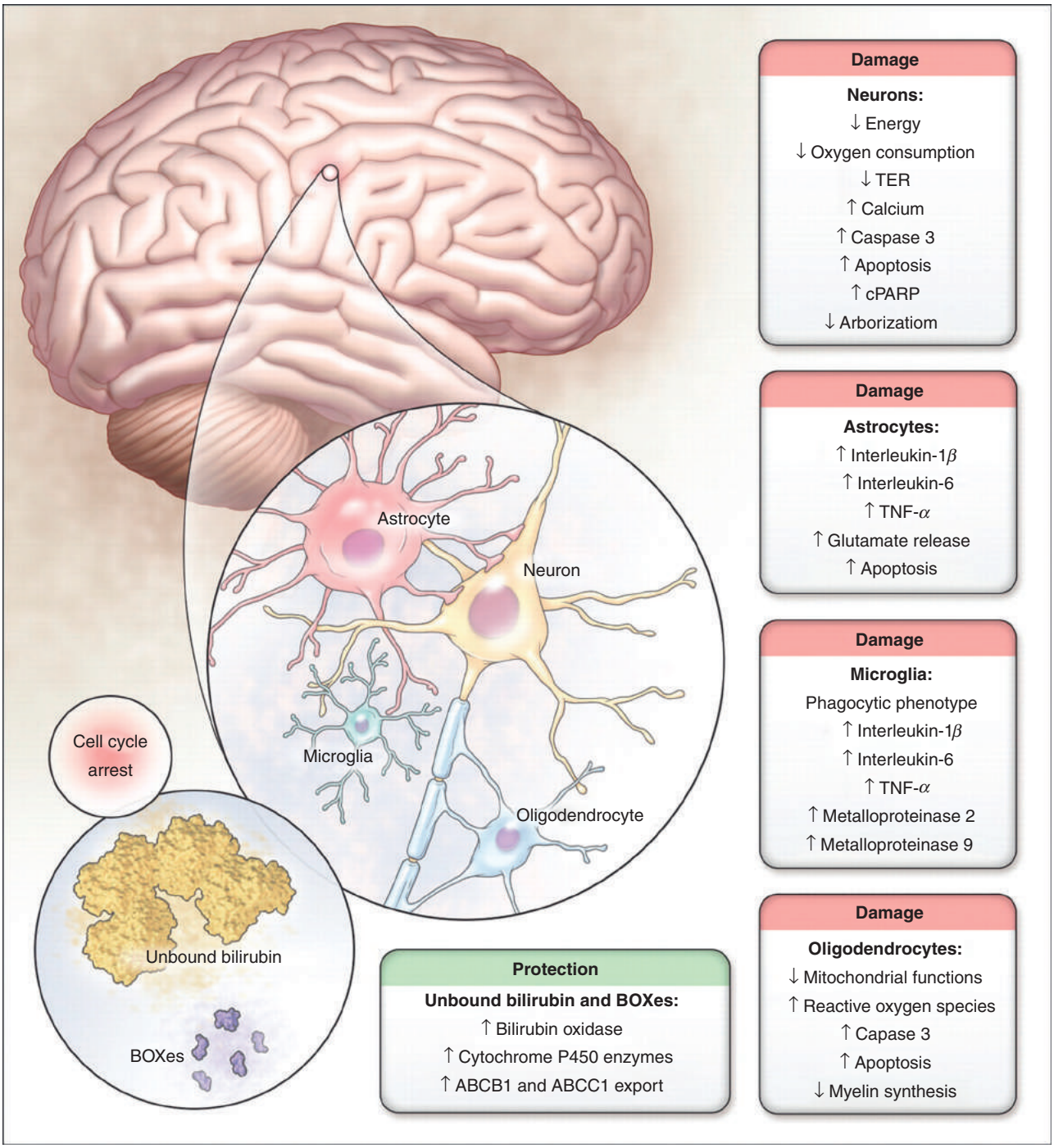
Preterm Neonates and Low-Bilirubin Kernicterus

Preterm newborns are more vulnerable to kernicterus secondary to CNS immaturity and concurrent adverse clinical conditions that may potentiate bilirubin neurotoxicity.[160] There remains some uncertainty, however, on how to quantify that risk and when to intervene with phototherapy or exchange transfusion in preterm neonates.[97,162,163] In this regard, low-bilirubin kernicterus, that is, bilirubin-induced neuronal damage at TSB levels generally thought to be nonhazardous (i.e., those below double volume exchange transfusion thresholds) continue to occur in preterm neonates. The CNS free bilirubin exposure resulting in neurotoxicity in premature infants suggests either (1) an albumin problem, that is, an abnormally low serum albumin and/or impaired albumin-bilirubin binding that results in a hazardous unbound unconjugated bilirubin concentration, and/or (2) a vulnerable neuronal pool resulting from cellular immaturity coupled with antecedent or concurrent insults that potentiate bilirubin neurotoxicity.[160,164–166] Hypoalbuminemia is common in preterm neonates[166] Conditions that predispose to a vulnerable neuronal pool are infection/inflammation, including chorioamnionitis and necrotizing enterocolitis, and comorbid CNS injuries including hypoxic–ischemic encephalopathy, intraventricular hemorrhage, and periventricular leukomalacia.[160,164,165] Oftentimes both an albumin problem and a vulnerable neuronal pool are evident in a given neonate with low bilirubin kernicterus, suggesting this injury is a two-hit (or multi-hit) phenomenon.[160,165]

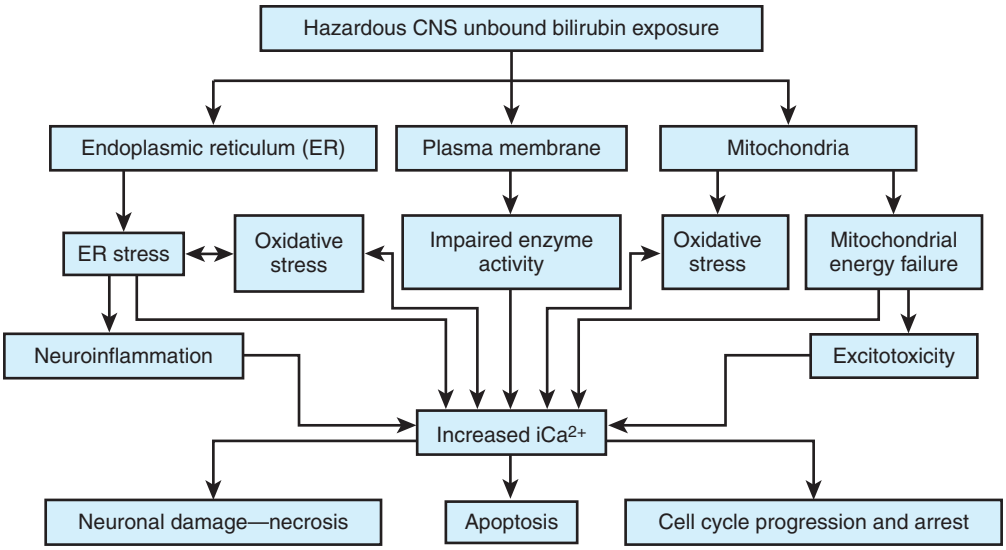
Important causes of hypoalbuminemia include leakage of albumin into the extravascular, extracellular interstitial space (reported in association with several conditions in sick preterm newborns), and significant albumin loss secondary to fetal perinatal hemorrhage. Marked neonatal hypoalbuminemia can be seen in (1) fetal–maternal transfusion, (2) the donor twin in twin–twin transfusion syndrome (TTTS) and the twin anemia–polycythemia sequence (TAPS), and (3) neonatal anemia associated with malformations of the placenta and cord.[160] Whenever anemia is present in the immediate neonatal period, measurement of the serum albumin concentration and bilirubin/albumin ratio are prudent.

Clinical Efforts at Kernicterus Prevention

The key elements to preventing kernicterus in term and late-preterm neonates are (1) hyperbilirubinemia risk assessment, (2) appropriate and timely birth hospitalization follow-up, and (3) timely and effective treatment of marked hyperbilirubinemia with phototherapy and/or exchange transfusion. Support of successful breastfeeding is also an important part of hyperbilirubinemia control.



• **Fig. 72.7** Cell Types and Metabolic Processes Affected by Bilirubin in the Central Nervous System. The main effects of bilirubin on neurons are decreased oxygen consumption and increased release of calcium and caspase 3, resulting in apoptosis. There is also decreased dendritic and axonal arborization. A similar pattern is observed in oligodendrocytes with increased apoptosis, impairment of the redox state (oxidative stress), and reduced synthesis of myelin. Microglia react to toxic injury associated with bilirubin by increased release of proinflammatory cytokines and metalloproteinase activity as cells manifest a phagocytic phenotype. A similar proinflammatory pattern is observed in astrocytes, with enhanced release of glutamate and apoptosis. At the same time, cells may reduce the intracellular concentration of bilirubin either by extruding the pigment through the ABC transporters and/or by increasing the formation of the less toxic products through bilirubin oxidation products (BOXes) and/or cytochrome P450 enzymes (1a1 and 1a2 in particular). These responses are protective, whereas all others result in cell damage; this suggests that once the intracellular concentration of bilirubin exceeds a toxic threshold (still to be defined) the polymorphic metabolic cascade leading to neurotoxicity ensues. *ABCB1*, ATP-binding cassette B1 transporter; *ABCC1*, ATP-binding cassette C1 transporter; *cPARP*, cleaved poly(adenosine diphosphate-ribose) polymerase; *TNF-α*, tumor necrosis factor α; *TER*, transcellular resistance. (From Watchko JF, Tiribelli C. Bilirubin-induced neurologic damage—mechanism and management approaches. *N Engl J Med*. 2013;369:2025.)



• **Fig. 72.8** Schematic of Several Hypothesized Pathophysiologic Mechanism(s) in Bilirubin-Induced Neuronal Injury. Hazardous unbound bilirubin exposure in the central nervous system *(CNS)* exerts direct effects at level of the plasma membrane, mitochondria, and/or endoplasmic reticulum *(ER)*, leading to ER stress, oxidative stress, impaired enzyme activity, and mitochondrial energy failure, culminating in neuroinflammation, excitotoxicity, and increased intracellular calcium levels *(iCa^{2+})*. If CNS free bilirubin exposure is of sufficient degree and/or duration than irreversible neuronal damage, i.e., necrosis, and/or cell cycle arrest may ensue.

A TSB or TcB measured 18 to 36 hours after birth interpreted according to the infant's age in hours significantly improves the prediction of subsequent severe hyperbilirubinemia[86,87,147,167] and is associated with a marked reduction in the incidence of extreme hyperbilirubinemia.[130–134] As outlined in Fig. 72.9,[43] the current AAP guideline recommends using the difference between the bilirubin concentration and the phototherapy threshold at the time of measurement to determine the interval between hospital discharge and follow-up and the need for additional TSB or TcB measurement. This approach incorporates both gestational age at birth and other hyperbilirubinemia neurotoxicity risk factors (see Box 72.4), which allows for formulation of a plan for birth hospitalization management and timely post-birth hospitalization follow-up (see Fig. 72.9).[43]

Clinicians are key to improving parental health literacy on neonatal jaundice, ensuring appropriate, timely follow-up and in navigating obstacles to parental care seeking when neonatal jaundice is present.[25,168–170] An example of specific written counselling and follow-up planning for parents during the birth hospitalization prior to discharge is shown in Fig. 72.10.[25]

Treatment Considerations

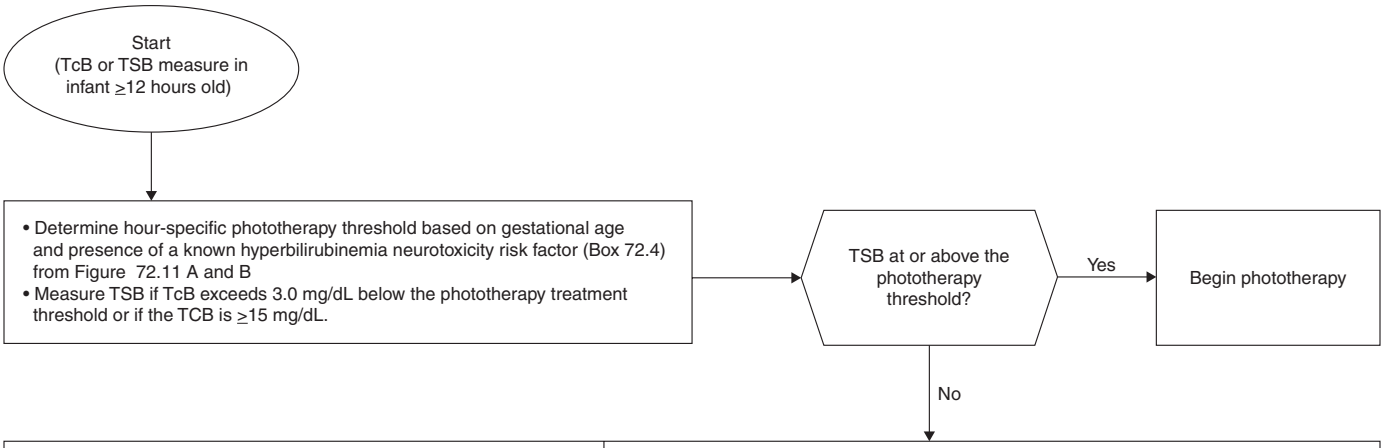
Phototherapy and exchange transfusion are the mainstays of intervention for neonatal hyperbilirubinemia. Current (2022) AAP phototherapy and exchange transfusion treatment thresholds for infants greater than or equal to 35 weeks' gestation are shown in Fig. 72.11 and 72.12, respectively.[43] These guidelines provide clinicians with TSB thresholds for both interventions in hyperbilirubinemic infants based on their gestational age (in weeks), chronologic age (in hours), and the presence or absence of hyperbilirubinemia neurotoxicity risk factors aside from gestational age. Notably, thresholds for phototherapy and exchange transfusion are incrementally higher in the 2022 AAP guideline based on evidence that neurotoxicity does not occur until bilirubin levels are well above the prior 2004 AAP guideline exchange transfusion thresholds.[43]

Tables 72.3 to 72.6[97,171] illustrate a range of TSB levels for intervention in varying circumstances for preterm neonates. Phototherapy is generally quite effective and capable of controlling the bilirubin levels in almost all infants, with the exception of the occasional infant with severe hemolysis. Indeed, a rising TSB on phototherapy should raise the concern that the neonate has significant hemolysis. The 2022 AAP Guidelines also provide direction for an escalation of care (Fig. 72.13), including preparations for exchange that should be made if the TSB is rising close to the exchange threshold.[43]

Phototherapy

The most effective phototherapy units deliver output in the blue to blue-green region of the visible spectrum, including the commercially available special blue fluorescent tubes and increasingly blue light-emitting diode (LED) units.[172–174] Phototherapy effectiveness is further enhanced by increasing the irradiance (the radiant flux [optical power] received by a surface per unit area) and the surface area exposed.[172,173] Irradiance increases dramatically as the distance between the light source and infant decreases. The efficacy of phototherapy is also closely related to the surface area of the infant exposed to the phototherapy lights. Previous clinical studies comparing intermittent versus continuous phototherapy have produced conflicting results, but in many circumstances, phototherapy does not need to be continuous.[172] As long as the serum bilirubin level is being controlled, phototherapy can be interrupted during feeding or short parental visits.[172]

Recent studies show that phototherapy converts bilirubin to more polar photoisomers quite rapidly, and it accounts for about 10% of TSB by 15 minutes of treatment, increasing to 20% to 25% of TSB by 2 hours of exposure.[175,176] In theory, these polar



Phototherapy threshold minus TcB or TSB		Discharge Recommendations
0.1-1.9 mg/dL	Age <24 hours	Delay discharge. Consider phototherapy, measure TSB in 4 to 8 hours
	Age ≥24 hours	Measure TSB in 4 to 24 hours[a] Options: • Delay discharge and consider phototherapy • Discharge with home phototherapy if all considerations in the guideline are met • Discharge without phototherapy but with close follow-up
2.0-3.4 mg/dL	Regardless of age or discharge time	TSB or TcB in 4 to 24 hours[a]
3.5-5.4 mg/dL	Regardless of age or discharge time	TSB or TcB in 1-2 days
5.5-6.9 mg/dL	Discharging <72 hours	Follow-up within 2 days; TcB or TSB according to clinical judgment[b]
	Discharging ≥72 hours	Clinical judgment[b]
≥7.0 mg/dL	Discharging <72 hours	Follow-up within 3 days; TcB or TSB according to clinical judgment[b]
	Discharging ≥72 hours	Clinical judgment[b]

• **Fig. 72.9** Flow diagram for infants during the birth hospitalization to determine postdischarge follow-up for infants who have not received phototherapy. [a]Use clinical judgment and shared decision making to determine when to repeat the bilirubin measure within this 4- to 24-hour time window. [b]Clinical judgment decisions should include physical examination, the presence of risk factors for the development of hyperbilirubinemia (see Box 72.3) or hyperbilirubinemia neurotoxicity risk factors (see Box 72.4), feeding adequacy, weight trajectory, and family support. (Adapted from Kemper AR, Newman TB, Slaughter JL, et al. Clinical practice guideline revision: management of hyperbilirubinemia in the newborn infant 35 or more weeks of gestation. *Pediatrics*. 2022;150[3]:e2022058859.)

stereoisomers should be less able to cross the blood-brain barrier and enter the CNS, reducing the risk for neurotoxicity.[175,176] Preliminary in vitro evidence also suggests that compared with unconjugated bilirubin, bilirubin photoisomers have no direct adverse effect on neuronal cell viability.[177]

Complications of phototherapy are exceptionally rare. Reported associations of neonatal phototherapy with an increased risk of subsequent seizures[178] and childhood cancers[179] have garnered attention. However, the absolute risk of seizures is small and limited to males,[178] and on balance not felt to be a contraindication to treatment when the TSB is at or above the phototherapy threshold.[43] The risk of childhood cancers is also small and tempered by data, suggesting that additional factors such as congenital and chromosomal anomalies, prematurity, or hyperbilirubinemia itself may contribute.[180–182] A more recent study from a large cohort of 139,100 children did not confirm associations between phototherapy and adjusted risk for any cancer, nonlymphocytic leukemia, or brain and/or central nervous system tumors in later childhood.[183] Another large study on early aggressive versus conservative phototherapy for infants with extremely low birth weight raised concerns that aggressive phototherapy may increase mortality while reducing neurological impairment in the birthweights of the 501 to 750 g subgroup.[184,185] Alternative approaches to bilirubin management, including cycling of phototherapy, are being tested in this vulnerable group of infants.[185,186] Collectively, these studies suggest that phototherapy should be used judiciously as set forth in established guidelines.[43,60,97]

The most overt complication of phototherapy is the dark, greyish-brown discoloration of the skin, serum, and urine (the "bronze baby syndrome") observed when treating neonates with direct hyperbilirubinemia or cholestatic jaundice.[187,188] The pathogenesis of this syndrome is unknown but is not related to copper porphyrins as previously hypothesized.[189] Although few deleterious consequences of the bronze baby syndrome have been described, there are case reports of infants with this syndrome who developed kernicterus.[190,191] Impaired binding of bilirubin to albumin has been detected in three infants with this syndrome[192,193] and in infants with cholestasis.[193] If there is a need for phototherapy, the presence of direct hyperbilirubinemia is not a contraindication to its use.[43,191] Treatment decisions

Parent Handout on Newborn Jaundice

Things you should know about neonatal jaundice:

- Jaundice is the yellowing of the skin and sometimes the whites of the eyes
- Severe, uncontrolled jaundice can cause permanent brain damage
- Severe jaundice can be prevented by early treatment with phototherapy

Important things you can do:

- Treat jaundice seriously
- Examine your baby for jaundice twice a day
- Call your provider if you observe jaundice anytime
- Seek care if the whites of the eyes become yellow
- Seek emergency help if jaundice extends to hands and feet or your baby becomes very fussy, sleepy, stops feeding, cries without being comforted, or stiffens when held
- Seek immediate care if jaundice recurs at home following hospital phototherapy

Avoid the following as they can produce severe jaundice in some babies:

- If breast feeding, mothers should not eat fava beans (broad beans)
- Do not use baby clothes, blankets, or bedding stored in moth balls (naphthalene)
- Avoid sulfa-containing antibiotics (e.g. Bactrim), henna, and herbal remedies (both for breastfeeding mothers and baby)

Review the result of your baby's bilirubin test (below) with your baby's provider, and ask whether a repeat test is necessary before leaving the hospital or in outpatient follow-up.

Pre-discharge bilirubin level:_____ mg/dL at_____ hours of life

Is a repeat bilirubin test necessary following discharge?	**Yes**	**No**
Late-preterm gestation ($34^{0/7}$-$36^{6/7}$ weeks)?	**Yes**	**No**
Positive direct antiglobulin test (DAT)?	**Yes**	**No**

Follow-up appointment: Dr. _____ on (date): _____; (time): _____
Office location:_____ Contact phone: _____

Take this report with you to your appointment with the baby's follow-up provider.

• **Fig. 72.10** An example of parental handout on neonatal jaundice to be reviewed with parents by hospital staff prior to birth hospital discharge. A copy should be given to the parents as it includes the birth hospital bilirubin screening result(s) and written details on the scheduled follow-up appointment with the baby's provider. Parents should be instructed to take this report with them to their appointment with the baby's follow-up provider. (From Okolie F, South-Paul JE, Watchko JF. Combatting the hidden health disparity of kernicterus in Black infants. A review. *JAMA Pediatr*. 2020;174(12):1199–1205.)

should be based on the TSB in cholestatic neonates when the direct fraction is < 50% of the total.[43,191] In the far less frequent circumstance where the direct bilirubin level is >50% of the total, consultation with an expert in the bilirubin field is recommended regarding treatment.[43,191] Rarely, purpuric bullous eruptions have also been described in infants with severe cholestatic jaundice who are receiving phototherapy.

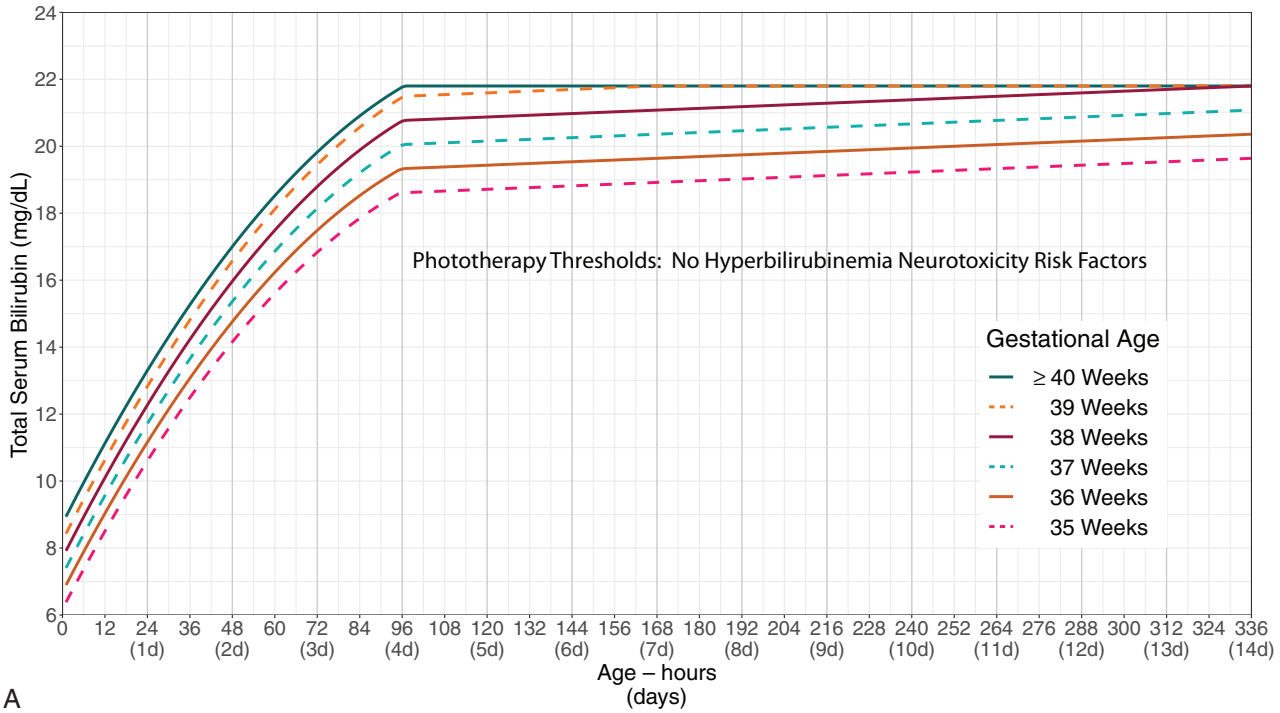
Intravenous Immunoglobulin

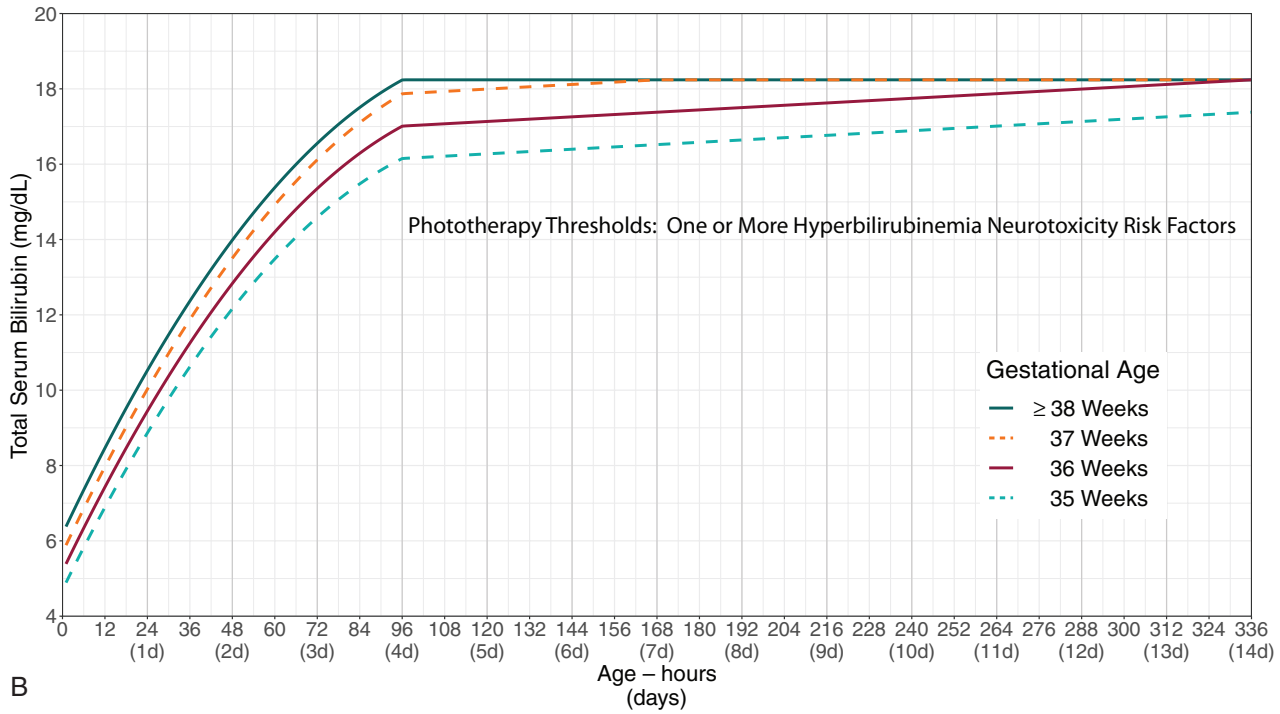
Intravenous immunoglobulin (IVIG) inhibits hemolysis in immune-mediated hemolytic disease possibly by blocking Fc receptors.[194] Although earlier studies and a systematic review suggested that IVIG administration to direct Coombs positive infants with alloimmune hemolytic disease reduces the need for exchange transfusion,[194–196] more recent investigations using early prophylactic IVIG do not support this benefit.[197–199] Routine IVIG administration in alloimmune hemolytic disease is not recommended; however, its targeted use in direct Coombs positive neonates in whom the TSB is rising—despite intensive phototherapy or the TSB level—that is within 2 to 3 mg/dL (34 to 51 μmol/L) of the exchange level remains prudent.[43]

Exchange Transfusion

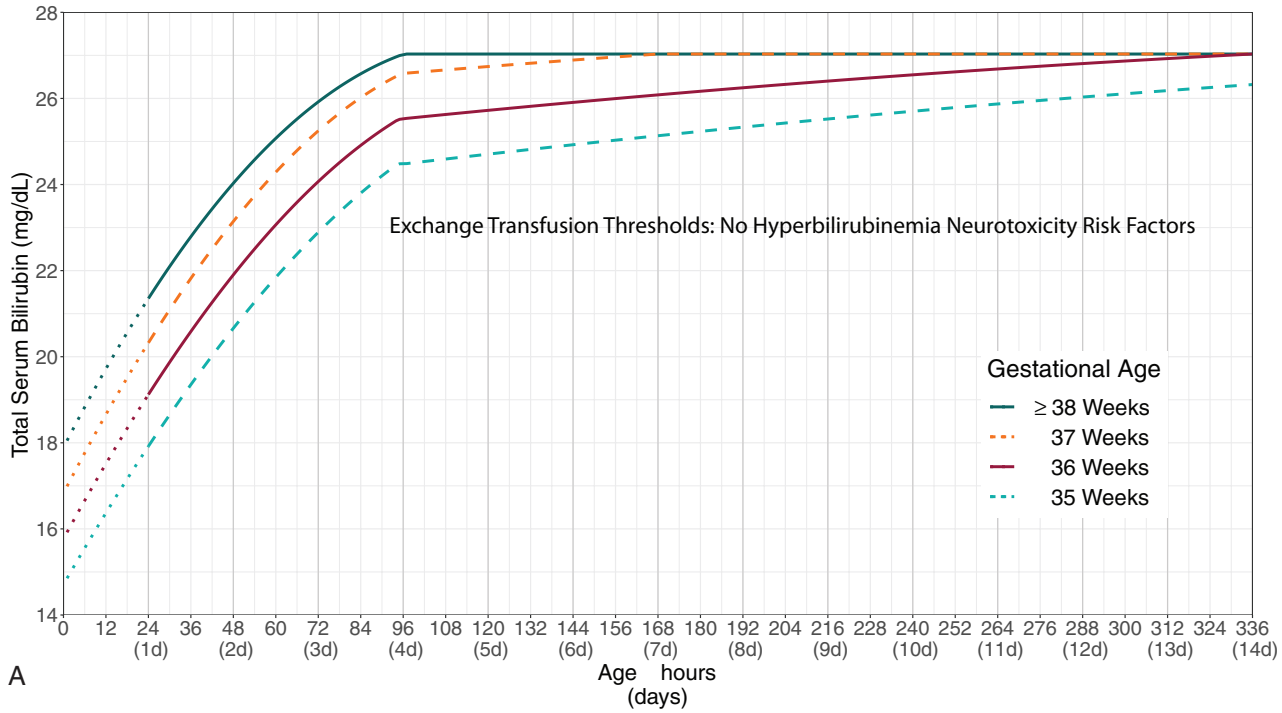
In addition to the TSB exchange transfusion thresholds outlined in Fig. 72.12, an exchange transfusion may be considered when the bilirubin-to-albumin ratio measured as TSB (in mg/dL) divided by serum albumin (in g/dL) exceeds the treatment thresholds outlined in Table 72.7.[43,171]

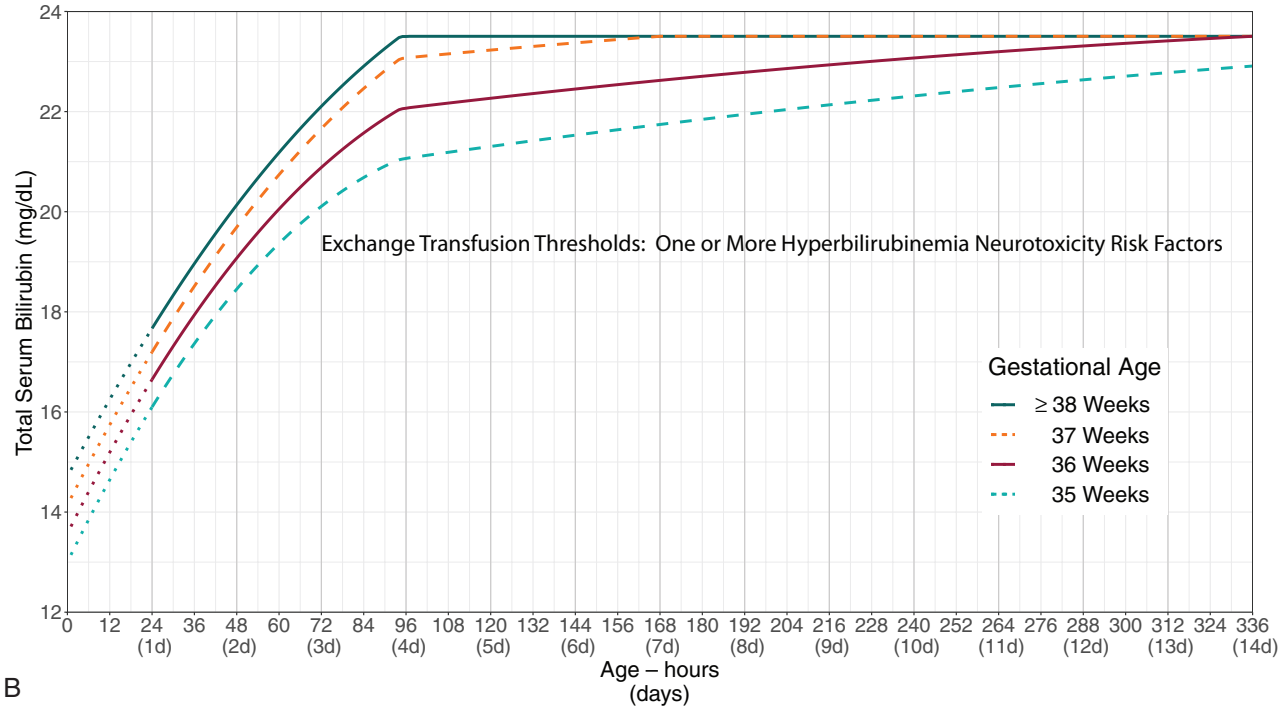
Historically, exchange transfusion was the first intervention to permit effective control of severe hyperbilirubinemia and prevent kernicterus. In addition to managing hyperbilirubinemia, an exchange transfusion in immune-mediated hemolytic disease also (1) removes antibody-coated red blood cells (a source of "potential" bilirubin), (2) corrects anemia (if present), and (3) removes maternal antibody. A "double volume" exchange refers to an exchange of twice the neonate's blood volume, or approximately 170 to 200 mL/kg, removing approximately 110% of circulating bilirubin (extravascular bilirubin





• **Fig. 72.11** Phototherapy thresholds by gestational age and age in hours for infants with no (A) and with any (B) recognized hyperbilirubinemia neurotoxicity risk factors other than gestational age. These thresholds are based on expert opinion rather than strong evidence on when the potential benefits of phototherapy exceed its potential harms. Use total serum bilirubin concentrations; do not subtract direct-reacting or conjugated bilirubin from the total serum bilirubin. In rare cases of severe hyperbilirubinemia in which the direct-reacting or conjugated bilirubin exceeds 50% of the total serum bilirubin (TSB), consult an expert. Note that infants less than 24 hours old with a TSB at or above the phototherapy threshold are likely to have a hemolytic process and should be evaluated for hemolytic disease. Hyperbilirubinemia neurotoxicity risk factors include gestational age less than 38 weeks; albumin less than 3.0 g/dL; isoimmune hemolytic disease, glucose-6-phosphate dehydrogenase (G6PD) deficiency, or other hemolytic conditions; sepsis; or any significant clinical instability in the previous 24 hours. (Adapted from Kemper AR, Newman TB, Slaughter JL, et al. Clinical practice guideline revision: management of hyperbilirubinemia in the newborn infant 35 or more weeks of gestation. *Pediatrics*. 2022;150:e2022058859.)





• **Fig. 72.12** Exchange transfusion thresholds by gestational age for infants with no (A) and with any (B) recognized hyperbilirubinemia neurotoxicity risk factors (other than gestational age). See Fig. 72.13, which describes escalation of care, including initiation of exchange transfusion. These thresholds are based on expert opinion rather than strong evidence on when the potential benefits of escalation of care exceed its potential harms. The stippled lines for the first 24 hours indicate uncertainty because of the wide range of clinical circumstances and responses to intensive phototherapy. Use total serum bilirubin concentrations; do not subtract direct bilirubin from the total serum bilirubin. In rare cases of severe hyperbilirubinemia in which the direct-reacting or conjugated bilirubin exceeds 50% of the TSB, consult an expert. Hyperbilirubinemia neurotoxicity risk factors include albumin <3.0 g/dL; isoimmune hemolytic disease, glucose-6-phosphate dehydrogenase (G6PD) deficiency, or other hemolytic conditions; sepsis; or any significant clinical instability in the previous 24 hours. (Adapted from Kemper AR, Newman TB, Slaughter JL, et al. Clinical practice guideline revision: management of hyperbilirubinemia in the newborn infant 35 or more weeks of gestation. *Pediatrics*. 2022;150[3]:e2022058859.)

TABLE 72.3 Guidelines for the Use of Phototherapy and Exchange Transfusion in Low Birth Weight Infants Based on Birthweight[a]

	Total Bilirubin Level (mg/dL [μmol/L][b])	
Birthweight (g)	Phototherapy[c]	Exchange Transfusion[d]
≤1500	5–8 (85–140)	13–16 (220–275)
1500–1999	8–12 (140–200)	16–18 (275–300)
2000–2499	11–14 (190–240)	18–20 (300–340)

[a]Note that these guidelines reflect ranges used in neonatal intensive care units. Lower bilirubin levels should be used for infants who are sick (e.g., presence of sepsis, acidosis, hypoalbuminemia) or have hemolytic disease.
[b]Consider initiating therapy at these levels. Range allows discretion based on clinical conditions or other circumstances. Note that bilirubin levels refer to total serum bilirubin concentrations. Direct reacting or conjugated bilirubin levels should not be subtracted from the total.
[c]Used at these levels and in therapeutic doses, phototherapy should, with few exceptions, eliminate the need for exchange transfusions.
[d]Levels for exchange transfusion assume that bilirubin continues to rise or remains at these levels despite intensive phototherapy.
From Maisels MJ. Jaundice. In: Avery GB, Fletcher MA, MacDonald MG (eds), *Neonatology: Pathophysiology and Management of the Newborn*. Philadelphia: Lippincott; 1999: 765–819.

enters the blood during the exchange); however, the procedure removes only 25% of total body bilirubin as the majority of the infant's bilirubin is in the extravascular compartment.[200] Post-exchange bilirubin levels are approximately 60% that of pre-exchange levels, but the rapid (~30 minutes) reequilibration of bilirubin between the vascular and extravascular compartments produces a rebound of serum bilirubin levels to 70% to 80% pre-exchange levels.[200,201]

Exchange transfusions are most readily performed via the umbilical vein using a 5- or 8-French umbilical catheter inserted just far enough to obtain free flow of blood (usually no more than the distance between the xiphoid process and umbilicus). The "push-pull" method with a single syringe and special *four-way stopcock* assembly permits a single operator to complete the procedure (Fig. 72.14).[202] Given that the efficacy of a double volume exchange is a direct function of the mass of albumin exchanged,[200,203] the ideal replacement fluid should have both a high plasma volume and a high albumin concentration to optimize the amount of bilirubin-free albumin introduced into the infant's circulation.[200,203,204] Accordingly, reconstituted whole blood, that is, packed red blood cells mixed with fresh frozen plasma to a hematocrit approximating 40%, is preferred.[200,203,204] The adult fresh frozen plasma ensures a high albumin concentration and the hematocrit a high plasma volume. Reconstituted whole blood

TABLE 72.4 Bilirubin/Albumin Ratio Trial Phototherapy and Exchange Transfusion Criteria[a]

	PHOTOTHERAPY				EXCHANGE TRANSFUSION			
	Standard Risk		High Risk		Standard Risk		High Risk	
BW (g)	TSB	B/A[b]	TSB	B/A[b]	TSB	B/A[b]	TSB	B/A[b]
<1000	5.8	2.3	5.8	2.3	9.9	3.9	9.9	3.9
1000–1250	8.7	3.5	5.8	2.3	12.8	5.1	9.9	3.9
1250–1500	11.1	3.7	8.7	2.9	15.2	6.1	12.8	5.1
1500–2000	12.8	4.2	11.1	3.7	16.9	6.8	15.2	6.1
2000–2500	14.0	4.6	12.8	4.2	18.1	7.2	16.9	6.8

[a]At 48 hours of postnatal age and older.
[b]*B/A*, Bilirubin/albumin ratio (mg/g); *BW*, birthweight; *TSB*, total serum bilirubin (mg/dL).
High risk: asphyxia, hypoxemia, acidosis, hemolysis, neurologic deterioration (sepsis, meningitis, intracranial hemorrhage >grade 2).
From Hulzebos CV, Diljk PH, van Imhoff DE, et al. The bilirubin albumin ratio in the management of hyperbilirubinemia in preterm infants to improve neurodevelopmental outcome: a randomized controlled trial—BARTrial. *PLoS One*. 2014;9(6):e99466.

TABLE 72.5 Guidelines for Exchange Transfusion in Low-Birth Weight Infants Based on Total Bilirubin (mg/dL) and Bilirubin/Albumin Ratio (mg/g)[a]

Birthweight (g)	<1250	1250–1499	1500–1999	2000–2499
Standard risk	13	15	17	18
Or bilirubin/albumin ratio	5.2	6.0	6.8	7.2
High risk[b]	10	13	15	17
Or bilirubin/albumin ratio	4.0	5.2	6.0	6.8

[a]Exchange transfusion at whichever comes first.
[b]Risk factors: Apgar less than 3 at 5 minutes; PaO_2 less than 40 mmHg at greater than 2 hours, pH less than 7.15 at greater than 1 hour; birthweight less than 1000 g, hemolysis; clinical or central nervous system deterioration; total protein less than or equal to 4 g/dL or albumin less than or equal to 2.5 g/dL.
From Ahlfors CE. Criteria for exchange transfusion in jaundiced newborns. *Pediatrics*. 1994;93(3):488–494.

TABLE 72.6 Suggested Use of Phototherapy and Exchange Transfusion in Preterm Infants Less Than 35 Weeks Gestational Age

	PHOTOTHERAPY	EXCHANGE TRANSFUSION
Gestational Age (Weeks)	**Initiate Phototherapy Total Serum Bilirubin (mg/dL)**	**Total Serum Bilirubin (mg/dL)**
$<28^{0/7}$	5–6	11–14
$28^{0/7}$–$29^{6/7}$	6–8	12–14
$30^{0/7}$–$31^{6/7}$	8–10	13–16
$32^{0/7}$–$33^{6/7}$	10–12	15–18
$34^{0/7}$–$34^{6/7}$	12–14	17–19

This table reflects the authors' recommendations for operational or therapeutic total serum albumin (TSB) thresholds—bilirubin levels at, or above which, treatment is likely to do more good than harm. These TSB levels are not based on good evidence and are lower than those suggested in recent UK and Norwegian guidelines.

The wider ranges and overlapping of values in the exchange transfusion column reflect the degree of uncertainty in making these recommendations.

Use the lower range of the listed TSB levels for infants at greater risk for bilirubin toxicity: e.g., (1) lower gestational age; (2) serum albumin levels less than 2.5 g/dL; (3) rapidly rising TSB levels, suggesting hemolytic disease; and (4) those who are clinically unstable. When a decision is being made about the initiation of phototherapy or exchange transfusion, infants are considered to be clinically unstable if they have one or more of the following conditions: (1) blood pH less than 7.15; (2) blood culture positive sepsis in the previous 24 hours; (3) apnea and bradycardia requiring cardiorespiratory resuscitation (bagging and or intubation) during the previous 24 hours; (4) hypotension requiring pressor treatment during the previous 24 hours; and (5) mechanical ventilation at the time of blood sampling.

Recommendations for exchange transfusion apply to infants who are receiving intensive phototherapy to the maximal surface area, but whose TSB levels continue to increase to the levels listed.

For all infants, an exchange transfusion is recommended if the infant shows signs of acute bilirubin encephalopathy (hypertonia, arching, retrocollis, opisthotonos, or/and high-pitched cry), although it is recognized that these signs rarely occur in very low birth weight infants.

Use total bilirubin. Do not subtract direct reacting or conjugated bilirubin from the total.

For infants less than or equal to 26 weeks' gestation, it is an option to use phototherapy prophylactically starting soon after birth. Use postmenstrual age for phototherapy: e.g., when a $29^{0/7}$ week neonate is 7 days old, use the TSB level for $30^{0/7}$ weeks.

Discontinue phototherapy when TSB is 1–2 mg/dL below the initiation level for the infant's postmenstrual age.

Discontinue TSB measurements when TSB is declining and phototherapy is no longer required.

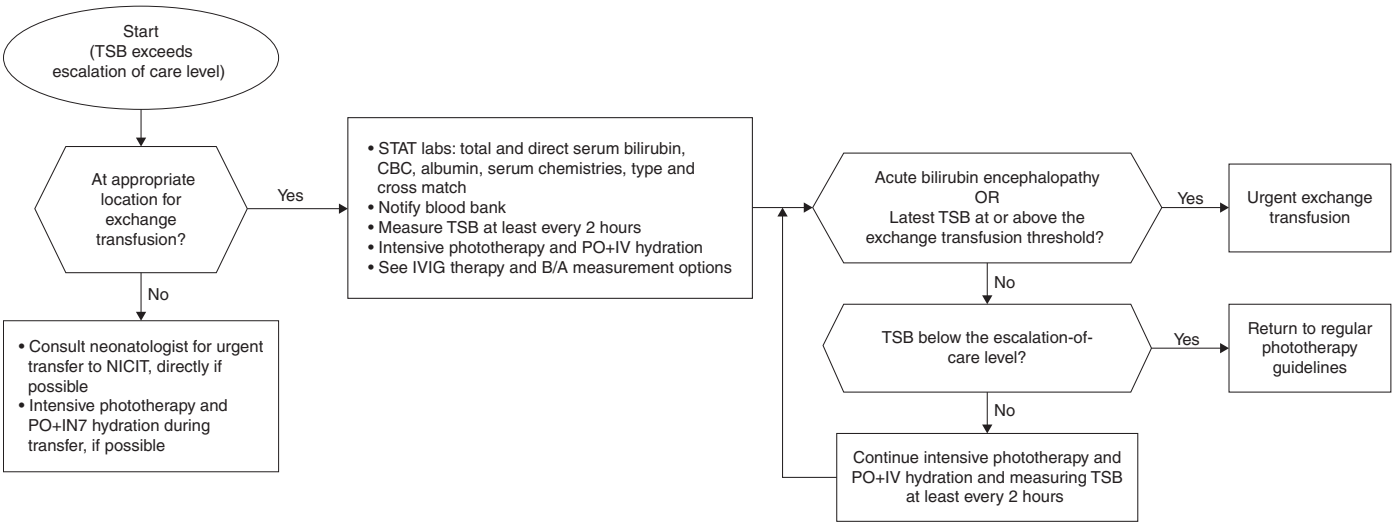
Measure the serum albumin level in all infants.

Measure irradiance at regular intervals with an appropriate spectroradiometer.

The increased mortality observed in infants who weigh less than or equal to 1000 g and are receiving phototherapy suggests that it might be prudent to use less intensive levels of irradiance in these infants. In such infants, phototherapy is almost always prophylactic—it is used to prevent a further increase in the TSB, and intensive phototherapy with high irradiance levels usually is not needed. In infants who weigh less than or equal to 1000 g it is reasonable to start phototherapy at lower irradiance levels. If the TSB continues to rise, additional phototherapy should be provided by increasing the surface area exposed (phototherapy above and below the infant, reflecting material around the incubator). If the TSB, nevertheless, continues to rise, the irradiance should be increased by switching to a higher-intensity setting on the device or by bringing the overhead light closer to the infant. Fluorescent and light-emitting diode light sources can be brought closer to the infant, but this cannot be done with halogen or tungsten lamps because of the danger of a burn.

On the other hand, in the first National Institute of Child Health and Human Development (NICHD) trial, irradiance levels were quite low, yet there was a 19% increase in mortality in infants who weighed less than 1000 g who received phototherapy ($P = .112$). One alternative to using lower irradiance is to decrease the length of exposure by using intensive phototherapy for a short period. Another option is to consider intermittent (cyclical) phototherapy. In one recent preliminary study, infants who weighed less than 1000 g who received phototherapy for 15 minutes in the hour had mean peak bilirubin levels (6.5 ± 1.6 mg/dL) that were virtually identical to those receiving continuous phototherapy (6.3 ± 1.3 mg/dL).

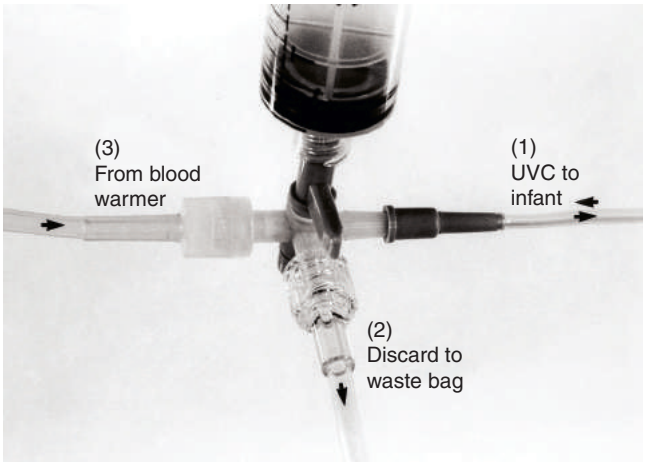
From Maisels MJ, Watchko JF, Bhutani VK, et al. An approach to the management of hyperbilirubinemia in the preterm infant less than 35 weeks of gestation. *J Perinatol*. 2012;32(9):660–664, with permission (footnotes modified).



• **Fig. 72.13** Approach to escalation of care when an infant's bilirubin level is approaching 2 mg/dL below the exchange transfusion threshold. *B/A*, Bilirubin to albumin ratio; *IVIG*, intravenous immunoglobulin. (Adapted from Kemper AR, Newman TB, Slaughter JL, et al. Clinical practice guideline revision: management of hyperbilirubinemia in the newborn infant 35 or more weeks of gestation. *Pediatrics*. 2022;150:e2022058859.)

TABLE 72.7 Bilirubin to Albumin Ratio Thresholds at Which to Consider Exchange Transfusion

Risk Category	B/A RATIO AT WHICH EXCHANGE TRANSFUSION MAY BE CONSIDERED	
	TSB (mg/dL)/ Alb (g/dL)	TSB (μmol/L)/ Alb (μmol/L)
Infants ≥$38^{0/7}$ week	8.0	0.94
Infants $35^{0/7}$–$37^{6/7}$ week and well or ≥$38^{0/7}$ week if at least one hyperbilirubinemia neurotoxicity risk factor present	7.2	0.84
Infants $35^{0/7}$–$37^{6/7}$ week if at least one hyperbilirubinemia neurotoxicity risk factor present	6.8	0.80



• **Fig. 72.14** Special Four-Way Stopcock Assembly. (1) Male adapter to umbilical venous line (*UVC*); (2) female adapter to waste bag; and (3) attachment to blood bag and warmer. The stopcock handle points to the port that is open to the syringe, and the stopcock handle is rotated in a clockwise fashion when correctly assembled (e.g., first, withdraw aliquot from infant; second, discard to waste container; third, draw fresh blood from bag; and fourth, infuse into infant to complete one cycle). (From Watchko JF. Exchange transfusion in the management of neonatal hyperbilirubinemia. In: Maisels MJ, Watchko JF, eds. *Neonatal Jaundice*. Amsterdam: Harwood Academic; 2000:169–176.)

should be less than 72 hours old and devoid of the offending antigen in the case of immune-mediated hemolytic disease.

The risk for graft-versus-host disease following an exchange transfusion is extremely rare; however, blood for exchange transfusion should be irradiated. The blood should be warmed to body temperature by a blood/fluid warmer. The actual exchange should be performed slowly in aliquots of 5 to 10 mL/kg body weight with each withdrawal-infusion cycle approximating a 3-minute duration.[205] Using this approach, a double volume exchange should take approximately 1.5 ± 0.5 hours and avoids deleterious hemodynamic changes.[205]

During the exchange transfusion, the infant's vital signs should be monitored closely, including electrocardiogram, respiration, oxygen saturation, temperature, and blood pressure. Supplemental calcium gluconate administration during the exchange transfusion has little effect on serum ionized calcium,[206–208] and too rapid infusion of calcium may cause bradyarrhythmias or cardiac arrest. If symptomatic hypocalcemia develops, temporary cessation of the procedure will allow recovery toward normal calcium levels as the citrate (which binds calcium) is metabolized by the liver. Postexchange studies should include bilirubin, hemoglobin, platelet count, ionized calcium, serum electrolytes, and serum glucose.

The unintended consequences of exchange transfusion include cardiovascular, hematologic, gastrointestinal, biochemical, and infectious hazards, among others.[202] Reported overall mortality rates approximate 0.5 to 1 per 100 exchange trasnfusions.[209,210] These rates may be higher in ill infants[211,212] and in those born prematurely,[212] but lower in healthy term neonates.[211] In one series of 81 hyperbilirubinemic but otherwise healthy term neonates, there were no procedure-related deaths.[211] Most reported that adverse events associated with exchange transfusion are treatable laboratory abnormalities.[212,213] Nevertheless, because significant morbidity (apnea, cyanosis, vasospasm, thrombosis) can occur, an exchange transfusion should be performed by experienced individuals in a neonatal intensive care unit with continuous monitoring prepared to respond to potential adverse events. Finally, the risks per tested packed red blood cell unit for known transfusion-transmitted viruses in the United States are 1:1,467,000 for the human immunodeficiency virus (HIV); 1:1,149,000 for the hepatitis C virus, and 1:282,000 for the hepatitis B virus.[214]

Acknowledgments

The contributions of Dr. Frank A. Oski, who authored the original chapter and Drs. Ashima Madan, James R. MacMahon, and David K. Stevenson, who coauthored earlier editions, are gratefully acknowledged.

Suggested Readings

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73 Congenital Malignant Disorders

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KEY POINTS

- The spectrum of malignancies in neonates differs from that in children.
- Malignancies in neonates are often associated with a genetic predisposition.
- Neuroblastoma and leukemia are the most common malignancies in neonates.
- Cancer treatment in neonates poses unique challenges, including the likelihood of significant late effects.
- Congenital solid tumors are often diagnosed in utero by ultrasonography.

Neonatal malignancies differ in incidence, clinical behavior, and heritable features from cancers seen in older children. While acute leukemia is the most common malignancy in young children, most neonatal tumors are solid tumors, many of which are detected prenatally during routine ultrasonography. Some childhood malignancies that carry excellent prognoses, such as acute lymphoblastic leukemia (ALL), are often fatal in neonates. In contrast, neuroblastoma, which responds poorly to treatment in older children, can spontaneously regress in newborns.

Treatment of cancer in the neonatal period presents special challenges. Among these are differences in drug metabolism in newborns, the sensitivity of rapidly growing normal tissues to chemotherapeutic agents and radiation, and the increased possibility of late effects, including neurocognitive sequelae, impaired reproductive capacity, growth disturbances, and secondary malignancies. The epidemiology, etiology, and diagnosis of neonatal malignancy are reviewed here, followed by a discussion of commonly encountered malignancies.

Epidemiology, Etiology, and Diagnosis of Neonatal Malignancy

Epidemiology: Incidence and Mortality

Neonatal tumors are rare, with an incidence of 1 per 27,500 live births in the United States; they compose 2% of childhood malignancies.[1] Although trend analyses suggest that the incidence of malignancy in the pediatric population may be increasing,[2] a number of factors affect incidence rates, including improvements in molecular methods of diagnosis, changes in population characteristics, screening fetal ultrasonography practices, and case ascertainment by cancer registries.[3]

The most common malignancy in infants is neuroblastoma, followed by leukemia, central nervous system (CNS) tumors, retinoblastoma, and germ cell tumors.[2] Female and male infants have similar cancer incidence rates. The distribution of the major types of cancers in newborns, infants, and children is depicted in Table 73.1. Incidence rates for the most common types of malignancy in infants are shown in Table 73.2.

The mortality rates for infants with cancer exceed those for older children, even among identical diseases.[4] Despite cure rates exceeding 85% for children older than 1 year with a diagnosis of ALL, newborns with ALL have cure rates of less than 50%.[5] Poorer survival patterns for infants are also seen with rhabdomyosarcoma (RMS) and CNS tumors, including primitive neuroectodermal tumor (PNET), atypical teratoid/rhabdoid tumor (ATRT), and ependymoma. Two notable exceptions are neuroblastoma, for which the 5-year survival rate in newborns with disseminated disease is more than 90%, and infantile fibrosarcoma, for which cure rates in newborns often exceed those achieved in older children or adults.

Etiology

Genetic Predisposition Syndromes and Congenital Defects

The cause of cancer in children is multifactorial, involving both genetic and environmental factors. However, in neonates, predisposing genetic factors more often play an important role. An acquired or inherited abnormality of a cancer-predisposing gene that is critical during embryogenesis underlies some cases of neonatal cancer, and the malignant transformation of normal cells results from the activation or suppression of these cancer-predisposing genes. The retinoblastoma gene at 13q is an example of a constitutional chromosomal abnormality that results in a high risk of malignancy.

A number of defined hereditary conditions and genetic defects are associated with an increased incidence of specific neoplasms; these are listed in Table 73.3.[1] Except for retinoblastoma, hepatoblastoma, and Wilms tumor, the neoplasms associated with these syndromes seldom manifest themselves in the neonatal period, but the associated abnormalities may be recognized early, allowing regular screening. A lack of a family history should not dissuade the clinician from investigating these syndromes, as both spontaneous germline mutations and parental mosaicism occur. The genetic defect in many of these neoplasms has been identified. For

TABLE 73.1 Distribution of the Major Types of Cancer in Newborns, Infants, and Children

Malignancy	Newborns Younger Than 30 Days (%)	Infants Younger Than 1 Year (%)	Children Younger Than 15 Years (%)
Leukemia	13	14	31
Central nervous system tumors	3	15	18
Neuroblastoma	54	27	8
Lymphoma	0.3	1	14
Renal tumors	13	11	6
Sarcoma	11	5	11
Hepatic tumors	0	3	1.3
Teratoma	0	6	0.4
Retinoblastoma	0	13	4
Other	5.7	5	6.3

From Reaman GH, Bleyer WA. Infants and adolescents with cancer: special considerations. In: Pizzo PA, Poplack DG, eds. *Principles and Practice of Pediatric Oncology*, 5th ed. Philadelphia: Lippincott Williams & Wilkins, 2006.

TABLE 73.2 Incidence of Malignant Tumors in US Infants Younger Than 1 Year

Malignancy	Number	Proportion of Total (%)	Incidence Rate*
Neuroblastoma	402	24	54.1
Leukemia	296	18	39.9
Central nervous system tumors	225	13	30.3
Retinoblastoma	196	12	26.4
Germ cell tumors	156	9	21.5
Wilms tumor	107	6	14.4
Hepatoblastoma	78	5	10.5
Soft tissue sarcoma (nonrhabdomyosarcoma)	76	5	10.2
Rhabdomyosarcoma	39	2	5.3

*Incidence rate per 1,000,000 person-years, age adjusted to the 2000 US Standard population.
From Linabery AM, Ross JA. Trends in childhood cancer incidence in the U.S. (1992–2004). *Cancer*. 2008;112:416–432.

TABLE 73.3 Hereditary Conditions With Associated Tumors

Syndrome	Gene	Locus	Inheritance Pattern	Most Common Tumors
Ataxia-telangiectasia	*ATM*	11q22-q23	Recessive	Leukemia Lymphoma Meningioma
Beckwith-Wiedemann syndrome	*IGF2*	11p15	Some autosomal dominant imprinting	Wilms tumor Hepatoblastoma Adrenal cortical carcinoma Rhabdomyosarcoma
Bloom syndrome	*BLM*	15q26	Autosomal recessive	Leukemia
Congenital central hypoventilation	*PHOX2B*	4p13	Autosomal dominant	Neuroblastoma
Congenital mismatch repair deficiency	*MLH1, MSH2, MSH6, PMS2*	Multiple	Autosomal recessive	Any malignancy
Cowden syndrome	*PTEN*	Multiple	Autosomal dominant	Gangliocytoma Gliosarcoma
Denys-Drash syndrome	*WT1*	11p13	Autosomal dominant	Familial Wilms tumor
Down syndrome		Trisomy 21	Sporadic	Leukemia
Familial adenomatous polyposis	*APC*	5q22.2	Autosomal dominant	Hepatoblastoma Medulloblastoma
Familial neuroblastoma	*ALK*	2p23	Autosomal dominant	Neuroblastoma
Fanconi anemia	*BRCA2, BRIP1, PALB2*	Multiple	Autosomal recessive	Leukemia Brain tumors Wilms tumor Neuroblastoma
Frasier syndrome	*WT1* intron 9	11p15	Autosomal dominant	Wilms tumor

continued

TABLE 73.3 Hereditary Conditions With Associated Tumors—cont'd

Syndrome	Gene	Locus	Inheritance Pattern	Most Common Tumors
Gonadal dysgenesis		45X/46XY	X-linked?	Gonadoblastoma Germinoma
Gorlin syndrome	*PTCH2, PTCH1*	1p33	Autosomal dominant	Medulloblastoma Basal cell carcinoma
Klinefelter syndrome		XXY	Sporadic	Teratoma Leukemia Breast cancer
Li-Fraumeni syndrome	*TP53*	17p13	Autosomal dominant	Sarcoma Central nervous system tumor Breast cancer
Medulloblastoma predisposition	*SUFU*	10q24	Autosomal dominant	Medulloblastoma
Multiple endocrine neoplasia, type 1	*MEN1*	Multiple	Autosomal dominant	Pituitary adenoma, pancreas, parathyroid and intestinal carcinomas
Multiple endocrine neoplasia, type 2B	*RET*	10q11	Autosomal dominant	Medullary thyroid carcinoma Pheochromocytoma
Neurofibromatosis	*NF1*	17q11.2	Autosomal dominant	Glioma Leukemia (JMML) Sarcoma
Noonan syndrome	*PTPN11, HRAS, KRAS, BRAF, SOS1*	Multiple	Autosomal dominant	Transient myeloproliferative disorder Leukemia (JMML) Neuroblastoma
Perlman syndrome	*DIS3L2*	2q37	Autosomal recessive	Wilms tumor
Pleuropulmonary blastoma and ovarian sex-cord stromal tumor predisposition	*DICER1*	14q32	Autosomal dominant	Pleuropulmonary blastoma Cystic nephroma Ovarian sex-cord stromal tumors
Retinoblastoma	*RB1*	13q14	Autosomal dominant	Retinoblastoma Pineoblastoma Osteosarcoma Rhabdomyosarcoma
Rhabdoid tumor predisposition	*SMARCB1, ATRT*	Many	Autosomal dominant	Atypical teratoid/rhabdoid tumor Renal rhabdoid tumor Extrarenal rhabdoid tumor
Trisomy 18		Trisomy 18	Sporadic	Wilms tumor
Turner syndrome		X0	Sporadic	Neuroblastoma
Von Hippel-Lindau syndrome	*VHL*	3p26	Autosomal dominant	Hemangioblastoma
WAGR syndrome	*WT1*	11p13		Wilms tumor
Wiskott-Aldrich syndrome	*WAS*	Xp11.23	X-linked	Non-Hodgkin lymphoma
X-linked lymphoproliferative disorders	*SAP*	Xq25	X-linked	EBV lymphomas

EBV, Epstein-Barr virus; *JMML*, juvenile myelomonocytic leukemia; *WAGR*, Wilms tumors, aniridia, genitourinary abnormalities, mental retardation.
Data from Orbach D, Sarnacki S, Brisse HJ, et al. Neonatal cancer. *Lancet Oncol.* 2013;14:e609–e620 and Jackson EM, Shaikh TH, Gururangan S, et al. High-density single nucleotide polymorphism array analysis in patients with germline deletions of 22q11.2 and malignant rhabdoid tumor. *Hum Genet.* 2007;122:117–127 and Mitchell SG, Pencheva B, Westfall E, et al. Cancer predisposition in neonates and infants: recognition, tumor types, and surveillance. *Clin Perinatol.* 2021;48(1):1–14.

example, the *NF1* gene, located at 17q11.2, encodes a protein, neurofibromin, that normally acts as a guanosine triphosphatase–activating protein that downregulates the Ras signaling pathway. Children with neurofibromatosis 1 (NF1) are at increased risk of developing juvenile myelomonocytic leukemia (JMML), a rare but aggressive myeloproliferative neoplasm that is treated with hematopoietic stem transplant but has also been seen to spontaneously regress.[6] In children with NF1 and JMML, the hematopoietic cells display loss of the wild-type *NF1* gene and duplication of the mutant allele, thus resulting in the complete loss of the normal

neurofibromin protein in the leukemia cells.[7] This promotes cell growth because there is no functional "off" switch.

A large number of childhood tumors occur in association with congenital defects. For instance, Down syndrome has an increased association with both leukemia and transient myeloproliferative disorders (TMDs). Children with congenital aniridia have an increased incidence of Wilms tumor. Although aniridia is found in only 1 in 75,000 persons, it is found in as many as 1 in 75 children with Wilms tumor. Children with abnormalities of the Wilms tumor 1 gene *(WT1)*, located at chromosome band 11p13, also have an increased risk of developing Wilms tumor.[8] Most individuals with constitutional *WT1* defects have associated phenotypic syndromes that include combinations of genitourinary abnormalities, renal dysfunction, and mental retardation. Beckwith-Wiedemann syndrome (BWS) and hemihypertrophy syndromes are associated with several neoplasms. This syndrome is typified by macroglossia, gigantism, and abdominal wall defects; patients may also have visceromegaly, flame nevus, neonatal hypoglycemia, microcephaly, and retardation.[8] Approximately 8% of infants with either the complete syndrome or the partial syndrome develop neoplasms, including Wilms tumor, adrenal cortical carcinoma, and hepatoblastoma—tumors of the same organs in which visceromegaly develops. Also reported are RMS, neuroblastoma, ganglioneuroma, and adenomas and hamartomas. BWS is linked with abnormalities of 11p15; this is the location of the insulin-like growth factor II gene *(IGF2)* and the tumor suppressor gene *H19*.[9]

Transplacental Tumor Passage

An exceedingly rare cause of cancer in neonates and infants is the transplacental passage of tumor cells from the mother. Rare cases of transplacentally transmitted cancer have been reported.[10] The malignancies transmitted include leukemia, melanoma, lymphoma, hepatic carcinoma, and lung cancer. The diagnosis of transplacentally acquired neoplasm usually occurs at birth but has been reported as late as age 8 months. The frequency of concurrent maternal malignancy in pregnant women is estimated at 1 per 1000 pregnancies,[11,12] and alternative treatment plans or delays in treatment are options for pregnant women. That transplacental transmission is so rare is attributed to the protective function of the placenta.

Twin-to-Twin Transmission

The risk of development of leukemia is increased in a monozygotic twin. If one monozygotic twin has leukemia, the cotwin has an approximately 25% chance of developing leukemia, usually within weeks or months of the diagnosis in the sibling. In contrast, a dizygotic twin has only a slightly increased risk of developing leukemia. This increased incidence is likely due to in utero twin-to-twin transmission of a preleukemic clone rather than the simultaneous development of a shared germline mutation facilitating the later development of leukemia.

Environmental Factors

Environmental factors are probably less important in the development of neonatal cancer compared with their role in the development of cancer in older children and adults. Nonetheless, there is evidence that environmental influences, including radiation exposure, maternal medication use, and various environmental exposures, may affect the incidence of neonatal cancer.[1]

Exposure to ionizing radiation during pregnancy is known to increase the risk of a number of tumors, including acute leukemia, in exposed offspring. There appears to be a dose-response relationship between the dose of ionizing radiation received by the fetus in utero and the subsequent development of cancer in childhood, with doses on the order of 10 milliGray sufficient to produce an increase in risk. Mixed evidence comes from atomic bomb survivors who were exposed to radiation in utero. Maternal exposure to ionizing radiation should be used sparingly and only for diagnostic purposes if required.[13]

Maternal exposure to some drugs during pregnancy has been associated with the subsequent development of cancer in offspring. Maternal use of diethylstilbestrol has been strongly associated with the development of clear cell adenocarcinoma of the vagina and cervix in daughters born from those pregnancies. Some substances and exposures known to be teratogenic may also be carcinogenic to offspring. Excessive maternal alcohol consumption may be linked to an increased risk of developing cancer in the newborn period, particularly acute myeloid leukemia (AML). The use of fertility drugs does not appear to increase the risk of cancer in the exposed offspring.[14]

Environmental exposures of the mother or father to hydrocarbons, dyes, and other chemicals and solvents may be related to the development of neonatal tumors, but there is only a weak association for most of the risk factors identified.[15] The association of neoplasms with other environmental factors, such as maternal use of tobacco, has not been conclusively proven.[16]

Diagnosis and Evaluation

The diagnostic evaluation of a newborn suspected of having cancer is guided by the signs and symptoms of the disease. Symptoms of malignancy in neonates can be nonspecific, such as irritability, poor feeding, failure to thrive, and fever. Table 73.4 lists clinical features associated with the more common malignancies found in the neonatal period. Most neonatal tumors present as a mass at birth; often the mass has previously been identified by prenatal ultrasonography. Postnatal imaging with magnetic resonance imaging (MRI) is usually required to better delineate the lesion.

Laboratory and pathologic evaluations should be directed at making the diagnosis efficiently, sparing the newborn unnecessary procedures that could result in acute and chronic morbidity. Routine laboratory studies, including a complete blood count (CBC) and liver and renal function tests, should be performed. Urine catecholamine excretion should be measured when neuroblastoma is being considered. Serum alpha fetoprotein (AFP) and beta human chorionic gonadotropin (β-hCG) levels should be measured in infants suspected of having a germ cell tumor or teratoma; these can serve as tumor markers, although the normally elevated levels in infancy can complicate the interpretation of these values (Table 73.5).[17] Surgeons and pathologists should submit biopsy tissue for histologic examination, immunoperoxidase staining, flow cytometry, cytogenetic analysis, and tumor banking.

Specific Neoplasms

Neuroblastoma

Overview

Neuroblastoma is the most common malignant tumor of infancy. It is of embryonal origin, derived from neural crest cells that have committed to the sympathoadrenal lines. The tumor can present in utero, in infancy, and in childhood; the age of presentation significantly affects the prognosis and treatment plans. Because,

TABLE 73.4 Differential Diagnosis of Malignant and Nonmalignant Conditions in Infancy

Feature	Malignancy	Nonmalignant Condition
Skin nodules	Neuroblastoma	Congenital viral infections
	Acute leukemia	Vasculitis
	Reticuloendothelioses	Fibromatosis
		Neurofibromatosis
		Xanthoma
Head and neck masses	Rhabdomyosarcoma	Brachial cleft cyst
	• Orbital	Thyroglossal duct cyst
	• Cervical	Cystic hygroma
	• Nasopharyngeal	Fibromatosis
	Neuroblastoma	Hemangioma
	Lymphoma	Abscess
	Infantile fibrosarcoma	Cellulitis
		Reactive hyperplasia of cervical nodes
		Granulomatous lesions (e.g., atypical tuberculosis)
Abdominal or pelvic masses	Neuroblastoma	Polycystic kidneys
	Wilms tumor	Hydronephrosis
	Sarcoma	Benign teratoma
	Malignant teratoma	Urinary retention
	Lymphoma	Gastrointestinal duplication
	Germ cell tumor	Intussusception
		Chordoma
		Meningomyelocele
		Horseshoe kidney
		Splenomegaly
		Hepatomegaly
Hepatomegaly	Neuroblastoma	Congenital viral infections
	Acute leukemia	Storage diseases
	Hepatoblastoma	Cavernous hemangioma
	Reticuloendothelioses	Hemangioendothelioma
Signs/symptoms of increased intracranial pressure	Brain tumors	Intracranial hemorrhage
	Acute leukemia	Communicating hydrocephalus
	Retinoblastoma	Dandy-Walker malformation
		Vascular malformations
Anemia	Acute leukemia	Short-term or long-term blood loss
	Neuroblastoma	Hypoproliferative anemia (nutritional, congenital)
		Dyserythropoietic anemias
		Hemolytic anemia
		Transient erythroblastopenia
Pancytopenia	Acute leukemia	Congenital viral infections
	Neuroblastoma	Immune-mediated neutropenia and thrombocytopenia
	Retinoblastoma (disseminated)	Congenital and acquired aplastic anemias

Modified from Reaman GH, Bleyer WA. Infants and adolescents with cancer: special considerations. In: Pizzo PA, Poplack DG, eds. *Principles and Practice of Pediatric Oncology*, 5th ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2006:455 [chapter 15].

TABLE 73.5 Age-Specific Alpha Fetoprotein (AFP) Values

Age	Mean (ng/mL)	AFP 95% interval (ng/mL)
Premature	134,734	51,846–217,622
Newborn	41,687	9120–190,546
Day of life 1	36,391	7943–165,959
Day of life 2	31,769	6950–144,544
Day of life 3	27,733	6026–125,893
Day of life 4	24,210	5297–109,648
Day of life 5	21,135	4624–96,605
Day of life 6	18,450	4037–84,334
Day of life 7	16,107	3524–73,621
Days of life 8–14	9333	1480–58,887
Days of life 15–21	3631	575–22,910
Days of life 22–28	1396	316–6310
Days of life 29–45	417	30–5754
Days of life 46–60	178	16–1995
Days of life 61–90	80	6–1045
Days of life 91–120	36	3–417
Days of life 121–150	20	2–216
Days of life 151–180	13	1.25–129
Days of life 181–720	8	0.8–87

Modified from Wu JT, Book L, Sudar K. Serum alpha fetoprotein (AFP) levels in normal infants. *Pediatr Res.* 1981;15:50–52. Blohm ME, Vesterling-Hörner D, Calaminus G, et al. Alpha 1-fetoprotein (AFP) reference values in infants up to 2 years of age. *Pediatr Hematol Oncol.* 1998 Mar–Apr;15(2):135–142.

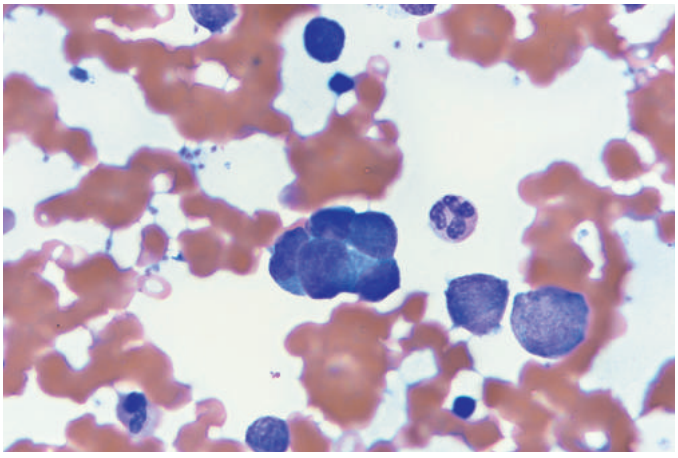
in part, of improvements in prenatal ultrasonography, neonatal neuroblastoma is now diagnosed in more children; neuroblastoma is diagnosed in approximately 100 children per year in North America prenatally or at age less than 3 months.[18] Neonatal neuroblastoma (defined as age younger than 28 days) represents 5% of neuroblastoma cases.

Etiology

Neuroblastoma can be associated with genetic disorders but does not have a single cause.[19] The incidence is increased in patients with Turner syndrome, Noonan syndrome (in 50% of Noonan syndrome patients the *PTPN11* gene is mutated, which is associated with an increased risk of leukemia and neuroblastoma), and Costello syndrome. While *NF1* mutations have been detected in neuroblastoma cell lines, patients with germline *NF1* mutations do not have a predisposition to neuroblastoma. Neuroblastoma is seen in BWS and other overgrowth disorders; abdominal ultrasonography is recommended quarterly until age 8 years for early detection. Congenital central hypoventilation syndrome (specifically the *PHOX2B* mutation) and Hirschsprung disease are associated with increased risk of neuroblastoma and ganglioneuroblastoma. There are cases of familial neuroblastoma; in 80% of these cases the anaplastic lymphoma kinase *(ALK)* receptor has been found to be mutated, although there are no phenotypic abnormalities.[20] In addition to germline mutations in the familial cases, somatic *ALK* mutations are found in up to 12% of sporadic neuroblastoma tumors.[21]

Presentation

In children, symptoms of neuroblastoma are often due to a mass effect in the compartment of tumor origin. Among all pediatric neuroblastoma cases, two-thirds of cases occur within the abdominal cavity; most of these occur in the adrenal glands. Abdominal distention is a common initial presentation. However, neuroblastoma can occur anywhere along the sympathetic chain and is sometimes incidentally found on chest x-rays. Neuroblastoma in the posterior mediastinum can present as bronchial obstruction. Neuroblastoma arising in the sympathetic paraspinal ganglia may invade the neural foramina, causing spinal cord compression with associated neurologic symptoms. Tumor cells can also rarely be found circulating on review of the peripheral blood smear (Fig. 73.1).



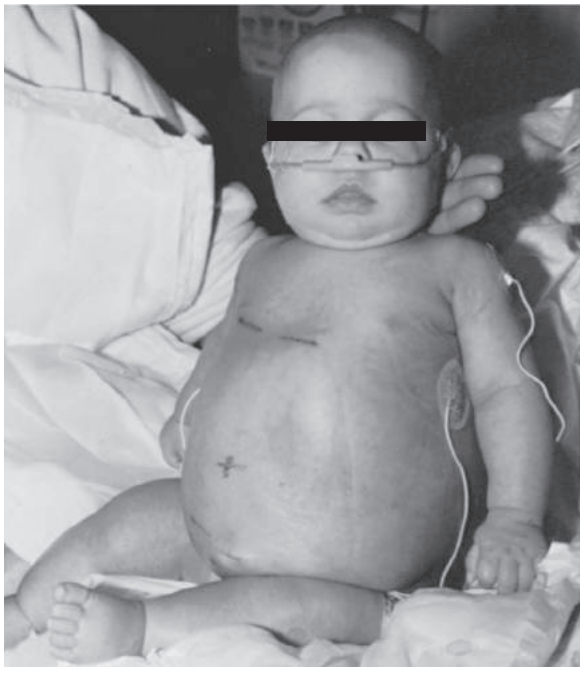
• **Fig. 73.1** Clump of Neuroblastoma Cells in Bone Marrow Aspirate. (Courtesy Kristie White, UCSF, San Francisco.)

In the newborn, neuroblastoma presents commonly as an asymptomatic adrenal mass found on routine ultrasonography in the third trimester, but it most often manifests as stage 4S (now referred to as stage MS and inclusive of patients up to 18 months of age) neuroblastoma with hepatomegaly, seen in 65% of cases, followed by subcutaneous metastases, seen in 32% of cases. This metastatic pattern is different from that seen in older infants and children. Metastases to the lungs, bones, skull, and orbit are rare in the newborn, although clumps of tumor cells are often found in the bone marrow. In the newborn the primary site of disease often cannot be identified or may be a small adrenal primary tumor. Liver involvement can cause massive hepatomegaly, which can be a cause of dystocia during vaginal delivery. This massive involvement can also cause abdominal distention, coagulopathy, heart failure, and life-threatening respiratory distress (Fig. 73.2), necessitating immediate chemotherapy.[22] Subcutaneous skin nodules can be present; these are typically bluish, and palpation of the nodules leads to transient erythema followed by blanching, presumably because of the vasoconstriction caused by the release of catecholamines from the tumor cell.

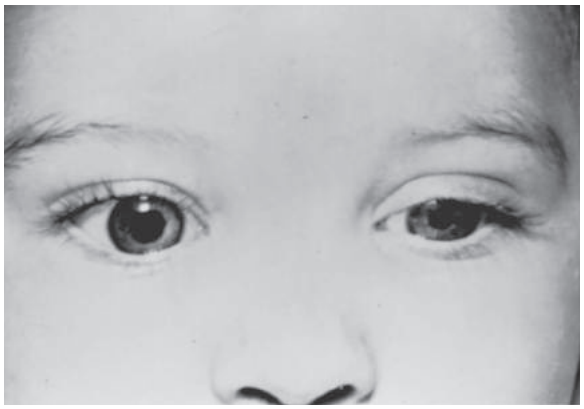
The neoplasm may also arise in the neck or pelvis. Involvement of the stellate ganglion may result in Horner syndrome, which includes ptosis of the upper eyelid, slight elevation of the lower eyelid, meiosis, narrowing of the palpebral fissure, anhidrosis, and enophthalmos (Fig. 73.3). Neuroblastoma arising from the paravertebral sympathetic ganglion has a tendency to grow into the intervertebral foramina, causing spinal cord compression and resultant paralysis. Careful periodic neurologic evaluation should be performed in a child with neuroblastoma in this region to evaluate the child for the onset of cord compression, which may necessitate emergency intervention with chemotherapy, surgery, or irradiation.

Unusual Presentations

Intractable diarrhea can be the sole presenting manifestation of neuroblastoma. Secretion of vasoactive intestinal peptide by the



• **Fig. 73.2** Stage 4S neuroblastoma causing abdominal distention and respiratory distress secondary to hepatic infiltration.



• **Fig. 73.3** Horner syndrome in an infant with neuroblastoma arising from the left cervical sympathetic ganglion.

tumor has been postulated to be the cause of the diarrhea, which resolves following surgical removal of the tumor.[23]

Opsoclonus and *myoclonus* ("dancing eyes, dancing feet") are associated with neuroblastoma, although this presentation is only rarely seen in the neonatal period.[24] Patients have rapid multidirectional eye movements (opsoclonus), myoclonus, and truncal ataxia (OMA) in the absence of increased intracranial pressure (ICP). The condition may be due to an autoimmune reaction, as the presence of antineuronal antibodies has been shown to be significantly more common in children with neuroblastoma and OMA than in case-controlled neuroblastoma patients.[25] Removal of the tumor usually results in a decrease in neurologic signs and symptoms, but the use of steroids, intravenous (IV) gammaglobulin, and other immunosuppressive therapy such as cyclophosphamide or rituximab is frequently required for complete resolution.[26,27] In general, the prognosis for survival of children with OMA is excellent, although long-term neurologic deficits and learning delays are common and can be quite debilitating.

Catecholamine Secretion

A hallmark of neuroblastoma cells is the ability to store and secrete catecholamines. Patients with neuroblastoma usually have elevated urinary levels of norepinephrine as well as its biochemical precursors and their metabolites. More than 90% of patients have an elevated urinary excretion of vanillylmandelic acid (VMA) or homovanillic acid (HVA) or both. VMA and HVA determinations can be made on random urine samples when values are normalized for creatinine concentration. In the occasional case with no elevation of catecholamine levels, a 24-hour urine collection is necessary. Catecholamine secretion can be used not only as a diagnostic aid but also as a means to assess the response to therapy and to detect tumor recurrence. Thus urine catecholamine levels should be measured before surgical removal of the tumor or before initiation of therapy.

Diagnosis

Clinical evaluation should include a physical examination with particular attention paid to detecting an abdominal mass, hepatomegaly, lymphadenopathy, Horner syndrome, and skin lesions; a baseline neurologic examination is also performed. Laboratory evaluation should include a CBC, tests for urine levels of VMA and HVA, and tests for serum ferritin and lactate dehydrogenase. While the initial imaging study in an infant is often abdominal ultrasonography, additional imaging to better delineate the tumor and to evaluate the infant for metastatic disease is needed; this should include computed tomography (CT) or MRI of the primary lesion. MRI of the spine should be performed for paraspinal and posterior mediastinal lesions. An [^{123}I]metaiodobenzylguanidine (MIBG) scan is particularly important for diagnosis and follow-up. MIBG, a norepinephrine analogue specifically taken up by neuroblastoma in bone and soft tissue, serves as a sensitive modality (90% sensitive) for disease localization.[28] Bilateral bone marrow aspiration (along with bilateral bone marrow biopsy in patients older than 6 months) is also part of the initial evaluation.

Histologic evidence provides confirmation of the diagnosis of neuroblastoma. Tissue may be obtained from a primary lesion or a metastatic site. Because tumor-specific biologic information plays a critical role in risk classification and treatment recommendations, obtaining adequate tissue for biologic studies is essential.

Pathologic Classification

Neuroblastoma is made up of small round blue cells that are uniformly sized and contain dense, hyperchromatic nuclei and scattered cytoplasm with stroma around it. Immunohistochemistry is positive for neurofilament protein, synaptophysin, neuron-specific enolase, ganglioside GD2, and chromogranin A, which distinguishes it from the other small round blue cell tumors of childhood. The histopathologic appearance of neuroblastoma ranges from undifferentiated neuroblasts, to more mature ganglioneuroblastoma, to fully differentiated and benign ganglioneuroma. The most widely used morphologic classification system is based on the system proposed by Shimada et al.[29] in which tumors are classified as favorable or unfavorable. It is based on age, the amount of stroma, degree of neuroblastic differentiation, and the mitosis-karyorrhexis index. Further clarification with international agreement followed with the International Neuroblastoma Pathology Classification, which separates neuroblastoma into four categories: (1) neuroblastoma that is undifferentiated, poorly differentiated (<5% exhibiting differentiation), or differentiating (>5%); (2) ganglioneuroblastoma, intermixed; (3) ganglioneuroblastoma, nodular; and (4) ganglioneuroma.

Genetic Prognostic Factors: Tumor Biology

In addition to clinical factors and histology, a number of biologic factors have been shown to correlate with prognosis (Table 73.6). Genomic data currently used in risk classification schemes include the status of the *MYCN* oncogene, tumor cell DNA content (ploidy), and the allelic status of chromosome arms 1p, 11q, 14q, and 17q.[30] More recently, it has been found that any segmental chromosomal abnormality indicates a less favorable outcome.[31]

Amplification of the *MYCN* oncogene is present in 16% to 25% of primary neuroblastomas and has been shown to correlate with poor prognosis independent of age, stage, and other genetic alterations.[32] Patients with stage 1, 2, or 4S disease demonstrate *MYCN* amplification only rarely; when present, it has been associated with rapid disease progression in these normally favorable stages. In a Children's Cancer Group study of stage 4 neuroblastoma in infants, the progression-free survival rate after 3 years was less than 10% in infants with tumors that demonstrated *MYCN* amplification, compared with 93% for those with single-copy tumors.

Total cellular DNA content also predicts response to therapy in infants with neuroblastoma.[33] Diploid DNA content is an unfavorable prognostic factor, particularly in infants younger than 12 months. Infants with hyperdiploid tumors have a significantly

TABLE 73.6 Features That Affect Prognosis in Neuroblastoma

Feature	Favorable	Unfavorable
Age at diagnosis	<18 months	>18 months
INRG stage	L1, L2, MS	M
MYCN status	Nonamplified	Amplified
Histologic appearance	Ganglioneuroma, ganglioneuroma maturing, ganglioneuroblastoma intermixed	Ganglioneuroblastoma nodular or neuroblastoma
DNA ploidy (DNA index)	>1 or <1	1
Allelic status of 11q	Normal	11q deletion or LOH at 11q or any segmental chromosome loss

INRG, International Neuroblastoma Risk Group; *LOH*, loss of heterozygosity.

TABLE 73.7 International Neuroblastoma Risk Group Staging System

Stage	Definition
L1	Localized tumor not involving vital structures as defined by the list of image-defined risk factors[a] and confined to one body compartment
L2	Locoregional tumor with the presence of one of more image-defined risk factors[a]
M	Distant metastatic disease (except stage MS)
MS	Metastatic disease in children younger than 18 months with metastases confined to the skin, liver, and/or bone marrow

[a]Image-defined risk factors are specific to each body compartment. For example, risk factors within the neck include tumor encasing the carotid and/or vertebral artery and/or internal jugular vein, tumor extending to the base of the skull, or tumor compressing the trachea.
Data from Monclair T, Brodeur GM, Ambros PF, et al. The International Neuroblastoma Risk Group (INRG) staging system: an INRG Task Force report. *J Clin Oncol*. 2009;27:298–303.

better response to therapy than those with diploid tumors. Diploidy often correlates with tumor *MYCN* amplification, although in rare cases of hyperdiploidy with *MYCN* amplification, the *MYCN* amplification portends an unfavorable outcome.

Tumor karyotype also influences outcome. Loss of heterozygosity (LOH) of 1p occurs in up to 36% of primary tumors, and LOH at 11q23 is seen in 44% of primary neuroblastomas.[34] Both are associated with poor outcomes, older age at presentation, and advanced-stage disease. Gain of 17q occurs in 60% of neuroblastomas and is associated with metastatic disease and unfavorable prognosis. Comprehensive genome-wide approaches such as comparative genomic hybridization are becoming increasingly useful in refining the prognostic accuracy of chromosomal alterations.[35]

Staging

Neuroblastoma has traditionally been staged according to the International Neuroblastoma Staging System (INSS). Staging is based on age, disease site(s), and degree of surgical resection. New guidelines for a pretreatment risk classification system have been developed by the International Neuroblastoma Risk Group (INRG) Task Force and are being used in addition to the INSS summarized in Table 73.7[36]; this system is undergoing evaluation in risk-based clinical trials. INRG stages include L1, localized tumor not involving vital structures (corresponds to INSS stages 1 and 2); L2, locoregional tumor with one or more image-defined risk factors (corresponds to INSS stage 3); M, metastatic disease (corresponds to INSS stage 4); and MS, metastatic disease in children younger than 18 months with metastases confined to skin, liver, and/or bone marrow (corresponds to INSS stage 4 S). Two important differences in the INRG system compared with the INSS are that it is a radiologic rather than a surgical staging system and that the upper age limit for stage MS has been extended from 12 to 18 months.

Stage 4 S (MS) comprises a unique group of patients with disseminated disease but a good prognosis. This combination occurs exclusively in infants. In this special group of patients, typical findings include a small primary tumor that does not cross the midline and remote spread involving the liver, skin, or bone marrow (<15% of marrow replacement by tumor), without radiographic evidence of skeletal metastases. There is lack of MYCN oncogene amplification in most INSS stage 4 S tumors, in contrast to INSS stage 4 tumors. Infants with INSS stage 4 S disease have a very good prognosis despite having disseminated disease (5-year survival rate >90%); spontaneous regression occurs without cytotoxic therapy in approximately 50% of cases.[37]

Treatment

Treatment modalities for neuroblastoma include observation alone, surgery, chemotherapy, and radiation therapy or a combination of these. Patients with INSS stage 1 and INSS stage 2 neuroblastoma have a 96% to 100% survival rate with surgery alone.[38] Isolated adrenal masses, the more common presentation among infants with neuroblastoma diagnosed prenatally, can be monitored closely for spontaneous regression if a tumor diameter meets the size criteria and if urine VMA and HVA levels are decreasing.[39] Infants with INSS stage 3 and INSS stage 4 disease have a poorer survival, even with aggressive chemotherapy, although the outcome, with better than 70% surviving overall, is far better than the survival rate of 10% to 20% reported for older children with disease of these stages.[22]

The unpredictable course of neuroblastoma, with its occasional spontaneous maturation or regression, not only makes this tumor unusual but also requires careful assessment of clinical and biologic risk factors in planning therapy. The type and intensity of treatment are determined by identification of infants with relatively good, intermediate, and poor prognoses on the basis of stage, international pathology classification, ploidy, segmental chromosomal abnormalities, and *MYCN* amplification. Patients who have localized disease (L1 or L2) without amplification of *MYCN* have an excellent prognosis, and such patients should undergo surgical resection or partial resection, but they likely will not derive any additional benefit from postoperative chemotherapy or radiation therapy. An exception to this is in the case of spinal cord compression, in which prompt decompression with chemotherapy (preferred), laminectomy, or local irradiation may be used to preserve function. The combination of extensive laminectomy with

postoperative irradiation should be avoided because later spinal deformity is almost inevitable. Infants with stage 3 and stage 4 disease are usually treated with a combination of chemotherapy and local surgery, with radiation therapy given only as necessary to eradicate residual disease. The active drugs that are most commonly used include cisplatin or carboplatin, etoposide, doxorubicin, cyclophosphamide, and vincristine. Infants with stage 4 disease with amplification of the *MYCN* oncogene have a very unfavorable prognosis; standard chemotherapy regimens are not sufficient for cure. In these high-risk patients, intensive chemotherapy followed by myeloablative therapy with stem cell support may offer additional benefit.[40] In addition, the use of the differentiation agent isotretinoin and the anti-GD2 antibody ch14.18 has been shown to improve outcome in patients with advanced-stage, high-risk neuroblastoma.[41,42]

Infants with INSS stage 4S disease have a highly favorable prognosis and may require minimal or no therapy. Because many patients undergo spontaneous regression, therapy should be directed toward supportive care, with use of chemotherapy and surgery restricted to relieving symptoms.[43] The main cause of death in these patients is massive hepatic involvement resulting in respiratory insufficiency or compromise of renal or gastrointestinal function. Symptomatic patients are treated with chemotherapy. When there is a risk of organ impairment due to tumor bulk not responding to initial chemotherapy, low-dose radiotherapy can be considered (450 centiGray given in three fractions; in some cases not all three fractions are needed).[22]

Prenatal Diagnosis

Neuroblastoma is increasingly being detected prenatally by screening ultrasonography. Newborns with adrenal or other mass lesions detected prenatally should be evaluated by urine catecholamine levels (although this has a low specificity) and follow-up ultrasonography. Careful observation may be adequate for infants with localized tumors, which frequently regress.

Congenital Leukemia

Epidemiology

Although leukemia is the second most common malignancy in infants, congenital leukemia, defined as leukemia diagnosed in the first 4 weeks of life, is quite rare. The incidence of leukemia in the first 3 months is approximately five cases per million.[44] Two-thirds of congenital leukemia cases are classified as AML, in contrast to older infants and children, in whom ALL predominates. Congenital leukemia is associated with a high mortality with an overall survival rate at 24 months of only 20%,[45] which is due to the aggressive biology of these leukemias and age-related treatment complications.

The cause of leukemia is unclear. In infants and older children a number of factors are associated with the development of leukemia; these include genetic factors, environmental influences, and immunodeficiencies. Genetic epidemiologic studies of infant leukemia indicate that most, if not all, cases are initiated in utero and involve acquired, noninherited genetic rearrangements; chromosome band 11q23 (*KMT2A*, previously known as *MLL*) is frequently involved. Leukemia-associated gene rearrangements have been retrospectively identified in archived newborn screen blood spots of children who subsequently developed leukemia.[46–48] The Children's Oncology Group has reported a trend toward higher incidence of AML, but not ALL, in infants of mothers who consumed larger amounts of naturally occurring topoisomerase 2 inhibitors, such as those in foods high in flavonoids and phytates.[49]

Clinical Manifestations

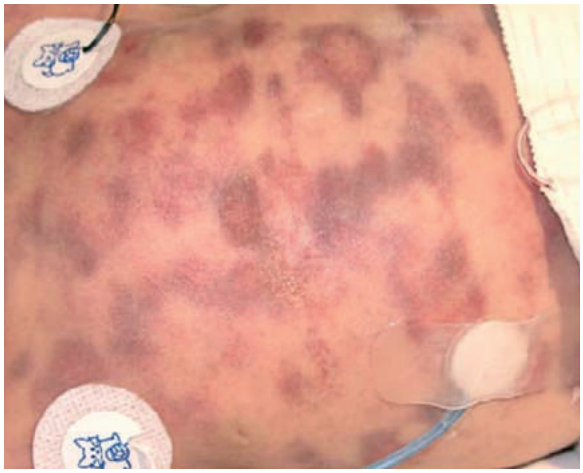
Clinical signs of leukemia may be evident at birth and include hepatosplenomegaly, petechiae, and ecchymoses. Myeloid leukemic cell infiltration of the skin *(leukemia cutis)* is present in 25% to 30% of patients with congenital leukemia; a skin nodule may be the first clinical sign of leukemia (Fig. 73.4). Patients typically have multiple nodules that are freely movable over the subcutaneous tissue with a greenish-blue or dark pink discoloration of the overlying skin. It is important to perform flow cytometry and cytogenetic studies on the skin biopsy specimen because infants found to have an *KMT2A* rearrangement have a poor prognosis even in the absence of bone marrow involvement and should be treated aggressively.[50] When chloromas are present on the head or neck, imaging studies should be performed to assess the patient for the presence of intracranial or skull involvement. Infants may present with respiratory distress from very high numbers of circulating leukemia blasts and resultant leukemic infiltration in the lungs. Some infants with leukemia may appear somnolent or have periodic apnea as a result of CNS leukostasis, caused by sludging of leukemic cells in blood vessels.

Laboratory Manifestations

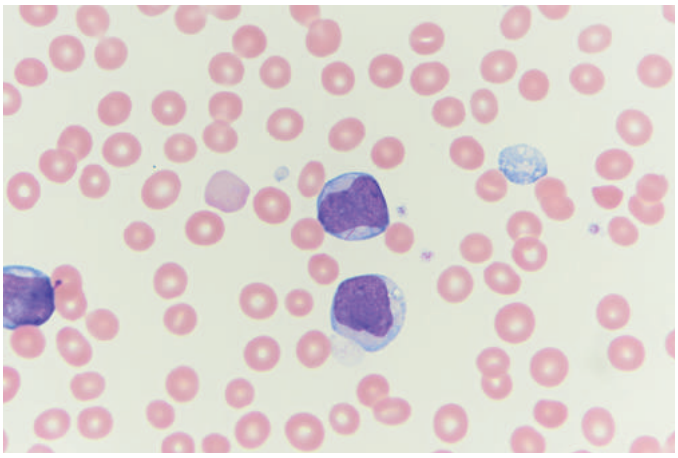
Hemoglobin levels may be normal initially, but anemia soon develops as the normal postnatal decrease in red blood cell production is combined with the leukemic proliferation and expansion within the bone marrow. Total white blood cell (WBC) counts may be normal or decreased, but leukocytosis is more often present. WBC counts of 150,000/mm^3 to 250,000/mm^3 or higher are common, and counts as high as 1,300,000/mm^3 have been reported. There is usually a predominance of blast cells on the CBC differential. Auer rods, characteristic intracellular inclusions, may be present and are pathognomonic of AML (Fig. 73.5).

Differential Diagnosis

A number of conditions can mimic congenital leukemia. Leukocytosis, hepatosplenomegaly, and thrombocytopenia can be seen in congenital infections such as syphilis, cytomegalovirus



• **Fig. 73.4** Extensive Leukemia Cutis in a Newborn. (From Zhang IH, Zane LT, Braun B, et al. Congenital leukemia cutis with subsequent development of leukemia. *J Am Acad Dermatol*. 2006;54:S22–S27.)



• **Fig. 73.5** Malignant Blast Cells with Auer Rods Present in Cytoplasm. This finding is pathognomonic of acute myelogenous leukemia. (Courtesy Kristie White, UCSF, San Francisco.)

(CMV) infection, herpes simplex virus infection, toxoplasmosis, and bacterial sepsis. Congenital human immunodeficiency virus infection may rarely be confused with leukemia. Clonal B-cell expansion in such patients may cause lymphadenopathy. A marked but transient leukemoid reaction may occur during the newborn period in the setting of suspected or proven infection, particularly in very low birthweight neonates.[51] Severe erythroblastosis fetalis can mimic leukemia. Affected infants usually have hepatosplenomegaly, large numbers of nucleated erythroblasts in the peripheral blood, and, occasionally, thrombocytopenia. Small infiltrates of extramedullary erythropoiesis in the skin are a rare manifestation, resembling leukemia cutis.

A transient leukemoid reaction *(TMD)* occurs in many infants with Down syndrome. The leukemoid reaction usually resolves, but these infants sometimes require low-dose chemotherapy to reduce the risk of death because of end organ injury and failure. Furthermore, infants diagnosed with transient myeloproliferative disorder are at higher risk of later development of acute leukemia (see later). Infants with neonatal neuroblastoma may have symptoms similar to those of congenital leukemia, with hepatomegaly and discolored skin nodules. CBCs are usually normal, without circulating blasts. Bone marrow biopsies and aspirates sometimes show clusters of neuroblastoma cells (see Fig. 73.1). Increased excretion of catecholamine metabolites and the presence of a primary tumor, most often intraabdominal, are other clues that point to the diagnosis of neuroblastoma rather than leukemia.

Cellular Morphology and Immunophenotype

The bone marrow of a newborn with leukemia shows extreme hypercellularity and a marked predominance of immature cells, either myeloid or lymphoid. AML and ALL are differentiated on the basis of typical morphologic characteristics, such as the presence of granules or Auer rods (in AML), histochemical stains, immunophenotyping by flow cytometry, and chromosomal analysis. Terminal deoxynucleotidyl transferase, a DNA polymerase that catalyzes the polymerization of deoxynucleotides in thymocytes, is usually present in lymphoblasts but is only rarely present in myeloblasts. Myeloblasts are usually positive for myeloperoxidase, whereas lymphoblasts are not. AML is primarily subclassified on the basis of recurrent genetic abnormalities as described in the most recent World Health Organization classification system.[52] Leukemias that do not have a genetic abnormality are classified according to the international French-American-British (FAB) classification based on morphology and histochemistry (Table 73.8).

TABLE 73.8 World Health Organization 2008 and Immunophenotypic Classification of Childhood Acute Leukemia

WHO 2008 Classification	Antigen Expression*
Acute Lymphoblastic Leukemia	
B-cell acute lymphoblastic leukemia	HLA-DR, CD10, CD19, CD20, CD24
T-cell acute lymphoblastic leukemia	CD2, CD5, CD7
AML and Related Neoplasms	
AML with recurrent genetic abnormalities†	
AML with t(9;11)(p22;q23); *MLLT3–MLL*	
AML, Not Otherwise Specified	
AML with minimal differentiation (M0)	CD13, CD33, CD34
AML without maturation (M1)	CD13, CD33, CD34
AML with maturation (M2)	CD13, CD33, CD34
Acute myelomonocytic leukemia (M4)	CD11b, CD13, CD14, CD15, CD33, CD34
Acute monocytic leukemia (M5)	CD11b, CD13, CD14, CD15, CD33, CD34
Acute erythroid leukemia (M6)	Glycophorin CD34
Acute megakaryoblastic leukemia (M7)	CD34, CD41, CD42, CD61
Myeloid Proliferations Related to Down Syndrome	
Transient myeloproliferative disorder	CD34, CD41, CD42, CD61
Myeloid leukemia associated with Down syndrome	CD34, CD41, CD42, CD61

*The indicated diagnosis may express some or all of the indicated antigens.
†Other genetic abnormalities omitted because of rarity in neonatal and infantile acute myeloid leukemia.
AML, Acute myeloid leukemia; *WHO*, World Health Organization.
Modified from the 2008 revision of the WHO classification of myeloid neoplasms and acute leukemia.

The immunophenotype, determined with a panel of fluorescently labeled monoclonal antibodies most often against cluster of differentiation (CD) antigens, is critical for differentiating AML and ALL.[53] Myeloid leukemia cells are usually positive for CD13/CD33 antigens, which are markers of myeloid and monocytic differentiation. A notable exception is acute megakaryoblastic leukemia, which expresses the CD41/CD42 platelet glycoproteins and CD61, and is most commonly seen in patients with Down syndrome. Most neonatal and infant lymphoblastic leukemia cells exhibit an early precursor B-cell phenotype and often are CD1a, CD19, CD24, and CD15 positive and CD10 negative.[54] In addition, coexpression of myeloid antigens is often present, suggesting that these leukemias arise from very immature lymphoid progenitors. Surface antigen expression is summarized in Table 73.8.

Genetics

A number of cytogenetic abnormalities have been found in association with congenital leukemia; many of these abnormalities are independent prognostic indicators. The most frequent abnormalities involve disruptions of the *KMT2A* gene at 11q23. Abnormalities of 11q23 are found in approximately 50% of infant AML cases and 70% to 80% of infant ALL cases.[45,55,56] These are nonhereditary, nonconstitutional abnormalities that occur in utero. In mice and in humans, the *KMT2A* protein positively regulates *HOX* genes, which are critical for hematopoietic development.[57] Rearrangements in the *KMT2A* gene confer a poor prognosis, which worsens with decreasing age.[5,58] Gene expression analysis studies demonstrate a unique early hematopoietic progenitor genetic signature that distinguishes infant ALL with *KMT2A* rearrangements from ALL and AML in older children.[57]

Treatment and Prognosis

The course of congenital leukemia is usually characterized by rapid deterioration and death from hemorrhage or infection. Although cure rates have increased significantly in older children with leukemia, improvements have been modest in neonates with ALL. Infants with leukemia frequently present with hyperleukocytosis (blast cell count in excess of 100,000/mm^3), which may result in sludging of blast cells in capillaries with resultant intracranial hemorrhage, respiratory distress, or tumor lysis syndrome, which is characterized by hyperkalemia, hyperphosphatemia, hypocalcemia, hyperuricemia, and acute renal failure. Disseminated intravascular coagulation is another common complication, especially with monocytic subtypes. Leukemic blast cells release procoagulants, causing a consumptive coagulopathy that places the neonate at risk of either bleeding or thrombotic sequelae and may be exacerbated by further leukemia cell lysis induced by chemotherapy.

Initial supportive care includes correction of metabolic and hemorrhagic complications. Transfusion of platelets and fresh frozen plasma is frequently required. Rasburicase, a recombinant urate oxidase enzyme, has been safely used in neonates with hyperuricemia[59,60] but is contraindicated if glucose 6-phosphate dehydrogenase deficiency is suspected or diagnosed as precipitation of severe hemolysis and death have been reported.[61]

Intensification of chemotherapy regimens has resulted in increased rates of remission and survival in both infants and older children diagnosed with AML. The contemporary chemotherapy regimens used in infants are similar to those used in older children and usually include daunorubicin and cytarabine, and outcomes for infants with AML are similar to those for older children, with overall survival rates of 65% to 75%.[62,63] Importantly, molecular features, in particular certain partner genes that fuse with *KMT2A* and a rare form of leukemia characterized by *CBFA2T3-GLIS2*, have been closely associated with poor survival outcomes in AML.[64]

Conversely, young infants with ALL fare worse than do older children with ALL, in whom chemotherapy results in a disease-free survival rate of more than 85%. Studies of infant ALL have reported rates of disease-free survival of 28% to 47%,[65] which is a significant improvement from previous trials and has been associated with the introduction of hybrid regimens that combine elements from standard ALL and AML protocols, addressing the more primitive nature of *KMT2A*-rearranged ALL. Unfortunately, patients with congenital ALL continue to have higher relapse rates, with a disease-free survival rate of less than 20%.[45]

Because CNS involvement is common in infants with AML and ALL, intrathecal chemotherapy is an important part of treatment. Radiation therapy, which can cause neurocognitive sequelae and secondary malignancies, is no longer routinely used. The use of hematopoietic stem cell transplant is controversial and remains an area of research in the treatment of congenital leukemia.

Transient Myeloproliferative Disorders and Leukemia in Patients With Down Syndrome

The incidence of acute leukemia in children with Down syndrome is 20-fold higher than in the general population. In children with Down syndrome younger than 3 years, a rare megakaryoblastic subtype of acute leukemia predominates and is classified by the World Health Organization as myeloid leukemia associated with Down syndrome (ML-DS). ML-DS is characterized by *GATA1* transcription factor mutations and is distinct from conventional acute megakaryoblastic leukemia, which occurs in older non–Down syndrome patients, on the basis of molecular features and prognosis.[52] The prognosis of myeloid leukemia associated with Down syndrome is favorable[66] and is associated with blast hypersensitivity to traditional chemotherapy agents such as cytarabine and daunorubicin.[67]

Transient Myeloproliferative Disorder

TMD, which occurs in 4% to 10% of neonates with Down syndrome, is clinically indistinguishable from AML.[68] Although most cases of TMD occur in patients with Down syndrome, TMD is also rarely seen in patients with no constitutional chromosomal abnormalities or with trisomy 21 mosaicism. TMD is a clonal disorder typically manifested by hepatomegaly, splenomegaly, and circulating myeloblasts. There may or may not be associated anemia or thrombocytopenia. In general, the blast count of the peripheral blood exceeds that of the bone marrow. Blast cells often have cell surface antigens characteristic of megakaryoblasts. Somatic mutations in *GATA1* have been detected in nearly all cases of TMD and myeloid leukemia associated with Down syndrome.[69]

Most neonates with Down syndrome and TMD experience complete clinical and hematologic recovery without systemic therapy, usually within 3 months. The blast count slowly decreases over a period of 2 to 3 weeks, and the hemoglobin and platelet counts normalize. However, in some cases, spontaneous resolution does not occur, and the neonate may experience clinical deterioration manifested by progressive hepatosplenomegaly, hepatic dysfunction, coagulopathy, ascites, and pleural or pericardial effusions. Approximately 20% of Down syndrome TMD cases are fatal, usually due to hepatic or cardiopulmonary failure.[70] Treatment with low-dose cytarabine has been shown to reduce the risk of early death in the setting of high-risk TMD. The indications for starting treatment differ but generally include a WBC count of more than 50,000/mm^3 to 100,000/mm^3, platelet count of less than 100,000/mm^3, renal or hepatic dysfunction, or cardiopulmonary compromise.[71,72] In some instances, complications from TMD occur in utero, and various degrees of hydrops fetalis can result.

Infants with Down syndrome and a history of TMD should undergo careful follow-up because TMD is considered to be a preleukemic syndrome: approximately 20% of neonates with TMD develop ML-DS within 4 years.[68] The administration of low-dose cytarabine does not appear to alter the risk of ML-DS development.

Germ Cell Tumors

Germ cell tumors are neoplasms derived from primordial germ cells. They are a heterogeneous group of tumors, differing in site, age at presentation, histopathologic features, and malignant potential. They can occur in both gonadal and extragonadal sites—although primarily in extragonadal sites in neonates.[73] Normal pluripotent primordial germ cells migrate from the yolk sac to the embryo, and persistence and abnormal migration of these cells or lack of normal erasure of parental methylating imprinting is thought to lead to these tumors.[74]

Germ cell tumors make up 35% to 40% of all neoplasms in the neonate, but only 5% of those contain a malignant component.[74] Benign germ cell tumors in the fetus and newborn are classified as either mature or immature teratomas.[75] However, one or more of the germ layer derivatives may develop malignant characteristics. Germ cell tumors may arise in a variety of locations in the body, usually along the axial midline. Common sites in children include the pineal gland, neck, mediastinum, retroperitoneum, and sacrococcygeal region. In the neonatal period, most teratomas occur in the sacrococcygeal region (40% of all germ cell tumors), followed next by tumors in the neck. Yolk sac tumor (endodermal sinus tumor) is the most common malignant germ cell tumor in neonates and young children. In the neonate it most often occurs within a teratoma, often in the sacrococcygeal region (see later). Of note, patients with undervirilization syndromes have a 5.5% prevalence of germ cell tumors which increases to 33% by puberty, so gonadectomy is recommended before puberty in these patients.

Pathology

By definition, teratomas contain tissue arising from all three layers of the embryonic disk. Ectodermal components, including glial tissue, are a major component of teratomas occurring at birth, in particular, sacrococcygeal tumors. There are often skin, hair, and tooth elements. Mesodermal components, including fat, bone, and muscle, also are present. Endodermal components include digestive tract tissue; this component generally forms a smaller portion of the tumor. The levels of hormonal markers, including serum AFP and β-hCG, are often elevated in the presence of malignant tissue within the teratoma and are useful to follow as therapy progresses. Elevated serum AFP level indicates the presence of immature endodermal sinus tissue or yolk sac elements, whereas elevated β-hCG level indicates the presence of embryonal carcinoma. Choriocarcinoma, which is rarely seen in newborns, manifests itself with an extremely elevated β-hCG level. Immature teratomas are defined and graded on a scale of 1 to 3 depending on the amount of embryonal tissue present.[74]

Evaluation

MRI or CT of the primary tumor is indicated to evaluate the extent of disease. Sacrococcygeal tumors should be imaged by MRI because of the possible involvement of the spinal cord. The entire abdomen is included in the imaging study to assess the extent of any local invasion, particularly involvement of the rectal wall. Chest imaging is performed to rule out metastasis. Baseline levels of serum AFP and β-hCG, which are normally elevated in newborns, are measured. Because of the variation in the levels at birth and the variation in the rates at which the levels decline to normal, these tumor markers can be difficult to interpret as measures of residual disease or recurrence. Normal AFP values for age are shown in Table 73.5. The half-life of AFP is 5 to 7 days; that of β-hCG is 24 to 36 hours.

Sacrococcygeal Teratomas

Sacrococcygeal teratomas are the most common solid tumors in newborns. The estimated incidence is 1 in 27,000 live births.[76] A minority are malignant: 10% to 17% of sacrococcygeal teratomas contain yolk sac tumor.[75] Malignancy is less common, only 7% to 10%, in infants younger than 2 months.[74] Females are affected 2 to 4 times more frequently than males, but the malignancy rate is similar. Half of sacrococcygeal teratomas are diagnosed prenatally by ultrasonography. Polyhydramnios, nonimmune fetal hydrops, and dystocia have all been described in association with sacrococcygeal teratomas. Lack of prenatal diagnosis has been reported to carry a 20% risk of death in the first hour after birth secondary to hemorrhage.[76] Congenital anomalies, including genitourinary, hindgut, and lower vertebral malformations, are present in 15% of patients.[75] In most cases the tumor manifests itself as a mass protruding between the coccyx and rectum; the mass may be quite large (Fig. 73.6). Approximately 10% of these tumors are found only by rectal examination. Nearly all arise at the tip or inner surface of the coccyx.

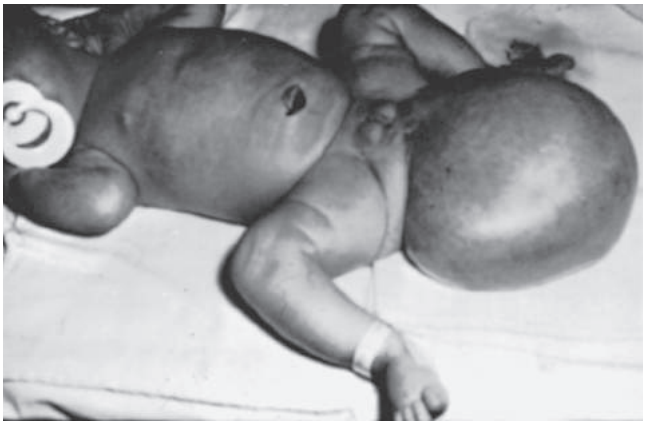
Differential Diagnosis

Sacrococcygeal teratomas may be confused with meningomyelocele, rectal abscess, pelvic neuroblastoma, pilonidal cyst, and a variety of very rare neoplasms that may occur in the sacral region. Most benign teratomas in this area produce no functional difficulties, even when marked intrapelvic extension is present. Bowel or bladder dysfunction, painful defecation, and vascular or lymphatic obstruction suggest that the lesion is malignant.

Treatment

Treatment of sacrococcygeal tumors is primarily surgical.[73] The tumor should be radically excised as soon as possible because small, undifferentiated foci may proliferate and become aggressive. Removal of the entire coccyx is required. Failure to remove the coccyx carries a 30% to 40% risk of local recurrence, which is sometimes accompanied by malignant elements. The survival rate for neonates with sacrococcygeal teratoma is 85%.

Sacrococcygeal teratomas diagnosed prenatally by ultrasonography (approximately 50% of cases) are associated with a worse outcome; the survival rate is only 30% to 50%.[75,77] Fetal hydrops and prematurity are the main factors contributing to the low survival rate. If hydrops occurs before fetal pulmonary maturity, open



• **Fig. 73.6** Large Sacrococcygeal Teratoma in a Newborn.

fetal surgical intervention to debulk and devascularize the tumor is an option.[77]

Infants with sacrococcygeal teratoma containing malignant yolk sac elements are treated with surgery followed by chemotherapy with cisplatin, etoposide, and bleomycin. Immediate and late complications of this regimen can be significant and include hearing loss, pulmonary fibrosis, and secondary malignancy.

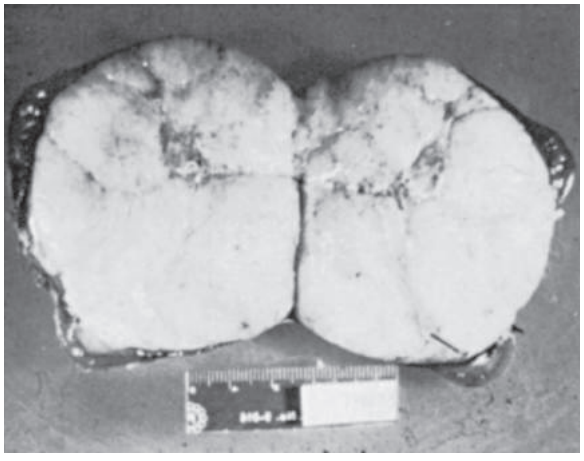
Renal Neoplasms

Approximately two-thirds of intra-abdominal masses in the neonatal period arise from the kidney. The vast majority of these neoplasms are nonmalignant and include polycystic or dysplastic kidney disease, hydronephrosis, renal vein thrombosis, and ectopic kidneys. The most common intrarenal neoplasm manifesting itself at birth is congenital mesoblastic nephroma (CMN), followed by Wilms tumor and nephroblastomatosis. Less common intrarenal neoplasms diagnosed during infancy include rhabdoid tumor, clear cell sarcoma of the kidney, renal cell carcinoma, RMS, and lymphoma.[78] The typical clinical presentation is an asymptomatic abdominal mass detected on physical examination or by ultrasonography.

Congenital Mesoblastic Nephroma

CMN is the most common intrarenal neoplasm in the neonate. It usually behaves as a benign neoplasm, but the histopathologic subset, cellular CMN, occasionally recurs or metastasizes. The typical clinical presentation is an asymptomatic abdominal mass detected on physical examination or by ultrasonography, but hypertension due to a mass effect on the renal vasculature and hypercalcemia due to elevated renin levels are also common findings. The tumor infiltrates into normal renal parenchyma and is not encapsulated (Fig. 73.7). Specific sonographic features can help to differentiate CMN from Wilms tumor. The tumor may be diagnosed prenatally by ultrasonography, which reveals a greatly enlarged kidney distorted by the tumor. There is an increased incidence of polyhydramnios (71%) and premature labor.[78]

Two histologic subtypes of CMN have been identified: the classic subtype and the cellular variant. The cellular variant usually manifests itself at an older age (>3 months) than the classic type (mean age at presentation of 1 month). Cytogenetic analysis of the cellular variant shows a translocation t(12;15)(p13;q25) that results in an *ETV6–NTRK3* gene fusion that is identical to that found in infantile fibrosarcoma.[78,79]



• **Fig. 73.7** Congenital Mesoblastic Nephroma Compressing and Nearly Totally Replacing the Kidney.

Complete surgical resection is usually an effective treatment for the classic form of CMN. Patients with the cellular variant are also treated with complete resection, but local and distant recurrences to the lungs and brain can rarely occur. Positive surgical margins or tumor rupture during resection are risk factors for recurrence, which usually occurs within the first year following surgery. Resection and chemotherapy can successfully treat recurrent disease.[78] The overall survival rate for CMN is 95% to 98%.[80]

Wilms Tumor

Wilms tumor, or nephroblastoma, is the most common intra-abdominal tumor of childhood, affecting 1 in 8000 children, but it is relatively rare in the neonatal period. In subsets of children with aniridia and hypospadias, the incidence is much higher. Wilms tumor is thought to arise from abnormal proliferation of metanephric cells, referred to as *nephrogenic rests*, without normal tubular and glomerular differentiation. Nephrogenic rests normally occur in 1% of newborn kidneys but often regress early in childhood.

Clinical Manifestations

Wilms tumor arises from one or both kidneys, and most children present with an abdominal or flank mass. It seldom extends beyond the midline, even though it may grow downward beyond the iliac crest. In 5% to 10% of all cases, tumors involve both kidneys. Gross hematuria is a rare presenting symptom, but microscopic hematuria is found in approximately 25% of cases. Wilms tumor is seldom diagnosed at birth or during the neonatal period. Characteristics associated with an earlier presentation include bilaterality, associated aniridia or hypospadias, and a family history.[81]

Hereditary Associations and Congenital Anomalies

Although most cases of Wilms tumor are sporadic, a number of conditions are associated with an increased risk of developing the tumor. These include *WT1*-associated phenotypes caused by deletions and mutations of the *WT1* gene (11p13), overgrowth syndromes, and constitutional chromosomal disorders.[8]

WAGR syndrome (*W*ilms tumor, *a*niridia, *g*enitourinary abnormalities, mental *r*etardation) predisposes to Wilms tumor because of a deletion in *WT1* and aniridia because of a deletion of *PAX6*, which are both encompassed within a deleted region at 11p13.[82] From a clinical perspective, if an infant has aniridia, chromosome analysis should be undertaken. If a deletion of chromosome 11p13 is found, the child should be monitored for the development of Wilms tumor with serial renal ultrasound examinations. Wilms tumor develops in approximately half of these patients. Denys-Drash syndrome, caused by missense mutations within the *WT1* gene, classically describes the triad of Wilms tumor, genitourinary anomalies, and nephropathy. The genitourinary anomalies may be severe enough to result in pseudohermaphroditism.

Hemihypertrophy, which can be either isolated or associated with various genetic syndromes, is associated with an increased risk of the development of Wilms tumor. BWS, an overgrowth disorder caused by dysregulation of imprinted genes

at chromosome 11p15, is associated with an increased risk of Wilms tumor, particularly bilateral disease. Between 1% and 8% of individuals with BWS develop Wilms tumor.[8] Other characteristic clinical findings include macroglossia, anterior abdominal defects, ear creases and pits, neonatal hypoglycemia, and hemihypertrophy.

Between 1% and 2% of Wilms tumor cases occur within families, but the underlying cause of familial Wilms tumor is heterogenous and mostly unknown.[8]

Prognostic Factors

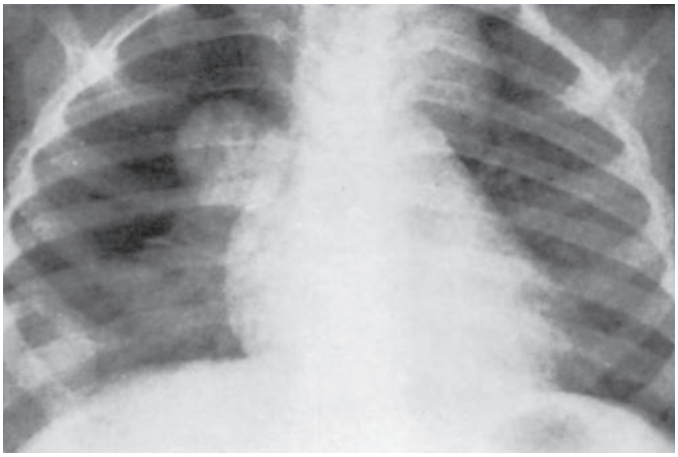
Important prognostic factors include the histologic assessment, the extent of disease, and chromosomal abnormalities. The presence of anaplasia is an important predictor of adverse outcome, and patients who have tumors with diffuse anaplasia fare worse than those with focal anaplasia. The presence of tumor-specific LOH for chromosome arms 1p and 16q is associated with a worse prognosis.[83] Patients younger than 2 years have fewer relapses, especially of distant sites, than older children.

Evaluation and Staging

Clinical staging, which includes a CT scan of the abdomen and chest, is an important factor in predicting survival; tumors with more extensive spread carry a poorer prognosis. The most common sites of metastasis are the liver and the lungs (Fig. 73.8). Tumor thrombus is occasionally noted in the inferior vena cava.

Treatment

Patients with low-stage Wilms tumor have a cure rate of more than 90%. Patients with small tumors limited to the kidney can be cured with surgery alone. Patients with intermediate-risk Wilms tumor are treated with a short course (12 weeks) of chemotherapy with vincristine and dactinomycin. More extensive disease or high-risk features, such as anaplasia, require the addition of doxorubicin and possibly other chemotherapy agents. Radiation therapy is indicated in children with diffuse abdominal disease; tumor spillage, including percutaneous biopsies, which is not a recommended diagnostic approach for pediatric renal tumors; or nonresponsive pulmonary metastases. Even patients with metastatic disease have a good prognosis, with a 70% long-term overall survival rate. Recurrent disease, which can be local or may involve metastases to the liver, lungs, or brain, is treated with additional chemotherapy and radiation therapy.



• **Fig. 73.8** Pulmonary Metastases from Wilms Tumor.

Persistent Nephrogenic Rests and Nephroblastomatosis

Nephrogenic rests are residual immature metanephric tissue within a fully developed mature kidney. They normally occur in 1% of newborn kidneys but typically regress during early childhood. Although benign, nephrogenic rests have been confused with Wilms tumor in the past and are believed to have the potential for neoplastic transformation to Wilms tumor. Histologically, nephrogenic rest cells may resemble Wilms tumor and occasionally present a diagnostic dilemma, although the presence of mitoses favors Wilms tumor. Radiographically, when imaged with MRI, nephrogenic rests tend to appear less heterogeneous, demonstrate less contrast enhancement, and are smaller than Wilms tumor. Bilateral or multicentric Wilms tumors are associated with a high incidence of synchronous nephrogenic rests. When nephrogenic rests are multifocal or diffuse, they are referred to as *nephroblastomatosis*. Children with massive bilateral nephroblastomatosis often respond to therapies for Wilms tumor.[84]

Rhabdoid Tumor of the Kidney

Rhabdoid tumor of the kidney (RTK) is an uncommon tumor of children that is one of the most lethal neoplasms of early neonatal life, with a mortality rate exceeding 80%. RTK is the second most common malignant neoplasm of the kidney in neonates, after Wilms tumor. It has a predilection for males and for infants, with median age at diagnosis of 11 months. It is often widely metastatic at diagnosis; metastatic sites include lung, abdomen, lymph nodes, liver, bone, skin, and brain. Homozygous inactivating deletions or mutations of the *INI1* gene, located in chromosome band 22q11.2, are responsible for most rhabdoid tumors[85] and can be detected immunohistochemically. The prognosis for infants with RTK is extremely poor. The survival rate at 2 years for infants with a diagnosis before 6 months of age is less than 15%.[86] Virtually all patients with distant metastases will have a fatal outcome. Treatment modalities have included surgical resection, chemotherapy, and radiation therapy.

Retinoblastoma

Retinoblastoma is a malignant ocular tumor that arises from embryonic retinal cells. It occurs in the setting of hereditary and nonhereditary disease. The incidence of retinoblastoma is approximately 1 in 18,000 live births; between 250 and 350 cases are diagnosed in the United States each year.[87,88] Bilateral involvement, which occurs in the setting of heritable disease, is observed in 20% to 35% of patients. Although the incidence of heritable retinoblastoma is constant among various population groups, the incidence of sporadic, nonhereditary, unilateral retinoblastoma is increased in poorer, tropical, and subtropical regions.[87]

Genetics

Approximately one-third of patients have hereditary disease, which is often bilateral. Patients with hereditary disease have a germline mutation in the retinoblastoma gene, *RB1*, a tumor suppressor gene located on chromosome band 13q14. The mutation is inherited from a parent or occurs during embryonic development. There is autosomal transmission with high penetrance, approximately 90%. Patients with nonhereditary disease tend to have unilateral retinoblastoma. Their disease is the result of acquired somatic mutations in both *RB1* alleles.

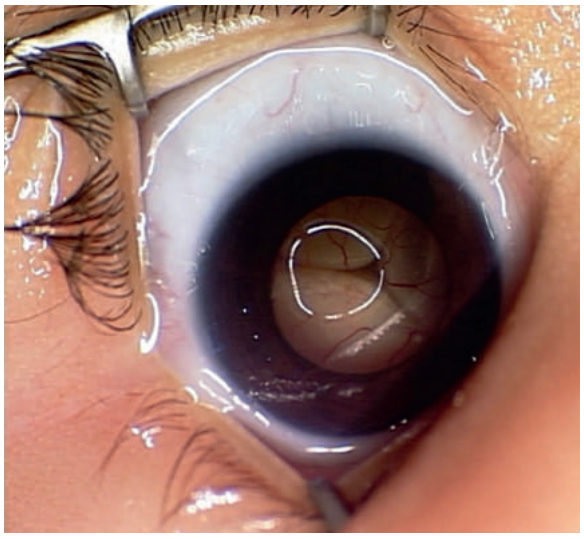
Approximately 5% of retinoblastoma patients are born with a constitutional deletion of chromosome 13, 13q−. These patients have associated constitutional anomalies, including

microencephaly, macrognathia, malformed ears and thumbs, hypertelorism, microphthalmia, ptosis, short stature, cleft palate, and developmental delay. In children with the bilateral and hereditary form, diagnosis is often at 1 year of age as opposed to on average at 2 years of age in children with the nonhereditary form.[88] This is due in part to early screening initiated because of the family history. In rare instances, a family history of retinoblastoma may be lacking; hereditary bilateral retinoblastoma may result from germline mosaicism in the parent.

Clinical Manifestations

Patients with retinoblastoma commonly present with leukocoria ("cat's eye"), squinting, or strabismus caused by loss of vision in the affected eye. Multifocal retinal involvement is common, occurring in 84% of cases. Intraocular spread may fill the vitreous body by extension or seeding, whereas exophytic tumors arise from the outer retinal layer and cause retinal detachment (Fig. 73.9). Extraocular spread is seen in less than 15% of patients, usually occurring by direct invasion of the optic nerve and eventually leading to subarachnoid involvement and intracranial spread. In such cases the cerebrospinal fluid may contain tumor cells. Rarely, tumors may spread by invasion of the orbit or by hematogenous dissemination to bone and bone marrow. Children with bilateral retinoblastoma are at risk of tumor dissemination to the pineal gland, a condition known as *trilateral retinoblastoma*. All patients with retinoblastoma should be evaluated initially by brain MRI for this condition. Patients with bilateral retinoblastoma are typically evaluated by brain MRI annually until age 5 or 6 years. Retinoblastoma can variably be detected in utero by ultrasonography or MRI in patients known to carry the mutated *RB1* gene.[88] Infants born to a parent with a known *RB1* mutation should be screened by an ophthalmologist from the time of birth.

The diagnosis of retinoblastoma is made by fundoscopic examination and orbital ultrasonography performed with the patient under general anesthesia. MRI of the orbit is useful to determine tumor extent and optic nerve involvement. A lumbar puncture for cerebrospinal fluid cytology is performed if there is optic nerve invasion, but more extensive evaluation with bone marrow biopsy or bone scan is not usually necessary. Tumors are staged according to the International Classification of Retinoblastoma, on the basis of tumor size and location and the extent of vitreus and subretinal seeding.[87]



• **Fig. 73.9** Retinoblastoma Filling the Orbit. (Courtesy Bertil Damato and Andrew Kao, UCSF, San Francisco.)

Treatment

Because extraocular spread and death from dissemination are rare, the main goal of treatment is local control and preservation of vision. Surgical enucleation is used only when there is no chance for useful vision, if glaucoma is present, or if conservative measures fail to control the tumor. Small tumors confined to the retina can often be controlled with focal consolidative therapies such as cryotherapy and laser photocoagulation. Systemic chemotherapy with agents such as carboplatin, vincristine, and etoposide is frequently used concurrently with cryotherapy and laser therapy. Intra-arterial chemotherapy with agents such as melphalan can provide directed therapy. External beam radiation therapy is effective, but because of the late effects of radiation on bone growth and the potential for second tumor induction, aggressive local therapy and systemic and/or intra-arterial chemotherapy are preferable. Decisions about management involve multidisciplinary discussion because of the various treatment modalities.

Prognosis

The prognosis for children with unilateral retinoblastoma is excellent, with cure rates of 85% to 90%. However, patients with bilateral disease have a much lower long-term survival rate because of the high incidence of second malignancies, which may occur at any point in the life span. Patients with hereditary disease can develop secondary sarcomas in the area treated with radiation therapy; they are also at increased risk of developing sarcomas in other, nonirradiated areas. Local extension of retinoblastoma confers a poor prognosis, with survival rates of less than 10% with orbital extension or distant dissemination.

Central Nervous System Tumors

Incidence and Epidemiology

Congenital brain tumors are rare (2% of childhood CNS tumors), with an incidence of approximately 1 to 3 per million live births.[89,90] The incidence is likely underreported as 30% of neonates with brain tumors are delivered stillborn. Most brain tumors in infants are supratentorial, in contrast to pediatric CNS tumors. Half are gliomas, including astrocytomas (low-grade gliomas). PNETs, choroid plexus tumors (associated with Aicardi syndrome), and medulloblastomas also occur. Atypical teratoid or rhabdoid tumor of the CNS is associated with a high mortality rate. In general, brain tumors presenting in the perinatal period carry a very poor prognosis. Mortality from congenital CNS tumors has been reported to be from 72% to 93%.[90] Discussion with the family regarding prognosis and alternatives to therapy should be undertaken prenatally if CNS tumors are identified.

Clinical Manifestations

Macrocephaly is often the first sign of a congenital CNS tumor if the tumor has not been identified in utero. Signs and symptoms of increased ICP may be present. In infants these include a bulging fontanelle, split sutures, or rapidly increasing head size. Poor feeding, vomiting, lethargy, and irritability can also be symptoms of increased ICP. Fundoscopic examination may or may not show papilledema. Specific neurologic abnormalities include Parinaud syndrome (impaired upward gaze secondary to increased pressure

in the dorsal midbrain), cranial nerve palsies, and nystagmus. Head tilting can occur in patients with posterior cerebellar masses secondary to cervical root irritation.

Treatment

Treatment depends on the pathologic characteristics of the tumor, which usually cannot be ascertained until after birth and further imaging. An important component of therapy in neonates is surgical resection of the tumor. The degree of surgical resection is the single most important predictor of survival.[89] Complete resection is often not possible because tumors in infants tend to be large, highly malignant, and invasive. They are also highly vascular, making it difficult to remove the tissue without significant morbidity. Many patients require ventriculoperitoneal shunt placement for relief of hydrocephalus.

Radiation therapy, a backbone of treatment for older children with malignant brain tumors, is avoided if possible in young infants because infants experience devastating late effects, including neurocognitive deficits and growth impairment. Adjuvant chemotherapy can play a role in treatment; this may allow necessary radiation therapy to be delayed until the child is older. Most tumor types are treated similarly to childhood CNS tumors, although the efficacy of these treatments is not clear especially given the desire to limit radiation therapy.

Sarcomas

Soft tissue sarcomas are rarely seen in newborns. The most commonly diagnosed soft tissue sarcoma in the neonatal age group is infantile or congenital fibrosarcoma, which is classified as a low-grade non-RMS soft tissue sarcoma. The incidence in infants between age 1 month and age 12 months is five cases per million infants.[4] In general, infantile fibrosarcoma is treated by complete surgical excision, although neoadjuvant chemotherapy with a variety of agents has been successfully used for tumor shrinkage, with subsequent reduction in the morbidity related to radical surgical procedures.[91] The cure rates for infantile fibrosarcoma approach 100%.[92,93]

The initial evaluation of a patient with a congenital fibrosarcoma includes imaging of the primary tumor by MRI or CT and a chest CT scan. Diagnosis is made by biopsy of the lesion. The chemotherapy regimens used successfully for treatment of this tumor include vincristine, dactinomycin, and cyclophosphamide, as well as etoposide and ifosfamide. Doxorubicin, while efficacious, is generally avoided because of the risk of cardiac toxicity. The duration of therapy depends on the size, location, and response of the tumor, but the general goal is to reduce the tumor size to maximize the chances of surgical local control. Radiation therapy is usually avoided to spare the infant the associated late effects of poor growth and secondary cancers.

RMS, the most common soft tissue sarcoma in children, is rarely seen in neonates: less than 1% of RMS cases are diagnosed in the first month of life. Congenital RMS often involves the genitourinary tract and is frequently of the embryonal subtype. Congenital embryonal RMS appears to be associated with a specific translocation, t(2;8)(q35;q13).[94]

Histiocytosis

The histiocytoses are a diverse group of disorders characterized by the accumulation and proliferation of cells derived from the monophagocytic system—macrophages and dendritic cells.[95] Taken as a whole, histiocytoses can occur throughout life but peak incidence is in 1- to 3-year-old patients. Class I disorders, which are dendritic cell related, include Langerhans cell histiocytosis (LCH) and pure cutaneous histiocytosis. Class II disorders, which involve macrophage-derived cells, include familial hemophagocytic lymphohistiocytosis (FHL) and infection-associated hemophagocytic syndrome (IAHS). Class III disorders are malignant disorders of mononuclear phagocytes and include acute monocytic leukemia, malignant histiocytosis, and histiocytic lymphoma. In addition to these three classes, other rare, noncategorized histiocytoses can occur in newborns, in particular, juvenile xanthogranuloma (JXG). The pathophysiology of the histiocytic disorders appears to be related to abnormal regulation of histiocyte activation resulting in cell proliferation and cytokine production.[95]

The most common histiocytic disease seen in the fetus and neonate is LCH. About half of newborns with LCH have disease confined to the skin, while the other half have disseminated disease with resultant organ dysfunction, usually involving bone marrow, liver, or lung. Presenting symptoms of LCH in neonates can include skin lesions, hepatosplenomegaly, lymphadenopathy, and respiratory distress. Natal teeth can be a sign of lesions in the maxilla or mandible. Histologically, both cutaneous and disseminated LCH are characterized by granuloma-like lesions. Diagnosis is made by biopsy; Langerhans cells are positive by immunohistochemistry for CD1a and S-100.

The course of LCH is unpredictable. The pure cutaneous form of LCH usually resolves spontaneously in 2 to 3 months, whereas disseminated LCH carries a poor prognosis, with 57% mortality;[96] prognosis depends on level of organ involvement, age of diagnosis, and progression of disease. To complicate matters, newborns with skin lesions may not develop the symptoms of disseminated disease for several weeks to months. Disseminated LCH is usually treated with chemotherapy, including vinblastine and prednisone. Recently, genetic findings in LCH include *BRAF* V600E mutations; treatment with vemurafenib (BRAF inhibitor) has been effective.[97]

FHL and IAHS are also seen in the newborn period. FHL has an incidence of approximately 1 in 50,000 liveborn children[98] and is more common in neonates than IAHS. The overall survival rate for newborns with FHL is only 9% unless a stem cell transplant is performed; the survival rate for newborns with IAHS is 59%.[95] Newborns with these disorders commonly have fever, hepatosplenomegaly, and cytopenias. Other symptoms include liver dysfunction, neurologic symptoms, hypertriglyceridemia, elevated serum ferritin levels, and hypofibrinogenemia. Diagnostic criteria are listed in Box 73.1. Hemophagocytosis is found in the bone marrow in 76% of patients. Natural killer cell and cytotoxic T-cell activity is reduced or absent. Thirty percent of patients with FHL harbor constitutional mutations in the *PRF1* gene, which encodes an essential protein for cellular immune activation. Other mutations, in *UNC13D* and *STX11*, have been reported, and additional genetic predispositions are being discovered, thus whole exome sequencing is indicated for patients without a known genetic predisposition.[99] Patients with FHL are usually well at birth, then develop clinical symptoms by age 2 to 6 months. Constant fever, cytopenias, marked hepatosplenomegaly, and progressive cerebromeningeal symptoms characterize the disease course. Initial treatment is with steroids and etoposide. Progressive disease usually leads to death within 4 months of diagnosis, but hematopoietic cell transplant can increase the 3-year survival rate to 64%.[100]

Patients with IAHS, whose symptoms are similar to those of patients with FHL, experience a high recovery rate (59%)

• BOX 73.1 Diagnostic Criteria for Hemophagocytic Lymphohistiocytosis

The diagnosis can be established if:

1. The patient has a molecular diagnosis consistent with hemophagocytic lymphohistiocytoses (pathologic mutations of *PRF1, UNC13D, STXBP2, RAB27A, STX11, SH2D1A*, or *BIRC4*).
2. The patient has five of the following eight diagnostic criteria:
   a. Temperature ≥38.5°C
   b. Splenomegaly
   c. Cytopenias (affecting at least two of three lineages in the peripheral blood): hemoglobin level <9.0 g/dL (<10 g/dL in infants younger than 4 weeks), platelet count $<100 \times 10^9$/L, neutrophil count $<1.0 \times 10^9$/L
   d. Hypertriglyceridemia and/or hypofibrinogenemia: fasting triglyceride level ≥3.0 mmol/L (i.e., ≥265 mg/dL), fibrinogen level ≤1.5 g/L
   e. Hemophagocytosis in bone marrow or spleen or lymph nodes; no evidence of malignancy
   f. Low or absent natural killer cell activity (according to local laboratory reference)
   g. Ferritin level ≥500 μg/L
   h. Elevated soluble CD25 level (i.e., alpha chain of soluble IL-2 receptor) ≥2400 IU/mL

Other findings consistent with the diagnosis include cerebromeningeal symptoms, lymph node enlargement, jaundice, edema, rash, hepatic enzyme abnormalities, hypoproteinemia, hyponatremia, elevated VLDL level, and decreased HDL level.

HDL, High-density lipoproteins; *IL-2*, interleukin-2; *VLDL*, very low-density lipoprotein.
From Henter J-I, Horne A, Arico M, et al. HLH-2004: diagnostic and therapeutic guidelines for hemophagocytic lymphohistiocytosis. *Pediatr Blood Cancer*. 2007;48:124–131.

when appropriate antibiotic or antiviral treatment is promptly administered.[95]

JXG, a disorder of dendritic-related cells, is less common in neonates than LCH. Two forms are recognized: cutaneous and extracutaneous. Patients have one or multiple cutaneous nodules, which are reddish to yellow-brown papules on the head, neck, and extremities. Three-quarters of neonates with cutaneous JXG have a solitary nodule. The nodules, which are present at birth in 34.5% of cases, typically resolve spontaneously within approximately 2 years.[101] Survival is excellent for neonates with JXG limited to the skin and subcutaneous tissue, with no deaths reported in one series.[95] Treatment is not generally indicated. Extracutaneous or disseminated JXG can involve the subcutaneous and soft tissues, liver, lung, spleen, eye, lymph nodes, and brain. Immunohistochemical stains may be required to differentiate this from LCH. Twenty-five percent of neonates with disseminated JXG do not have cutaneous lesions. Jaundice, hepatosplenomegaly, and thrombocytopenia can be seen. Although most cutaneous and extracutaneous lesions resolve spontaneously without treatment, disseminated JXG has an 11% death rate.[95] Ocular and CNS involvement can cause significant morbidity. Systemic JXG is usually treated with chemotherapy and/or corticosteroids.

Hepatoblastoma

Hepatoblastoma, the most common malignant tumor of the liver in children, is an embryonal neoplasm composed of malignant epithelial tissue. Besides hepatoblastoma, the differential diagnosis of hepatic lesions in neonates includes metastases from other malignant tumors (a more common cause than hepatoblastoma) and benign vascular tumors of the liver (see later). The incidence of hepatoblastoma in infants is 11.2 cases per million; premature infants with low birthweights have a particularly high incidence.[102] In neonates the female-to-male ratio is 1:6.[103] Treatment of hepatoblastoma in neonates is similar to that in young children. Histologic subtype is associated with outcome, with pure fetal histology having the best prognosis. Hepatoblastoma is associated with a number of genetic abnormalities and malformation syndromes, including BWS and trisomy 18.[104] Patients with sporadic hepatoblastoma have tested positive 10% of the time for a germline mutation in the tumor suppressor *APC*, the gene associated with familial adenomatous polyposis.[105]

The most common presenting symptom in neonates is abdominal distention. Even in the absence of distention an abdominal mass can sometimes be palpated. Anemia, fetal hydrops, and respiratory distress are other initial findings. Serum AFP level is elevated above neonatal norms in half of patients. Hepatoblastoma is occasionally diagnosed prenatally by screening ultrasonography. Tumor rupture can occur during birth, resulting in massive hemorrhage, often necessitating birth by cesarean delivery.[105]

Ultrasonography is useful to distinguish cystic and solid masses from diffuse hepatic enlargement. CT scan of the abdomen demonstrates the extent of tumor involvement, anatomic landmarks, and operability; MRI most accurately shows tumor margins and vessel involvement. Use of the PRETEXT system, which uses imaging findings to stage hepatoblastoma, helps guide decisions about resection and need for liver transplant.

The goal of therapy is complete surgical resection. Infants with pure fetal histology whose tumors are completely resected have a 92% 24-month survival rate; the overall survival rate in a case series of 52 patients was 86%.[105] A lobectomy of the involved portion of the liver is performed if one lobe is free of malignancy and there is no evidence of distant metastases. For unresectable but nonmetastatic tumors, initial treatment consists of chemotherapy with cisplatin and vincristine. If adequate tumor shrinkage results, the tumor is then resected. Orthotopic liver transplant has been used in patients with unresectable, nonmetastatic hepatoblastomas in conjunction with chemotherapy.

Hepatic Hemangioendothelioma

Hepatic hemangioendothelioma, also referred to as *hepatic hemangioma*, is a benign vascular tumor of the liver; it is the most frequent liver tumor of infants.[106] The disease is classified into three subtypes: focal, multiple, and diffuse hepatic lesions. Some patients also have cutaneous hemangiomas. While some infants with small tumors are asymptomatic, infants with larger tumors can have multiple symptoms, including abdominal distention, respiratory distress, high output cardiac failure, and consumptive coagulopathy. The diagnosis is often suspected prenatally when a hepatic mass is detected by ultrasonography. The diagnostic evaluation usually includes abdominal ultrasonography and CT or MRI. If imaging studies fail to provide a diagnosis, then a biopsy can be considered. Infants with focal or multifocal disease without evidence of cardiac failure can usually be carefully observed with periodic physical examinations and ultrasound examinations. Various modalities have been used for symptomatic infants, including use of corticosteroids, use of corticosteroids with vincristine, sirolimus, embolization or artery ligation, surgical excision, and use of antiangiogenic chemotherapy agents.[107,108] Propranolol, which is used for treatment of cutaneous hemangiomas, may also be effective for treatment of hepatic hemangiomas.[109] The overall

survival rate for focal hemangioma is 86%; the overall survival rate for multifocal disease is 71%.[103]

Treatment Considerations in Infants

Chemotherapy Dosing

Neonates and infants experience more frequent and more severe side effects and late effects from chemotherapy compared with older children and adults. This is likely due to age-related differences in body composition, drug bioavailability, and drug metabolism. Infants have an increased amount of total body water, decreased activity of drug-metabolizing enzymes, particularly cytochrome P450, and less efficient renal function. To compensate for these differences, specific chemotherapy dosing protocols have been developed for neonates and infants. The doses for systemic chemotherapy are reduced overall and are frequently calculated by weight instead of by body surface area. Paradoxically, the volume of the cerebrospinal fluid in relation to body surface area is much greater in young children than in adults. Accordingly, the doses of intrathecal chemotherapy are adjusted for age to avoid the underdosing of young infants with leukemia.

Radiation Effects

The use of radiation therapy in newborns is reserved for acute life-threatening situations in which the benefits of radiation therapy clearly outweigh the risks of adverse late effects, which include growth impairment, cognitive impairment, and the risk of secondary malignancies. The goal is to minimize the use of radiation as much as possible, while not compromising disease-related outcomes, to spare the infant potentially morbid treatment-related side effects.

Pain Management

Infants experience pain and should be treated with adequate pain medication. Signs of pain in the neonate can be subtle and can include crying, grimacing, poor feeding, tachycardia, and high blood pressure. Acetaminophen can be an effective analgesic in infants, but it must be used judiciously to avoid the masking of a fever that could signify an infection, particularly if a central line is in place or in the setting of neutropenia. Use of nonsteroidal antiinflammatory medications is usually avoided in patients with cancer because of the risks of bleeding due to interference with platelet function in the setting of chemotherapy-induced thrombocytopenia. Narcotics can be used as needed provided that adequate monitoring of side effects such as respiratory depression is in place. Narcotics can produce physical dependence when used for more than 1 week, and the doses may need to be tapered to avoid withdrawal symptoms.

Nutrition

Adequate nutrition is particularly important for neonates and infants. Patients might require supplemental nutrition via a nasogastric tube or IV parenteral nutrition. A nutritionist should be consulted to help assess the infant's nutritional needs. Breastfeeding can usually be continued in neonates with malignancy.

Intravenous Access

Most neonates and infants with diagnosed cancer will require a central venous catheter to facilitate delivery of chemotherapy, blood product support, and parenteral nutrition. Some chemotherapy agents, such as vincristine and doxorubicin, are vesicants and can cause severe skin and subcutaneous burns if inadvertently infiltrated underneath the skin, which is a more common occurrence with peripheral IV access. The type of central line inserted depends on the size of the infant and the specific needs associated with the chemotherapy regimen.

Transfusions

Cancer patients frequently require blood product support to correct life-threatening cytopenias and coagulopathies, which can be secondary to the underlying malignancy or treatments directed against the malignancy. To minimize the risk of transfusion-associated infections and complications, a number of guidelines exist. These include the use of CMV-negative blood to prevent transmission of CMV, which is potentially life-threatening in an immunocompromised infant; the use of irradiated blood products to prevent the possibility of life-threatening graft-versus-host disease; and the use of leukocyte-depleted blood products to minimize nonhemolytic febrile and allergic reactions. Use of donor-designated blood is usually discouraged in infants with congenital leukemia, given the possible need for future related-donor hematopoietic stem cell transplant.

Immunizations

Immunizations are generally avoided until the patient has not been receiving chemotherapy for at least 6 months. In addition, close contacts of the patient should receive the inactivated polio vaccine rather than the live oral polio vaccine. There is no contraindication to immunization of first-degree relatives with the varicella vaccine. However, any person in whom a rash develops after the vaccination should be kept away from the patient.

Psychosocial Considerations

Social services and psychological support are essential for families. These services are usually best coordinated through the efforts of a multidisciplinary team composed of nurses, social workers, case managers, and the pediatric oncologist.

Late Effects

Infants who receive treatment for cancer during the first year of life are at risk of many late effects directly related to chemotherapy, surgery, and radiation therapy. Infants in the neonatal period will be particularly susceptible to therapies affecting normal growth and development. Information about late effects of treatment and suggested screening after childhood cancer may be found on the Children's Oncology Group website (http://www.childrensoncologygroup.org). Infants require follow-up evaluations at routine intervals by a pediatric oncologist (or multidisciplinary cancer survivor team), who can help identify appropriate screening tests and support.

Conclusion

Cancer in the neonatal period is rare but must be diagnosed and treated promptly with careful attention to the epidemiologic and clinical features that differ from those of older children with similar malignancies. There are special challenges in treating the neonate with cancer, including the newborn's unique physiologic status, which results in marked susceptibility to immediate and late adverse effects of treatment. Careful teamwork is necessary among the neonatologist, pediatric oncologist, surgeon, radiation therapist, nurse, and social worker to support and treat the patient and the family.

Suggested Readings

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Developmental Abnormalities of the Kidneys

RACHEL M. ENGEN AND SANGEETA HINGORANI

KEY POINTS

- Kidney malformations account for 20% to 30% of all prenatally diagnosed developmental anomalies and are responsible for 31% of all childhood end-stage kidney disease.
- Patients with unilateral kidney agenesis are at risk for hypertension in childhood and chronic kidney disease in adulthood.
- Multicystic dysplastic kidney typically presents as a collection of large kidney cysts on ultrasound, and patients generally do well. Autosomal recessive polycystic kidney disease presents as bilaterally enlarged hyperechoic kidneys, often without cysts, and patients may have significant pulmonary and kidney complications.
- The outcome for patients with kidney dysplasia depends significantly on the amount of functioning kidney tissue and the associated congenital anomalies.
- Infant dialysis has improved rapidly over recent years, and 2-year survival outcomes as high as 80% are now reported by multinational dialysis databases.

Normal kidney development begins with two precursor organs, the pronephros and mesonephros, followed by the definitive metanephros at approximately 5 weeks' gestation. The metanephros develops from an interaction between a branch of the mesonephric (wolffian) duct, called the ureteric bud, and the surrounding metanephric mesenchyme. The repeatedly branching ureteric bud will become the collecting system, including the collecting ducts, kidney pelvis, ureter, and bladder trigone. The metanephric mesenchyme will form the glomerular podocytes, proximal tubule, loop of Henle, and distal convoluted tubule. Nephrogenesis begins at 9 weeks' gestation and is complete by 32 to 36 weeks' gestation. A complex and carefully timed web of signaling "crosstalk" between the mesonephric duct, metanephric mesenchyme, and surrounding tissue is necessary to coordinate proper kidney development. Any interruption of this process can lead to abnormalities in kidney structure and function.[1]

Developmental abnormalities of the kidney and the urinary tract affect 3 to 6 per 1000 births[2] and account for 31% of all children with end-stage renal disease (ESRD) in the United States.[1] Renal malformations represent 20% to 30% of all prenatally diagnosed developmental anomalies; prenatal ultrasound allows malformations that are asymptomatic at birth to be detected. Long-term outcomes for children with development anomalies of the kidney depend on the amount of functional renal tissue at birth, associated urinary tract anomalies, degree of prematurity, urine output, and presence of additional congenital anomalies.

With advances in neonatal critical care, nutritional therapy, and peritoneal dialysis techniques, many children who previously would have died in early infancy are surviving, growing, and receiving a kidney transplant. Reviews of multinational dialysis databases have reported 2-year survival rates as high as 80% in infants who start dialysis.[3] Successful provision of care requires a multidisciplinary team approach that includes the neonatologist or pediatrician, nephrologist, surgeon, urologist, nutritionist, nurse, and social worker. Above all, it requires close communication with and strong support of the child's family, who may bear enormous emotional and financial burdens in caring for their child.

Abnormalities of Kidney Number

Unilateral Kidney Agenesis

Unilateral kidney agenesis, or a congenital solitary kidney, results from a unilateral early and complete failure in the signaling interaction between the ureteric bud and metanephric mesenchyme. The estimated incidence is 1 in 500 to 1 in 1000 children.[4] Approximately 30% to 40% of children with unilateral kidney agenesis will have other developmental anomalies; approximately 25% will have vesicoureteral reflux, with the remainder having ureter-pelvic junction obstruction and gastrointestinal, cardiac, and musculoskeletal anomalies.[5]

Kidney agenesis was once thought to be a benign condition if the contralateral kidney appeared healthy. However, there is now increasing concern that unilateral kidney agenesis increases the risk for chronic kidney disease in adulthood.[6,7] The "hyperfiltration" hypothesis suggests that compensatory hyperfiltration in the healthy kidney leads to single-nephron hypertension, glomerulosclerosis, and nephron loss over time.[8] Recent studies of outcomes for children with congenital solitary kidney identified proteinuria and hypertension in 5% to 25% of patients during childhood,[9,10] while chronic kidney disease affects approximately 13% to 30% of adults with a solitary kidney.[4,9] The presence of other urinary tract abnormalities or any dysplasia in the remaining kidney worsens the prognosis.

TABLE 75.1 Syndromes With Kidney Agenesis and Ectopia

Syndrome	Inheritance	Genes	Kidney Disease (%)	Type of Kidney Involvement	Key Features
Fraser	AR	*FRAS1, FREM1, FREM2, GRIP1*	67	Bilateral or unilateral kidney agenesis	• Failure of eyelid formation • Syndactyly • Genital anomalies • Laryngeal stenosis
Kallman	XR	*KAL-1*	30	Unilateral or bilateral kidney agenesis Horseshoe kidney Vesicoureteral reflux	• Hypogonadism • Olfactory defects • Mirror movements of arms • Sensorineural hearing loss
Mayer-Rokitansky-Küster-Hauser	Sporadic or familial	*WNT4*	20–40	Unilateral kidney agenesis	• Congenital absence of uterus and upper two-thirds of vagina • Vertebral, cardiac, skeletal, ocular, and ear involvement
OEIS complex	Sporadic	Unknown	36	Kidney agenesis Vesicoureteral reflux	• Omphalocele • Cloacal extrophy • Imperforate anus • Spinal defects
Turner syndrome	Sporadic	XO karyotype	33	Kidney agenesis Horseshoe kidney Ectopic kidney	• Coarctation of aorta • Bicuspid aortic valve • Short webbed neck • Ovarian failure
Thrombocytopenia-absent radius	AR	*RBM8A*	23	Horseshoe kidney	• Bilateral absent radii • Bilateral thumbs present • Thrombocytopenia • Cardiac malformations

AR, Autosomal recessive; *OEIS*, omphalocele-exstrophy-imperforate anus-spinal defects; *XR*, X-linked recessive.[96–103]

Unilateral kidney agenesis is asymptomatic in infants with a healthy, nonobstructed contralateral kidney. The routine use of voiding cystourethrogram (VCUG) to screen for vesicoureteral reflux in infants without evidence of a urinary tract infection is now controversial. Children with unilateral kidney agenesis should be monitored for appropriate compensatory hypertrophy of the healthy kidney during infancy and should have annual screening for proteinuria and hypertension during childhood (Table 75.1).[9]

The same embryologic insult that led to failure of kidney development can also cause abnormalities of other mesonephric duct derivatives, including the seminal vesicles, vas deferens, and epididymis and müllerian duct organs.[4] Approximately 32% to 50% of girls with unilateral kidney agenesis will have genital tract abnormalities, including unicornate or didelphic uterus and vaginal obstruction.[11,12] The syndrome of obstructed hemivagina and ipsilateral kidney agenesis (OHVIRA), presumed to be due to the arrest of müllerian and mesonephric ducts around week 8 of gestation, can lead to unnecessary surgeries for evaluation of misdiagnosed pelvic mass and complications such as abscess formation and endometriosis.[13] Therefore uterine ultrasound screening for genital tract abnormalities in girls with a solitary kidney is recommended[11] but may be technically challenging.

Bilateral Kidney Agenesis

Bilateral kidney agenesis occurs in 1 in 3000 births. It may be an isolated finding or part of a syndrome, such as the brachio-oto-renal dysplasia syndrome or a hereditary kidney adysplasia. It is typically diagnosed prenatally in a pregnancy complicated by severe oligohydramnios or anhydramnios and nonvisualization of the fetal kidneys and bladder. Bilateral kidney agenesis is responsible for approximately 20% of cases of the oligohydramnios sequence (Potter syndrome),[14] in which decreased amniotic fluid causes compression of the fetus. Patients have the classic low-set ears, wide-set eyes with epicanthal folds, flat nose, and receding chin (Fig. 75.1). Historically, infants were typically born alive but died within the first hours to days of life because of pulmonary hypoplasia. There are now multiple case reports of prenatal treatment with serial amniotransfusion that resulted in the birth of infants who survived and received peritoneal dialysis followed by a kidney transplant with minimal respiratory support.[15,16] As of writing, there is an ongoing clinical trial in the United States to evaluate amnioinfusions with normal saline or lactated ringers solution for management of bilateral kidney agenesis (https://clinicaltrials.govNCT03101891).

Abnormalities of Kidney Position

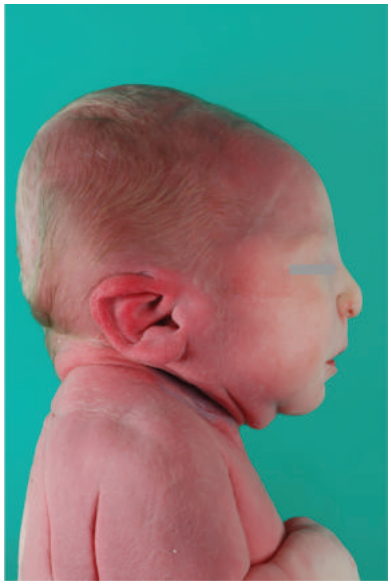
Ectopic Kidney

Kidney ectopia occurs when the kidney fails to ascend from its embryologic position in the fetal pelvis to its final position in the kidney fossa. Ectopia can be simple, with the kidney located ipsilateral to its ureteral insertion, or crossed, with the kidney

located contralaterally. Crossed ectopic kidneys typically fuse to the orthotopic kidney.[17] It is found in 1 in 1000 individuals on autopsy and is typically asymptomatic; approximately 90% of patients are never diagnosed, and ectopic kidneys with ectopic ureters can become obstructed or associated with incontinence.[18] Approximately 20% to 30% of patients will have vesicoureteral reflux usually into the orthotopic kidney,[17,19] which may be associated with infections. Ectopia is not associated with hypertension, proteinuria, or chronic kidney disease. Patients are typically not followed closely if there is no evidence of reflux or obstruction.

Horseshoe Kidney

A horseshoe kidney occurs when the two kidneys are fused, typically at the lower poles, by a parenchymal or fibrous isthmus. This fusion impedes the embryologic ascent of the horseshoe kidney past the origin of the inferior mesenteric artery. It occurs in 1 in 400 children. Twenty-two percent of cases are associated with other systemic abnormalities, including vertebral anomalies, anorectal malformations, and Turner syndrome, and 20% to 30% of children will have vesicoureteral reflux. Urinary tract infections and kidney stones are common. The risk of progression to chronic kidney disease is primarily dependent on the amount of kidney damage suggested by proteinuria, hypertension, and kidney scarring.[20]



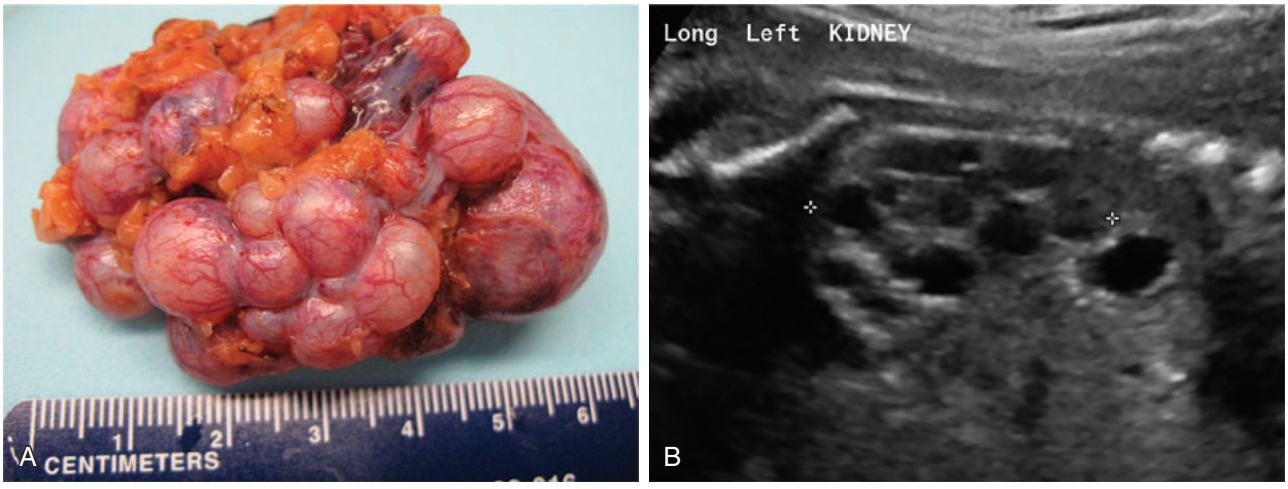
• **Fig. 75.1** Potter Syndrome. Potter syndrome facies with low-set ears, wide-spaced eyes with epicanthal folds, flattened nose, and receding chin. (Courtesy Dr. Laura Finn, Seattle Children's Hospital, Seattle.)

Kidney ectopia occurs when the kidney fails to ascend from its embryologic position in the fetal pelvis to its final position in the kidney fossa. Ectopia can be simple, with the kidney located ipsilateral to its ureteral insertion, or crossed, with the kidney located contralaterally. Crossed ectopic kidneys typically fuse to the orthotopic kidney.[17] It is found in 1 in 1000 individuals on autopsy and is typically asymptomatic; approximately 90% of patients are never diagnosed, and ectopic kidneys with ectopic ureters can become obstructed or associated with incontinence.[18] Approximately 20% to 30% of patients will have vesicoureteral reflux usually into the orthotopic kidney,[17,19] which may be associated with infections. Ectopia is not associated with hypertension, proteinuria, or chronic kidney disease. Patients are typically not followed closely if there is no evidence of reflux or obstruction.

Abnormalities of Kidney Organization

Multicystic Dysplastic Kidney

A multicystic dysplastic kidney (MCDK) develops when there is an impairment of nephrogenesis, resulting in branched ducts surrounded by connective tissue and undifferentiated cells. Occasionally there will be areas of recognizable kidney tissue, but the ureter is not patent and the kidney is nonfunctional.[21] MCDK is typically a sporadic and unilateral finding that occurs in 1 in 4300 births. It is the second most common cause of a flank mass in newborns. MCDK can be accurately diagnosed on ultrasound as a collection of large cysts that do not communicate with the kidney pelvis[22]; a nuclear medicine scan is not necessary for diagnosis (Fig. 75.2).[23–25] Bilateral MCDK is functionally



• **Fig. 75.2** Multicystic Dysplastic Kidney. (A) Gross pathology specimen of a multicystic dysplastic kidney. (B) Prenatal ultrasound image of a multicystic dysplastic kidney. Note the many large cysts. (A courtesy Dr. Laura Finn, Seattle Children's Hospital, Seattle; B courtesy Jennifer McBroom, Seattle Children's Hospital, Seattle.)

similar to bilateral kidney agenesis, with similar management and outcomes.

Approximately 20% of patients with MCDK have vesicoureteral reflux in the contralateral kidney; 40% of these children will have severe (grade III to V) reflux.[21] However, given the low rate of clinical intervention in some recent cohorts, the need for routine screening VCUG is controversial. If VCUG is not performed, the parents should be given instructions on monitoring for urinary tract infections.[26] Five percent of MCDKs are associated with a contralateral ureteropelvic or ureterovesical junction obstruction; approximately 15% will have other developmental abnormalities.[21]

MCDKs undergo spontaneous involution over time, with 5% of prenatally detected MCDKs no longer visible at birth and 40% to 55% involuted by age 5 years. If the contralateral kidney is normal, MCDK is usually asymptomatic, with a prognosis similar to unilateral kidney agenesis (see earlier). In rare cases MCDK may be a bilateral finding; these infants generally die soon after birth because of pulmonary hypoplasia. Patients should have routine ultrasound monitoring in infancy to ensure involution of the MCDK and appropriate compensatory hypertrophy of the contralateral kidney, with annual screening throughout childhood for hypertension and proteinuria. Lack of compensatory hypertrophy of the contralateral kidney is a risk factor for progressive kidney disease.[25] Nephrectomy is generally only performed if there is a clear indication. Older studies associated MCDK with an increased incidence of Wilms tumor, but more recent cohorts have not found an association.[27,28] Currently, routine tumor monitoring is not recommended for MCDK.[25]

Isolated Kidney Dysplasia

Kidney dysplasia occurs when either failure of ureteric bud-metanephric mesenchyme signaling or early urinary flow obstruction disrupts the normal development and differentiation of the fetal kidney.[29] The tissue is made up of primitive ducts, branches of the ureteric bud, surrounded by a ring of fibromuscular tissue and disorganized lobar development.[30] Kidney dysplasia may be unilateral or bilateral, isolated or syndromic, and sporadic or genetic. Mutations in *ITGA8*, an integrin important to cell structure and signaling, and *FGF20*, a fibroblast growth factor with a variety of functions in growth and development, have been associated with dysplasia and/or agenesis. Kidney dysplasia occurs in 0.1% to 3% of births and is the most common cause of childhood ESRD.[29]

Kidney dysplasia is typically diagnosed prenatally or postnatally with the appearance of large, bright kidneys on ultrasound. Cysts may or may not be present. Treatment and prognosis depend on the degree of dysplasia and associated findings. Children with unilateral kidney dysplasia and a normal contralateral kidney may have outcomes similar to children with unilateral kidney agenesis. Children with bilateral kidney dysplasia have variable outcomes depending on the degree of residual kidney function. Mild bilateral dysplasia may result in adequate amniotic fluid production for lung development; however, in general, there is a progressive decline in kidney function in infancy or childhood. Severe bilateral dysplasia has significantly worse postnatal outcomes, particularly if children develop the oligohydramnios sequence (Potter syndrome) with pulmonary hypoplasia.[31] Treatment may include dialysis, but the appropriateness of dialysis is typically determined on a case-by-case basis after discussion of the multidisciplinary care team and parents.

Prognosis for children with kidney dysplasia associated with genetic syndromes often depends on the patient's other developmental abnormalities. Some of the more common syndromes are presented as follows and in Table 75.2.

Renal Coloboma Syndrome

Renal coloboma syndrome (a.k.a. papillorenal syndrome) is an autosomal dominant disorder caused by mutations in *PAX2*, a transcription factor involved in development. Affected children have optic nerve coloboma (dysplasia) and small dysplastic kidneys.[32] Vesicoureteral reflux, high-frequency hearing loss, and central nervous system anomalies may also be present. Most patients have progressive kidney dysfunction, although the timing is highly variable, even in families with the same *PAX2* mutation.[32,33] *PAX2* mutations have also been associated with congenital anomalies of the urinary tract and autosomal dominant childhood-onset focal segmental glomerulosclerosis (FSGS).[34]

Brachio-Oto-Renal Syndrome

Branchio-oto-renal syndrome is an autosomal dominant condition affecting 1 in 40,000 newborns. It is caused by mutations in *EYA-1, SIX1*, or *SIX5*, which interact in the development of the branchial arches, inner ear, and kidney.[35] Clinical manifestations include branchial arch anomalies (clefts, fistula, cysts), preauricular pits, hearing impairment (conductive or sensorineural), and kidney anomalies ranging from unilateral dysplasia to bilateral agenesis.[36]

Hypothyroidism-Deafness-Renal Dysplasia Syndrome

Hypoparathyroidism-deafness-renal dysplasia syndrome (a.k.a. Barakat syndrome) is an autosomal dominant disorder with variable penetrance caused by mutations in *GATA3*, a transcription factor involved in embryologic development.[37] Patients can present at any age with symptomatic hypocalcemia secondary to hypoparathyroidism or early-onset bilateral sensorineural hearing loss that worsens with age. The associated kidney abnormalities include unilateral or bilateral kidney dysplasia or agenesis, although vesicoureteral reflux, proteinuria, and progressive chronic kidney disease have been described.[38,39]

VACTERL

The VACTERL association consists of vertebral defects, anal atresia, cardiac anomalies, trachea-esophageal fistula, kidney malformations, and limb abnormalities; diagnosis is made by the presence of three of the component features. The genetics of VACTERL are heterogeneous, and many of the currently identified mutations are related to the sonic hedgehog signaling cascade.[40] Kidney anomalies are one of the most common component features of VACTERL, found in approximately 65% to 80% of affected children, and typically consist of unilateral kidney agenesis or dysplasia. There is an increased risk of chronic kidney disease in childhood. Twenty-seven percent of those with kidney anomalies and 12% of children without visible kidney anomalies will have vesicoureteral reflux.[41]

TABLE 75.2 Syndromes With Kidney Dysplasia

Syndrome	Inheritance	Genes	Kidney Disease (%)	Type of Kidney Involvement	Key Features
Alagille	AD	*JAG1, NOTCH2*	40 with *JAG1*	Kidney dysplasia Kidney tubular acidosis Vesicoureteral reflux	• Paucity of intrahepatic bile ducts • "Butterfly" vertebrae
Brachio-oto-renal	AD	*EYA-1, SIX1, SIX5*	67	Unilateral dysplasia Bilateral kidney agenesis	• Branchial arch anomalies • Hearing impairment
Cornelia de Lange	AD, XD	*NIPBL, SMC1A, SMC3, RAD21, HDAC8*	36	Kidney dysplasia Kidney ectopia Vesicoureteral reflux	• Short stature • Hirsutism, low hairline • Limb abnormalities • Hearing loss
DiGeorge	AD	22q11.2 deletion	30	Kidney dysplasia Kidney agenesis MCDK Vesicoureteral reflux	• Facial dysmorphia • Cardiac malformations • Congenital hypoparathyroidism • Absent thymus
Ecterodactyly, ectodermal dysplasia, and cleft lip/palate (EEC1)	AD	7q11.2–q21.3	20	Kidney dysplasia Ureterocele Vesicoureteral reflux	• Absent digits, "split" hand/foot • Fair hair • Hyperkeratotic skin • Cleft palate, cleft lip
Fanconi anemia	XR	Heterogeneous	5	Kidney dysplasia Kidney agenesis	• Microcephaly • Café au lait spots • Absent radii • Thumb malformations
Fryns	AR	Unknown	35	Kidney dysplasia	• Congenital diaphragmatic hernia • Pulmonary hypoplasia • Distal finger hypoplasia • Craniofacial anomalies
Hypoparathyroidism-deafness-renal dysplasia	AD	*GATA3*	>60	Kidney dysplasia Kidney agenesis Nephrotic syndrome Vesicoureteral reflux	• Hypoparathyroidism • Bilateral hearing loss
Pallister-Hall	AD	*GLI3*	21–36	Kidney agenesis Kidney dysplasia Vesicoureteral reflux	• Hypothalamic hamartoma • Polydactyly • Imperforate anus
Prune-belly	AR	Unknown	>97	Hydronephrosis Kidney dysplasia	• Absent abdominal wall muscle • Bilateral cryptorchidism
Renal coloboma	AD	*PAX2*	>90	Kidney dysplasia Vesicoureteral reflux	• Optic nerve coloboma • Hearing impairment • Central nervous system anomalies
Townes-Brocks	AD sporadic	*SALL1*	27–42	Kidney dysplasia Chronic kidney disease	• Imperforate anus • Dysplastic ears • Hearing loss • Thumb anomalies
VACTERL	Sporadic	Unknown	65–80	Kidney dysplasia Kidney agenesis Vesicoureteral reflux	• Vertebral defects • Anal atresia • Tracheoesophageal fistula • Limb abnormalities
Wolf-Hirschhorn	Sporadic	4p16 deletion	40	Kidney dysplasia Kidney hypoplasia	• Seizures • Cognitive disabilities • Growth delay

AD, Autosomal dominant; *AR*, autosomal recessive; *MCDK*, multicystic dysplastic kidney; *XR*, X-linked recessive.[32,36,38,41,44,104–112]

• **Fig. 75.3** Eagle-Barrett Syndrome.

Eagle-Barrett Syndrome

Eagle-Barrett (prune-belly) syndrome is the triad of deficient abdominal wall musculature, bilateral undescended testes, and urinary tract abnormalities including kidney dysplasia and an enlarged, hypotonic bladder (Fig. 75.3). It affects 3.7 per 100,000 live male births; females comprise fewer than 5% of cases. The etiology is unknown, but familial recurrence suggests a genetic cause. Autosomal copy number variants involving candidate genes involved in mesodermal, muscle, and urinary tract development have been identified[42] but not reproducibly.[43] Cardiac, gastrointestinal, and musculoskeletal abnormalities are common; 10% of children have respiratory insufficiency related to oligohydramnios and pulmonary hypoplasia. Ten to 25% of children die in the neonatal period of respiratory failure or prematurity. Because of poor bladder tone, most patients have vesicoureteral reflux and develop urinary tract infections. Urologic management with intermittent catheterization or surgery is important to allow adequate bladder drainage and preserve kidney function. Forty to 50% of children develop chronic kidney disease, and 15% will ultimately require dialysis or kidney transplantation, often before school age.[44]

Abnormalities With Kidney Overgrowth

Beckwith-Wiedemann syndrome is an overgrowth disorder caused by abnormal methylation in two gene regulation regions, *IC1* and *IC2*, or paternal uniparental disomy of chromosome 11p15.5.[45] It occurs in 1 in 10,000 live births, and most cases are sporadic; in rare cases there is autosomal dominant inheritance. Patients are large at birth with hemihyperplasia, abdominal wall defects, enlarged tongues and internal organs, hyperinsulinemic hypoglycemia, *nevus flammeus* capillary malformations, and dysmorphic facial features. Nephromegaly is the most common kidney finding, although kidney cysts, nephrolithiasis, and urinary tract malformations have also been reported.[46] The kidneys contain nephrogenic rests, areas of embryonic kidney tissue that are at high risk for malignant transformation. Ten percent of patients with Beckwith-Wiedemann syndrome develop embryologic cancers before 10 years of age, and 40% to 60% of these cases are Wilms tumor (nephroblastoma). Tumor screening by abdominal ultrasound is recommended for all patients every 3 to 4 months until age 8 years.[47]

Simpson-Golabi-Behmel syndrome is an X-linked condition associated with mutations in *glypican3*, which may be involved in regulating embryologic growth. Affected children have many of the same findings as in Beckwith-Wiedemann syndrome, although hemihypertrophy and *nevus flammeus* are typically absent. There is a similar risk for Wilms tumor, and a similar screening schedule is typically recommended.[48]

Perlman syndrome is an autosomal recessive congenital overgrowth disorder caused by mutations in *DIS3L2*, a gene involved in ribonucleic acid processing, that leads to upregulation of insulin-like growth factor 2 (IGF-2).[49] Patients are large at birth with poor muscle tone and characteristic facial features. There is often polyhydramnios in utero and nephromegaly. In one case series, 29% of children with Perlman syndrome developed Wilms tumor, half of those tumors were bilateral, and the average age at diagnosis was less than 2 years.[50] More than half of patients with Perlman syndrome die of respiratory or kidney failure within the first month of life.[51]

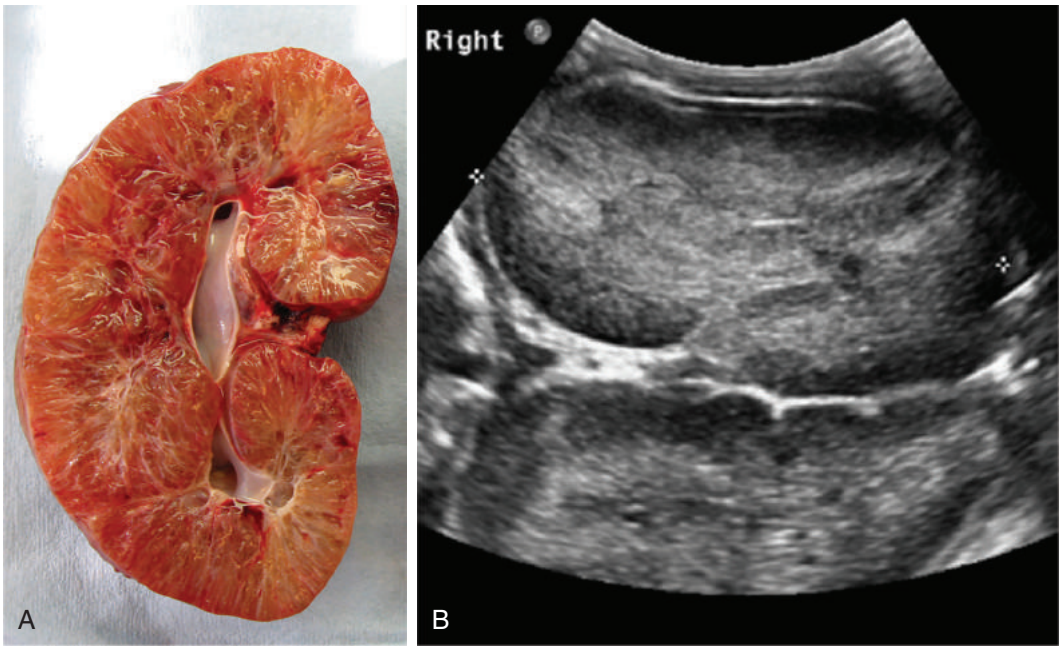
Abnormalities Predominated by Kidney Cysts

Ciliopathies

Primary cilia are protrusions of the cell membrane overlying a microtubule-based axon core. At the base of the cilium is a basal body, which is one of the two centrioles of the centrosome. Primary cilia sense the extracellular environment and transmit signals to the center of the cell, and they are critical to maintaining coordinated cell polarity.[52] Failure to maintain cell polarity in kidney tubular cells results in the development of kidney cysts, which is why diverse kidney cystic syndromes are now being grouped as ciliopathies. Except for a few diseases, including autosomal recessive and autosomal dominant polycystic kidney disease (ADPKD), the ciliopathies are highly heterogeneous genetically. Many syndromes are associated with a dozen or more genes, and single genes are often associated with multiple syndromes depending on the exact mutation and the presence of other cilia gene abnormalities. This genetic diversity can also lead to a high degree of clinical variability within any one syndrome and significant overlap between genetically related syndromes.

Autosomal Recessive Polycystic Kidney Disease

ARPKD is an autosomal recessive hepatorenal fibrocystic syndrome associated with mutations in fibrocystin *(PKHD1)*, which encodes a transmembrane protein of unknown function that localizes to the primary cilia, mitotic spindle, and apical membranes of kidney tubule and biliary duct cells. *PKHD1* is expressed in a number of tissues during fetal development, including the ureteric bud, mesonephric tubules, and immature hepatocytes and probably has a role in organogenesis and tubule formation. In the kidney the abnormality causes dilation of collecting ducts, forming cysts. Recently, mutations in a second gene, *DZIP1L*, have also been associated with an ARPKD phenotype with a more moderate clinical course.[53,54]



• **Fig. 75.4** Autosomal Recessive Polycystic Kidney Disease. (A) Autosomal recessive polycystic kidney disease. Many microcysts without large cysts. (B) Ultrasound image of an autosomal recessive polycystic kidney measuring 9.1 cm in length, twice the normal length for age. Note the significant increased echogenicity. (A courtesy Dr. Laura Finn, Seattle Children's Hospital, Seattle.)

ARPKD affects 1 in 20,000 live births, and carrier rates as high as 1:70 have been suggested.[55] Most patients present at or before birth with enlarged echogenic kidneys with poor corticomedullary differentiation; macrocysts are generally not visible on ultrasound at birth but do develop later in life (Fig. 75.4). Severely affected patients may have the oligohydramnios sequence with pulmonary hypoplasia, but oligohydramnios alone is not predictive of a poor neonatal outcome.[56] A small subset of patients present in childhood or adolescence with liver disease.

The kidneys may be so enlarged as to compromise respiratory function after birth, and 20% to 30% of affected infants will die due to respiratory insufficiency. The kidneys are often palpable, and the abdomen may be grossly distended. Most patients have abnormal kidney function from birth. Hypertension can be severe. As blood flow to the kidney increases after birth, polyuria may develop due to a urinary concentrating defect. All patients with ARPKD have liver disease due to bile duct malformation, and some will develop congenital hepatic fibrosis. Portal hypertension and ascending cholangitis are common complications, and some infants will have fat malabsorption due to abnormal bile flow. The enlarged kidneys may also compress other abdominal organs, further complicating enteral nutrition.[56] Hepatic synthetic and metabolic functions generally remain normal until late in the disease course.[55]

Initial management is focused on respiratory support and the initiation of peritoneal dialysis, if necessary.[57] Polyuric patients require high levels of daily fluid intake to match their excessive urine output, and some may require sodium supplementation.[57] Hypertension is often treated with angiotensin-converting enzyme inhibitors.[58] Infants with fat malabsorption may require supplementation of their fat-soluble vitamins (A, D, E, and K).

If the enlarged kidneys are causing respiratory failure, intractable hypertension, or feeding intolerance, simultaneous or staged bilateral nephrectomy may be performed. However, bilateral nephrectomy necessitates dialysis, which can be challenging in neonates. It may cause refractory hypotension due to the absence of renin production,[56] which animal models suggest is a significant early regulator of blood pressure while the sympathetic nervous system matures postnatally.[53] One cohort study of children with ARPKD and bilateral nephrectomy within the first 3 months of life reported a higher incidence of neurologic complications (63%), including seizures, hypoxic brain damage, severe neurodevelopmental disorder, and optic neuropathy with vision loss, compared with those whose second nephrectomy was performed at 4 to 15 months of age (22%) or who had initiation of dialysis within the first 3 months of life (17%). This association was seen despite similar total kidney volumes between groups and was independent of the incidence of severe hypotensive episodes.[53] There is one case report of using midodrine to treat an infant with refractory hypotension after bilateral nephrectomy,[59] but this has not been widely studied. In addition, kidney function in ARPKD may improve over the first several months of life. Therefore the decision to perform bilateral nephrectomy should be approached with caution based on individual clinical circumstances.[60]

Approximately 70% to 75% of patients with ARPKD survive the newborn period; among those survivors, the 15-year survival rate is 67% to 79%.[57] More than 50% of affected patients require kidney replacement therapy, either dialysis or a kidney transplant, during childhood, and 7% to 10% require a liver transplant.[61]

Autosomal Dominant Polycystic Kidney Disease

ADPKD is the most common inherited kidney disease, affecting 1 in 500 to 1 in 1000 live births. It is associated with mutations in *PKD1* and *PKD2*, with *PKD1* mutations being more frequent and associated with earlier onset of symptoms. *PKD1* encodes polycystin-1, a membrane protein involved in cell-matrix interactions and possibly calcium homeostasis. *PKD2* encodes polycystin-2, a

nonselective cation channel that increases membrane permeability to calcium and interacts physically with polycystin-1 at the primary cilia. ADPKD has an autosomal dominant inheritance pattern but is recessive at the molecular level; it is now generally understood that cells must develop a second, somatic mutation to begin forming a cyst.[57]

ADPKD generally presents with bilateral kidney cysts in early adulthood followed by progressive cyst development, kidney enlargement, hypertension, and kidney function decline. Patients may also have cysts in the liver, pancreas, spleen, and seminal vesicles and are at increased risk for vascular abnormalities, including cerebral aneurysms, aortic dilatation, and aortic dissection. Two to 5% of patients with ADPKD present prenatally or in early childhood with symptoms ranging from asymptomatic kidney cysts on ultrasound to the oligohydramnios sequence. A history of early-onset ADPKD in a family member portends a high risk of early-onset disease in the patient.[57] Biallelic abnormalities in *PKD1* or *PKD2* or a mutation in one allele of *PKD1* or *PKD2* with a mutation in a second kidney cystic disease gene may also cause early, severe disease, suggesting a gene-dosage effect.[62] Most infants diagnosed with ADPKD are asymptomatic at birth and remain so throughout childhood. Chronic kidney insufficiency has been reported in 8% of children and hypertension in 19% of children by adolescence.[63]

It may be difficult to differentiate severe early ADPKD from ARPKD by ultrasound. There are no diagnostic criteria for ADPKD in children younger than 15 years old; the kidneys of affected infants may be enlarged and hyperechoic with or without visible cysts and have increased corticomedullary differentiation. Children rarely have liver or pancreatic cysts, but 10% will have inguinal hernias. Screening the patient's parents for kidney cysts may be helpful in determining the correct diagnosis, although normal parental ultrasound does not rule out a diagnosis of ADPKD, because 8% to 10% of cases are associated with new mutations. Management of early ADPKD is guided by clinical presentation; severe cases may require nephrectomy and dialysis similar to children with ARPKD, whereas asymptomatic cases may need only outpatient follow-up. There is one case report of a 31-day-old infant with early, severe ADPKD who was treated with tolvaptan, a selective vasopressin V_2-receptor antagonist, for hyponatremia and edema, but this drug is not generally approved for use in children in the United States.[62]

Tuberous Sclerosis Complex

Tuberous sclerosis complex (TSC) is an autosomal dominant condition associated with mutations in either *TSC1*, encoding hamartin, or *TSC2*, encoding tubulin. *TSC1* and *TSC2* are important regulators of the mechanistic target of rapamycin (mTOR) kinase, a key signaling molecule involved in cell proliferation. Patients develop uncontrolled cellular proliferation, leading to hamartomas of the brain, skin, heart, lungs, and kidneys. Hypomelanotic skin macules (ash leaf marks) may be the only skin manifestation in infants, and the presence of three or more ash leaf marks at birth is suggestive of TSC. Shagreen patches, angiofibromas, and nail fibromas tend to develop later in childhood.[64] Seizures and kidney angiomyolipomas are common; simple kidney cysts are a minor feature of TSC but can be seen. *TSC2* is physically located near *PKD1* on chromosome 16, and 2% of patients with TS will also have ADPKD secondary to a contiguous gene deletion.[65,66] The cysts in ADPKD-TSC appear earlier, are larger, and are more numerous than the simple kidney cysts of isolated TSC. Enlarged kidneys also raise the concern for ADPKD-TSC.[67] ESRD is rare in patients with isolated TSC but may occur before age 30 years in patients with ADPKD-TSC.

Bardet-Biedl Syndrome

Bardet-Biedl syndrome is an autosomal recessive ciliopathy caused by mutations in any of at least 17 genes whose products are involved in the "BBsome," a complex involved in signaling receptor trafficking to the primary cilia. It affects 1 in 125,000 to 1 in 175,000 children. The abnormal primary cilia signal trafficking that leads to nonspecific glomerular and tubulointerstitial changes in the kidney, with or without kidney cysts, and reduced nephron mass. Retinitis pigmentosa, obesity, kidney anomalies, learning disabilities, polydactyly, and hypogonadism are the cardinal features.[68] Hearing loss, developmental or behavioral problems, diabetes, hypertension, cardiac defects, and limb anomalies have also been reported.

Kidney anomalies may be both structural and functional. Fetal ultrasound may show enlarged hyperechoic kidneys without corticomedullary differentiation. Cysts and abnormalities of the kidney pyramids may be seen. By 3 months of age there is inversion of the corticomedullary differentiation with a hyperechoic medulla. Kidney size normalizes over time, and kidney cysts may disappear. In childhood, one-third of patients will have polyuria and polydipsia; kidney tubular acidosis and other signs of tubular dysfunction are less commonly seen. Hypertension is also common. Approximately 10% of children will develop ESRD, and 25% will require dialysis or transplant by 40 years of age.[69]

Jeune Syndrome

Jeune syndrome, also called asphyxiating thoracic dystrophy, is an autosomal recessive skeletal ciliopathy affecting 1 in 126,000 live births and is associated with mutations in a large number of genes, including *IFT80*, *DYNC2H1*, *WDR19*, and *TTC21B*, that are involved in transport along the axon of primary cilia. Since 2006 it has been considered one of the short-rib thoracic dysplasias. Patients have characteristic skeletal abnormalities, including a small narrow chest with short ribs, short squared iliac wings, and short digits and extremities that can typically be diagnosed on prenatal ultrasound. Liver and eye abnormalities may be present. Approximately 40% of patients will have kidney anomalies, including cystic dysplasia, kidney hypoplasia, or hydronephrosis causing a tubulointerstitial nephropathy and tubular dysfunction.

The abnormal chest development in Jeune syndrome prevents the intercostal muscles from contributing to respiration, and half of children with Jeune syndrome die before 6 months of age secondary to respiratory failure. Surgical techniques for chest wall reconstruction, such as using a vertical expandable prosthetic titanium rib, which allows for ongoing chest growth, are currently being explored.[70] In limited case series, outcomes appear good if surgery is performed after 1 year of age but demonstrate 50% mortality before 1 year of age.[71] If affected children have mild respiratory involvement and live past infancy, they develop a urinary concentrating defect, polyuria, and polydipsia. After 3 years of age, kidney failure is the leading cause of death among children with Jeune syndrome.[72]

Nephronophthisis

Nephronophthisis is an autosomal recessive ciliopathy associated with at least 14 different genes involved in primary cilia structure and function, the most common being *NPHP1*. Nephronophthisis

is a tubulointerstitial nephropathy with tubular atrophy, corticomedullary cysts, and interstitial fibrosis that presents as polydipsia and polyuria around age 6 years, progressing to kidney failure in early adolescence. Infantile nephronophthisis progresses to ESRD around 1 year of age. It has similar pathologic findings as polycystic kidney disease, including cysts seen throughout enlarged kidneys,[73] and is typically associated with mutations in *NPHP2* and *NPHP3*.[74] It has been reported in isolation but is more common as a component of a number of genetic syndromes, including Meckel-Gruber syndrome, Joubert syndrome, and the Joubert-related disorders.

Meckel-Gruber Syndrome

Meckel-Gruber syndrome is an autosomal recessive disorder associated with mutations in any of at least 11 genes, all of which are associated with proper functioning of the primary cilia. It occurs in 2.6 per 100,000 live births and is more common if there is parental consanguinity. Patients have the characteristic triad of bilateral kidney cystic dysplasia, occipital encephalocele, and polydactyly. Hepatic fibrosis may also be present. More than 90% of cases are diagnosed prenatally by ultrasound, and 80% of cases result in fetal death or termination of pregnancy. Outcomes are poor; only about one-third of patients survive the first week of life, and the longest recorded survival is 28 months.[75]

Joubert Syndrome and Joubert-Related Disorders

Joubert syndrome is an autosomal recessive disorder associated with mutations in any of 35 genes that are associated with the primary cilia and basal body.[76] Patients have developmental delay, hypotonia, an irregular neonatal breathing pattern (episodic apnea and/or tachypnea), and abnormal eye movements including nystagmus and oculomotor apraxia. Facial features may be dysmorphic with a broad forehead, arched eyebrows, ptosis, wide-spaced eyes, and polydactyly. Magnetic resonance imaging shows the characteristic "molar tooth sign," an unusual combination of cerebellar vermis hypoplasia/dysplasia, elongated superior cerebellar peduncles, and a deep interpeduncular fossa. The molar tooth sign has now been associated with seven other conditions, including some cases of Senior-Løken syndrome, COACH syndrome, Dekaban-Arima syndrome, orofaciodigital (OFD) syndrome type VI (Varadi-Papp) syndrome, and Malta syndrome, all of which have variable levels of ocular and kidney involvement.

Kidney disease in Joubert syndrome and Joubert-related disorders may present as cystic dysplasia or nephronophthisis, with corticomedullary cysts and tubulointerstitial nephritis. Ultrasound shows increased kidney echogenicity in normal-sized kidneys with poor corticomedullary differentiation. Affected infants may be asymptomatic at birth but develop urinary concentrating defects, polyuria, and progressive kidney dysfunction during childhood. ESRD is common in the teenage years. Rarely, patients will present with a clinical picture similar to ARPKD; this is more common in patients with COACH syndrome and *MKS3* mutations.

Orofaciodigital Syndrome

OFD syndrome is a heterogeneous ciliopathy of 14 subtypes that are grouped together by the common finding of malformations of the face, oral cavity, and digits. OFD1 is an X-linked dominant condition that is lethal in males and occurs in 1 in 50,000 to 1 in 250,000 live Caucasian births;[77] the other 13 OFD subtypes are autosomal recessive and linked to a variety of cilia-associated genes. Tooth and tongue abnormalities, a buccal frenulum, agenesis of the corpus colosseum, and brachydactyly are common findings. Cleft lip and/or cleft palate occur in approximately one-third of patients. Kidney cysts have been reported in 6% of children with OFD syndrome and in up to 70% of adults, and patients develop progressive kidney insufficiency that results in ESRD in early adulthood.[78] Case series suggest that kidney cysts are strongly associated with neurological structural anomalies in patients with OFD1.[77]

Cranioectodermal Dysplasia

Cranioectodermal dysplasia, also called Sensenbrenner syndrome, is an autosomal recessive or sporadic ciliopathy associated with mutations in *IFT122, WDR35, IFT43*, *WDR19*, and *IFT52*, all of which encode proteins that localize to the cilia; *IFT43* and *WDR35* are involved in axonal transport within the primary cilia.[79] Patients have frontal bossing, dolichocephaly, low-set ears, wide-spaced eyes with epicanthal folds, small and widely spaced teeth, sparse hair, abnormal nails, short stature, a narrow thorax, short humeri, and brachydactyly. The liver and eyes may also be affected. More than half of patients will develop nephronophthisis that progresses to ESRD.

Renal-Hepatic-Pancreatic Dysplasia

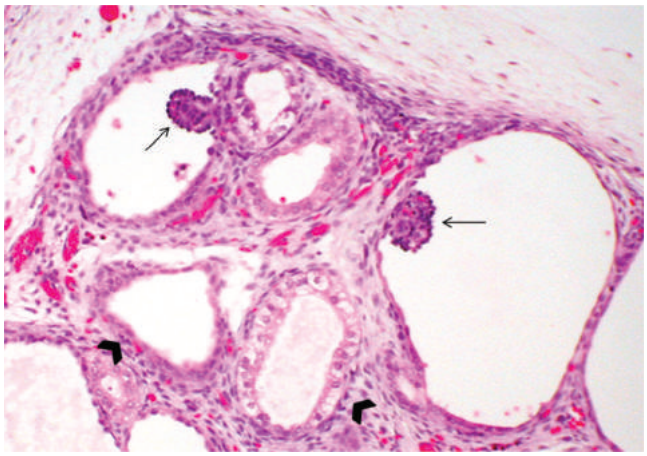
Renal-hepatic-pancreatic dysplasia (RHPD), also called Ivemark syndrome, is an autosomal recessive disorder associated with mutations in *NPHP3*, which encodes nephrocystin 3, a protein that localizes to primary cilia.[80] Patients often present in utero with oligohydramnios caused by kidney dysplasia with peripheral cortical cysts. Hepatic and pancreatic fibrosis is also present, and presentation may appear clinically similar to Alagille syndrome.[81] Most patients develop the oligohydramnios sequence and die of respiratory failure soon after birth, but there are a few reports of survival among patients who received a combined liver-kidney transplant in early childhood.[82]

Glomerulocystic Kidney Disease

Glomerulocystic kidney disease is defined as dilation of the Bowman capsule around the glomeruli with or without tubular dilatation and kidney cysts (Fig. 75.5). It is an autosomal dominant disorder associated with mutations in *UMOD* or *HNF1β* but may also be seen in ciliopathies, kidney dysplasia, or associated with severe fetal kidney damage. *HNF1β* encodes a transcription factor critical to embryologic development of the kidney, pancreas, liver, and müllerian duct. Mutations in *HNF1β* are also associated with maturity-onset diabetes of the young, as in the "renal cysts and diabetes syndrome." *UMOD* encodes uromodulin, which is linked to urinary tract defense but probably has other, currently unknown, functions in the kidney. Glomerulocystic kidney disease often presents in young children with kidney hyperechogenicity on ultrasound, but presentation may be delayed into adulthood. Patients may be hyperuricemic. The kidneys generally progress to ESRD, but the rate of progression is highly variable.[83–85]

Renal Tubular Dysgenesis

Renal tubular dysgenesis is an autosomal recessive disorder associated with mutations in genes for the renin-angiotensin system (*AGT, REN, ACE*, and *AGTR1*) that result in failure of kidney



• **Fig. 75.5** Glomerulocystic Kidney Disease. *Arrows* show glomeruli inside dilated Bowman capsule; *arrowheads* indicate normal tubules. (Courtesy Dr. Laura Finn, Seattle Children's Hospital, Seattle.)

proximal tubule development. Similar pathology may develop if kidney blood flow is interrupted in utero, such as in a major cardiac anomaly, kidney artery stenosis, twin-twin transfusion syndrome, or use of angiotensin-converting enzyme inhibitors or angiotensin receptor blockers during the second or third trimesters.

Patients present with severe oligohydramnios by 20 to 22 weeks' gestation, leading to the oligohydramnios sequence. The kidneys may appear normal on ultrasound, or there may be hyperechogenicity or decreased corticomedullary differentiation. The skull may have large fontanelles and sutures. Diagnosis is confirmed by kidney biopsy showing the absence or dramatic reduction in the number of differentiated proximal tubules.

Most patients die in utero or in the neonatal period because of pulmonary hypoplasia and hypotension that is not responsive to standard medical therapy. In cases of refractory hypotension, fludrocortisone may improve the blood pressure; fresh frozen plasma may also be helpful in patients with an angiotensinogen mutation. Of the 150 patients reported with kidney tubular dysgenesis, there are at least 10 reported cases of survivors past 18 months of age, 4 of whom have ESRD and 5 of whom have chronic kidney disease.[86]

Kidney Teratogens

A number of commonly used medications have been associated with abnormal kidney development. Angiotensin-converting enzyme inhibitors and angiotensin receptor blockers inhibit nephrogenesis when taken in the second and third trimesters, and they have been associated with kidney tubular dysgenesis, ureteropelvic junction obstruction, and kidney agenesis. Nonsteroidal antiinflammatory drugs, especially indomethacin, are linked to fetal and neonatal kidney failure, probably because of their effects on the ductus arteriosus. Mycophenolate mofetil is associated with a variety of organ anomalies, including kidney anomalies. Tacrolimus may cause a mild and transient decline in kidney function, but the placenta prevents an estimated 60% of the dose from reaching the fetus.[87] The breast cancer drug trastuzumab has been associated with oligohydramnios when used in the second or third trimester and is a US Food and Drug Administration pregnancy category D medication. Gentamicin has been associated with nephrotoxicity in animal studies and two human cases of cystic dysplasia; however, these findings were not confirmed by a large Swedish study.[87] Maternal diabetes is associated with kidney and urinary tract abnormalities, especially if it is present in the first trimester.[88]

Inborn Errors of Metabolism

Multiple Acyl-CoA Dehydrogenase Deficiency

Multiple acyl-CoA dehydrogenase deficiency (MADD), also called glutaric acidemia type II or glutaric aciduria type II, is an autosomal recessive defect in the mitochondrial electron transfer chain caused by mutations in *ETFDH, ETFA*, or *ETFB*. This causes degeneration of cells that use fatty acids as a primary source of energy, including kidney tubular epithelial cells. Affected children present in the neonatal period with lethargy, vomiting, a "sweaty feet" odor, nonketotic hypoglycemia, metabolic acidosis, and organic aciduria. Some will have other congenital anomalies, including a high forehead, low-set ears, wide-spaced eyes, a small midface, kidney dysplasia, and kidney cysts. Most patients who present near birth die within weeks to months despite attempts at treatment with dietary interventions, riboflavin, and L-carnitine. There is a milder, late-onset form of the disease, not associated with kidney anomalies, which may be treated with riboflavin.[89]

Smith-Lemli-Opitz Syndrome

Smith-Lemli-Opitz syndrome is an autosomal recessive disorder of cholesterol synthesis associated with mutations in *DHCR7* affecting 1 in 20,000 to 1 in 70,000. The clinical spectrum is broad, ranging from asymptomatic cases to perinatal lethality. Affected children may have delayed growth, microcephaly with bitemporal narrowing, ptosis, a short nose with anteverted nares, micrognathia, epicanthal folds, and low-set ears. Mental retardation, clef palate, heart defects, genital anomalies, and syndactyly of the second and third toes are common. One-fourth of patients have kidney anomalies, including kidney hypoplasia or agenesis, kidney cortical cysts, or urinary tract anomalies. Due to inability to synthesize steroids, patients may have varying levels of adrenocortical hormone deficiency; however, presentation with acute adrenal crisis is rare.[90]

Zellweger Syndrome

Zellweger syndrome, also called cerebrohepatorenal syndrome, is an autosomal recessive condition associated with mutation in any of 12 pexin genes, usually *PEX1*, leading to peroxisome dysfunction. Affected infants have severe hypotonia, absent reflexes, a high forehead and large anterior fontanelle, small supraorbital ridges, a triangular mouth, and low-set ears with abnormal lobes. There are usually small kidney cysts and calcific stippling of the epiphyses. Severe liver dysfunction typically appears after 3 months of age. Some patients present with seizures caused by abnormal neuronal migration. Death before age 1 year is common, although some patients survive longer with varying developmental outcomes.[91,92]

Congenital Disorders of Glycosylation

The congenital disorders of glycosylation (CDG) are a family of autosomal recessive defects in the synthesis of the glycans of

glycoproteins.[93] Patients can present with a variety of symptoms, including developmental delay, seizures, hypotonia, liver disease, protein-losing enteropathy, and dysmorphic facial features. Patients may have enlarged hyperechoic kidneys on ultrasound and kidney tubular microcysts. Congenital nephrotic syndrome, associated with diffuse mesangial sclerosis pathology, has also been reported.[94] Due to the variability of the condition, management is individualized to a patient's specific clinical presentation.[95]

Acknowledgments

Thank you to Dr. Laura Finn, Seattle Children's Hospital Department of Pathology, for providing kidney pathology images. Thank you to Jennifer McBroom, Seattle Children's Hospital Department of Radiology, for providing ultrasound images.

Suggested Readings

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76

Developmental Abnormalities of the Genitourinary System

COURTNEY K. ROWE AND PAUL A. MERGUERIAN

KEY POINTS

- A voiding cystourethrogram should be performed in infants with high-grade hydronephrosis or bilateral hydronephrosis.
- Vesicoureteral reflux is more common in male infants and has a high rate of spontaneous resolution.
- Bladder exstrophy repairs are currently delayed to 3 months of age and performed with a dedicated team.
- Nadir creatinine predicts future renal function in posterior urethral valves.
- Current American Urological Association guidelines do not recommend ultrasound for the evaluation of undescended testicles; instead, refer children to a pediatric urologist at approximately 6 months of age.
- Infants with bilateral nonpalpable testicles should be evaluated for possible congenital adrenal hyperplasia; infants with hypospadias and unilateral or bilateral undescended testicles should be evaluated for disorder of sexual development.
- Hydroceles are common in male infants and most resolve by one year of age.

Congenital anomalies of the kidney and urinary tract (CAKUTs) are found in around 0.5% of pregnancies and account for 20% to 30% of all prenatally diagnosed congenital anomalies. They are responsible for about two thirds of all children with chronic kidney disease in developed countries.[1] Ureteropelvic junction obstruction (UPJO) and vesicoureteral reflux (VUR) are the most common CAKUTs. Other forms of CAKUTs include multicystic dysplastic kidneys (MCDK), primary megaureter, duplicated collecting systems, ureterocele, renal dysplasia, and bladder outlet obstruction such as posterior urethral valves (PUVs).[2] There are well-recognized features related to these anomalies in infants, and they include higher incidence of UPJO and VUR in males; UPJO and MCDK are often unilateral; most of these anomalies are associated with VUR; most infants with high-grade reflux have renal dysplasia; and primary megaureter is overwhelmingly found in males. On the other hand, in older children there is a female preponderance of several of these CAKUTs. These anomalies are thought to be a result of defects in the same molecular pathways involved in kidney development. According to the Genitourinary Molecular Anatomy Project, there may be several hundred genes involved in the process of kidney development.[3] The phenotypic spectrum of these CAKUTs is shown in Box 76.1.

• BOX 76.1 Spectrum of Congenital Anomalies of the Kidney and Urinary Tract

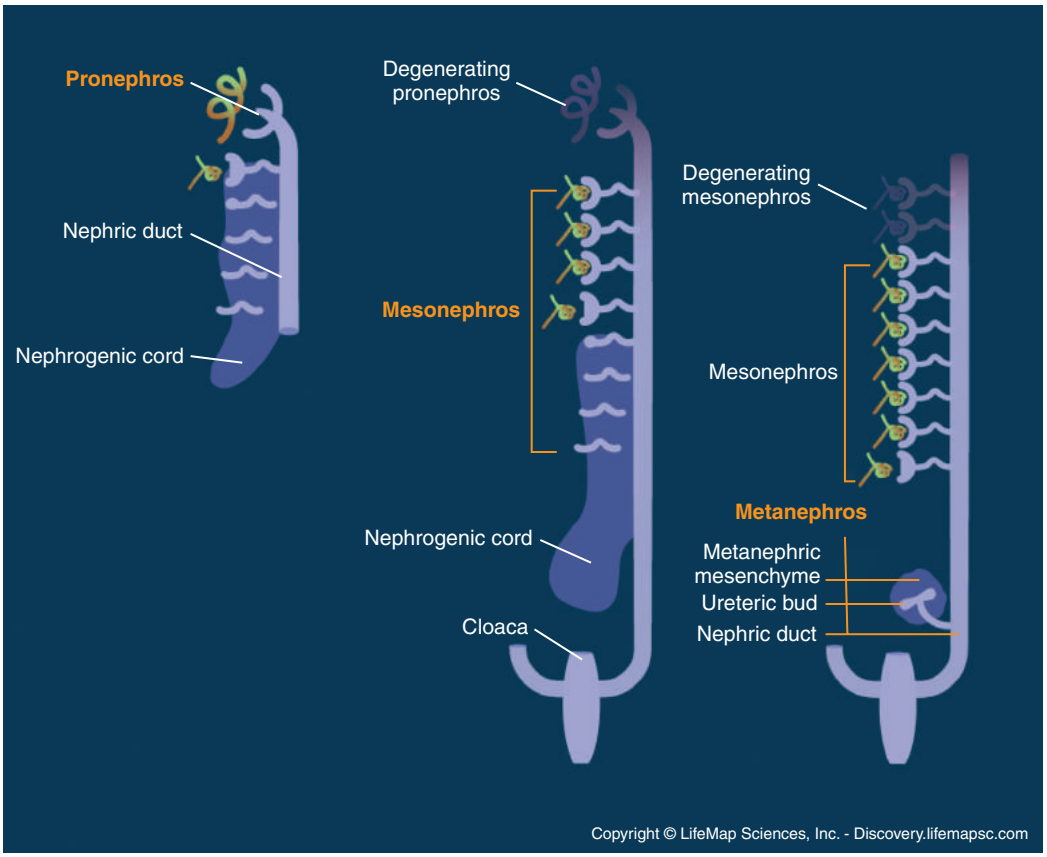
Renal agenesis
Renal dysplasia
Renal hypoplasia
Duplex kidney and ureter
Horseshoe kidney and other fusion anomalies (crossed fused ectopia)
Ureteropelvic junction obstruction
Megaureter (obstructing, refluxing, nonobstructing, nonrefluxing)
Ectopic ureter
Ureterocele

Early Kidney and Urinary Tract Embryologic Development

The development of the kidney and urinary tract begins when the nephric duct (ND) is formed from the intermediate mesoderm (Fig. 76.1). The ND extends caudally and induces the adjacent mesoderm to form two transient kidneys, the pronephros and the mesonephros. The pronephros contains nonfunctional tubules that open into the pronephric duct and disappears at the end of the fourth week of gestation.[4]

The mesonephros then begins to develop and contains well-developed nephrons with vascularized glomeruli connected to proximal and distal tubules draining into the mesonephric duct. The mesonephric duct then fuses with the cloaca and contributes to the formation of the bladder trigone. In the male, it also forms part of the genital system, including the vas deferens, seminal vesicles, epididymis, ejaculatory ducts, and the efferent ductules of the testis.[5] In females, the mesonephros forms vestigial structures, the epoophoron and the paroophoron.[6]

The metanephros is the final stage of renal development and is identified at around 5 to 6 weeks' gestation. This structure consists of two components: the ureteric bud and the metanephric mesenchyme. The ureteric bud forms from the nearby caudal mesonephric (Wolffian) duct and grows to penetrate the metanephric blastema. The reciprocal interaction between the ureteric bud and the metanephric mesenchyme results in branching of the ureteric bud to form the collecting system of the kidney.



• **Fig. 76.1** Development of the Human Kidney.

A mesenchymal-to-epithelial transition of the metanephric mesenchyme at each of the newly formed ureteric bud tips results in the development of the nephrons. With each division of the ureteric bud, a new layer of nephrons is induced from stem cells in the periphery of the organ.[4,7] As development proceeds, the metanephros extends laterally and cranially, ascending from the sacral to the lumbar position by 8 weeks' gestation.[8]

The main ureteric duct (future ureter) undergoes a process of temporary obliteration followed by recanalization of the lumen as the embryo grows. This process begins in the middle zone of the ureter and progresses proximally and distally. In embryos of approximately 17 mm in length the primary ureter forms a solid cord, and in a 23-mm embryo it is totally patent. These observations have given rise to the theory that UPJO and ureterovesical junction obstruction arise from incomplete recanalization of the ureter at its most proximal and distal ends.[9,10]

The ureteral orifice is also transposed from its original budding site on the Wolffian duct into the bladder. This transposition occurs with expansion of the terminal part of the duct and its incorporation into the base of the bladder as the hemitrigone. If, for example, the bud arises caudally on the duct, the orifice becomes incorporated onto a long cornu of the hemitrigone and is therefore laterally displaced. This lateral displacement causes the submucosal tunnel to be short, leading to VUR.[11]

All the branches of the ureteric bud and the nephrons are formed by 34 weeks' gestation.[7] However, these structures will continue to mature after birth. Once matured, humans have an estimated 210,000 to 2.7 million nephrons per kidney.[12]

Anomalies of the Kidney

Renal Agenesis

Renal agenesis is the congenital absence of the kidneys. Renal agenesis can be bilateral or unilateral. Bilateral renal agenesis occurs in roughly 1 in 4000 births, with male predominance.[13] Prenatally, it is discovered in the second or third trimester because of severe oligohydramnios on ultrasound. Findings of absent kidneys, no bladder filling, and lack of renal arteries on color Doppler ultrasonography support the diagnosis.[14] Postnatally, infants are noted to have the characteristic Potter facies with a prominent skin fold from the eye to the cheek, blunted nose, low-set ears, and orthopedic abnormalities, including clubbed legs or fused lower extremities. Lack of adequate amniotic fluid results in pulmonary hypoplasia and respiratory distress.[15] These findings, or lack of urine output within 24 hours, should prompt renal ultrasound. Bilateral renal agenesis is almost universally fatal, with 40% demise in utero due to cord compression, and those born alive surviving for less than 48 hours because of pulmonary compromise. There have been reports of survival of monoamniotic twins discordant for bilateral renal agenesis[16] and reports of bilateral renal agenesis managed with serial amnioinfusion with survival through delivery with successful initiation of peritoneal dialysis,[17] but in general prognosis remains poor.[18] There is an increased prevalence of congenital renal anomalies in relatives, and screening ultrasound has been recommended for parents and siblings of children with renal agenesis.[19]

Unilateral renal agenesis occurs in approximately 1 in 500 to 1000 births. The true incidence can be difficult to measure because of the frequent involution of multicystic dysplastic kidneys. In fetal ultrasounds with a unilateral empty renal fossa, 47% have an absent kidney, while most of the rest have renal ectopia.[20] Unilateral renal agenesis is associated with abnormalities of the paramesonephric and mesonephric ducts, including absence of the ipsilateral uterine horn or vas deferens. Due to the risk of obstruction at the time of menarche, screening for OHVIRA syndrome (obstructed hemivagina with ipsilateral renal agenesis) at puberty should be considered.[21] About a third of children with unilateral renal agenesis will have extra-renal anomalies including gastrointestinal, cardiac, and musculoskeletal;[22] there is also an association with a number of multisystem syndromes.[23] There is an increased risk of contralateral renal anomaly, including UPJO and VUR.[24,25]

Patients with unilateral renal agenesis have a higher risk of end stage renal disease than those with solitary kidney from donor nephrectomy or MCDKD, with 50% requiring dialysis by 30 years of age.[26] Because of the low risk of renal injury, the American Academy of Pediatrics recommends participation in sports for those with solitary kidney, particularly non-contact sports.[27] Children with unilateral renal agenesis should be followed regularly to monitor urine protein, blood pressure, and kidney function blood pressure.[28]

Renal Ectopia and Fusion

Renal ectopia describes a kidney that fails to reach its standard location. The kidney can be pelvic, iliac, abdominal, thoracic, and contralateral or crossed. Renal ectopia occurs in about 1 in 700 infants on ultrasound screenings.[29] Abnormalities of renal ascent during fetal development are thought to be the cause of ectopia. Most patients with renal ectopia are asymptomatic.

Half of ectopic kidneys are hydronephrotic, of which 50% are due to obstruction, 25% are due to vesicoureteral reflux, and 25% are due to abnormal rotation without obstruction.[30] There is a strong association with extrarenal anomalies, which are seen in almost half of patients.[31] An association with genital anomalies is common, and a large number of girls with müllerian anomalies, including cloacal anomalies, are also noted to have ectopic kidneys.[32,33] Because of this, the finding of an ectopic kidney in a female should prompt further investigation. Prognosis for the ectopic kidney is good, with no evidence of adverse effects on blood pressure or renal function.[34]

When the ectopic kidney is located on the opposite side from where its ureter enters the bladder, this is called *crossed renal ectopia*. This is found in 7.5 per 10,000 newborns, and 90% of these kidneys are also fused to the adjacent kidney.[35] The cause of crossed renal ectopia is unknown. The condition is found more commonly in males, and left-to-right crossover is most frequent. Approximately 20% to 40% of patients with crossed renal ectopia also have VUR, and there is a high incidence of associated cardiac, skeletal, genital anomalies with solitary crossed renal ectopia, although it is not known if this is due to the ectopia or the agenesis.[36]

The most common renal fusion abnormality is the horseshoe kidney, which consists of two renal masses on either side of the midline connected by an isthmus of tissue. Horseshoe kidney is found in approximately 1 in 600 on autopsy study.[37] There is male predominance, an association with syndromes such as Edwards syndrome, Down syndrome, and Turner syndrome, and an increase in associated genitourinary anomalies, including hypospadias, undescended testes, bicornuate uterus, septate vagina, and duplication of the ureter.[38] Up to half of children with horseshoe kidney have associated urologic anomalies including VUR and UPJO.[39]

Most patients with horseshoe kidney are asymptomatic, although the incidence of Wilms tumor is higher in patients with horseshoe kidney than in the average population,[40] and about a third develop stones in adulthood.[41] Neither risk is significant enough to justify routine screening of these patients.

Supernumerary Kidney

A supernumerary kidney is a separate or loosely attached renal mass with its own blood supply and collecting system. It is exceedingly rare, with only about 100 cases reported.[42] About 50% have a structural abnormality such as a duplicated system or hydronephrosis, and this condition is usually diagnosed due to infection, obstruction, or urinary incontinence.[43]

Cystic Disease of the Kidney

Cystic diseases of the kidney are classified as having inheritable or noninheritable causes (Box 76.2). Most cases present during the neonatal period.

Autosomal Recessive Polycystic Kidney

Autosomal recessive polycystic kidney disease presents as symmetric enlargement of the kidneys bilaterally due to collecting duct cysts and is associated with biliary dysgenesis and portal fibrosis. This disease used to be referred to as *infantile*; however, mild cases can present later in life. The incidence is estimated at 1 in 20,000 live births, with a mutation of *PKHD1* located on chromosome 6 responsible for this disease.[44] Ultrasound findings of bilateral hyperechoic kidneys with poor corticomedullary differentiation are suggestive, and infants often have Potter facies and respiratory issues due to oligohydramnios. Survival rates have improved overtime; despite this, about 20% of patients die shortly after birth of pulmonary complications.

• BOX 76.2 Cystic Diseases of the Kidney

Inheritable

- Autosomal recessive (infantile) polycystic kidney disease
- Autosomal dominant (adult) polycystic kidney disease
  - Juvenile nephronophthisis and medullary cystic disease complex
  - Juvenile nephronophthisis (autosomal recessive)
- Medullary cystic disease (autosomal dominant)
- Congenital nephrosis (familial nephrotic syndrome) (autosomal recessive)
- Familial hypoplastic glomerulocystic disease (autosomal dominant)
- Multiple malformation syndromes with renal cysts (e.g., tuberous sclerosis, von Hippel–Lindau disease)

Noninheritable

- Multicystic kidney (multicystic dysplastic kidney)
- Benign multilocular cyst (cystic nephroma)
- Simple cysts
- Medullary sponge kidney
- Sporadic glomerulocystic kidney disease
- Acquired renal cystic disease
- Calyceal diverticulum (pyelogenic cyst)

Modified from the American Academy of Pediatrics, Section on Urology.[218]

Those who survive have a high risk of developing childhood renal insufficiency, systemic hypertension, and portal hypertension, with 50% requiring early renal replacement, and many undergoing renal and/or liver transplants.[45]

Autosomal Dominant Polycystic Kidney

Autosomal dominant polycystic kidney disease involves progressive cystic enlargement of bilateral kidneys due to an abnormal form of the protein polycystin. It is the most commonly inherited renal disease, occurring in 1 in 1000 live births, and is attributed to a mutation in the genes *PKD1* and *PKD2* in 90% of cases.[46] While it used to be diagnosed late in life, advances in radiographic imaging have allowed early detection in fetuses and infants.[47] Renal failure progression is variable but typically occurs in adulthood; about 20% of children will have hypertension or proteinuria despite a normal glomerular filtration rate (GFR).[48] Because of this, children with family history or confirmed disease should have regular clinical screening with blood pressure monitoring and urinalysis; routine ultrasounds or "cyst counting" should be avoided in asymptomatic children.[49] There is no need to screen children for associated findings such as cerebral aneurysm or hepatic involvement. Presymptomatic testing to obtain a diagnosis in at risk children is controversial. Tolvaptan was recently approved to slow disease progression in adults and has been used for severe neonatal disease.[50]

Tuberous Sclerosis

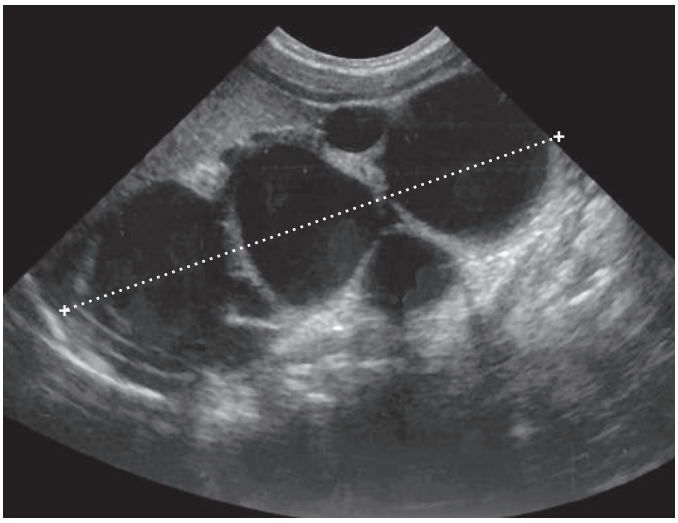
Tuberous sclerosis complex is a neurocutaneous disease that presents with benign hypopigmented skin lesions and is associated with epilepsy or autism. Renal manifestations are the primary source of morbidity in adult patients, and include angiomyolipoma, renal cysts, and oncocytomas.[51] Renal ultrasound should be performed at the time of diagnosis and then every 1 to 3 years to avoid missing a growing angiomyolipoma at risk of spontaneous hemorrhage.[52] First line treatment is with the mammalian target of rapamycin (mTOR) inhibitor everolimus, with surgery reserved for emergent situations.

Multicystic Dysplastic Kidney

Multicystic dysplastic kidney disease is the most common type of renal cystic disease, occurring in 1 in 4300 live births. It is diagnosed based on ultrasound findings of a collection of cysts of various sizes without any evidence of renal parenchyma (Fig. 76.2). There is a male and a left-sided predominance. The cause is unknown. Since 60% of MCDK will involute spontaneously with associated contralateral hypertrophy, early nephrectomy is rarely needed.[53] While vesicoureteral reflux is common in the contralateral kidney, it is rarely clinically significant and so a screening voiding cystourethrogram (VCUG) should be considered only if there are anomalies on the contralateral kidney.[54] Poor neonatal outcomes are generally only found when there are contralateral or extrarenal anomalies. Isolated multicystic dysplastic kidneys typically follow a benign course with hypertension and malignancy occurring at a similar proportion to general population.[55]

Renal Tumor

Malignancies are rare in neonates, and renal tumors make up 7% to 12% of neonatal malignancies.[56] The most common finding of renal tumor is a palpable abdominal mass, but more commonly they are noted on prenatal ultrasound, after which a fetal MRI can help characterize the lesion.[57] The first line of treatment is radical nephrectomy for all unilateral renal tumors. Biopsy before surgery is generally reserved for complex cases such as those with bilateral disease or metastases at presentation.



• **Fig. 76.2** Sonographic view of a multicystic dysplastic kidney in which multiple cysts that do not communicate with one another are grouped together, giving the typical "bunch of grapes" appearance. No function was noted on the patient's renal scan, confirming this diagnosis.

The most common renal tumor in neonates is congenital mesoblastic nephroma, generally diagnosed before 3 months of age. There is a male predominance of 1.5:1. The mainstay of treatment is a radical nephrectomy, and surgery is generally curative in stage I/II tumors, with rare need for chemotherapy. The overall survival rate for congenital mesoblastic nephroma is excellent, around 95%.[58]

Wilms tumor is the second most common neonatal renal tumor. It affects males and females equally and is associated with several syndromes, including Beckwith-Wiedemann syndrome, WAGR syndrome, Denys-Drash syndrome, and Perlman syndrome.[59] Treatment generally begins with radical nephrectomy, although there is increasing evidence that partial nephrectomy in appropriately selected patients is safe and may be preferred for those with bilateral masses or predisposing syndromes.[60,61] After surgery, treatment is based on risk stratification and may include chemotherapy and radiation therapy based on protocols from two international oncologic groups, the Children's Oncology Group and the International Society of Pediatric Oncology. The overall survival rate is high at 90%, but long-term survivors are at risk of cardiac disease, adverse pregnancy outcomes, and renal dysfunction, as well as secondary primary tumors in 16%.[62]

Malignant rhabdoid tumor of the kidney is a rare and aggressive cancer that generally presents at advanced stages. The overall survival rate in neonates is 16%. Other renal tumors in neonates include clear cell carcinoma of the kidney, which presents at advanced stage but for which there is an overall survival rate of 50%, ossifying renal tumor of infancy, which is generally benign, nephroblastomatosis, which is a premalignant condition requiring observation, and cystic nephroma, which is benign but indistinguishable from rare malignancies and therefore generally surgically removed.[63,64]

Renal Vein Thrombosis

The risk factors for renal vein thrombosis include umbilical vein catheterization, perinatal asphyxia, maternal diabetes, and dehydration, as well as the presence of prothrombotic states. The classic presentation is gross hematuria, a palpable flank mass, and thrombocytopenia.[65] Contrast angiography is the gold standard for diagnosis, but because of concerns regarding radiation, Doppler ultrasound is often used as an alternative. This will show enlarged and echogenic kidneys with either absent flow in the renal vein or increased resistance in the renal artery.[66] The current standard is treatment with anticoagulation; thrombolysis can be considered with life-threatening renal vein thrombosis.[67] Optimal targets for anticoagulation have not been established, so adult ranges are generally used.[68] Even with anticoagulation, over 60% of affected kidneys will become atrophic.[69]

Adrenal Hemorrhage

Adrenal hemorrhage occurs after birth in approximately 2 in 1000 live births. While presentation can include prolonged jaundice, palpable abdominal mass, anemia, or scrotal hematoma, these symptomatic cases are less common in the era of ultrasound diagnosis.[70] Predisposing factors include vaginal birth, macrosomia, perinatal hypoxia, birth asphyxia, and sepsis.[71] Treatment is generally supportive and adrenal insufficiency rare. Serial ultrasounds should be obtained to confirm resolution given the rare chance of a fetal neuroblastoma mimicking an adrenal hemorrhage.[72]

Anomalies of the Ureters

Duplication of the Ureters

Ureteral duplication can develop if there are duplicate ureteral buds or early division of these buds. Ureteral duplication occurs in between 0.8% and 1.67% of the population.[73] Ureteral triplication and even quadruple ureters have also been reported in the literature, but are much rarer. Complete duplication results in two separate ureters, while partial duplication results in a bifid renal pelvis with distal confluence into a single ureter. There is a strong genetic link to duplication of the ureter, which occurs in 12% of screened siblings and parents of affected patients.[74] Diagnosis is generally by ultrasound. Some clues can indicate a duplex kidney prenatally, including renal length greater than the 95th percentile with a cyst-like structure in the upper pole surrounded by a rim of parenchyma, two noncommunicating renal pelvises, or a cystic structure in the bladder consistent with a ureterocele.[14] The location of the ureters in the bladder of patients with complete ureteral duplication generally follows the Weigert-Meyer law, with the upper pole ureter found caudal to the lower pole ureter.

About half of patients with duplex kidneys are otherwise asymptomatic, and the anomaly by itself is not thought to have any clinical significance. Duplex kidneys is, however, associated with other conditions; a quarter of patients have upper pole obstruction, usually associated with a ureterocele, 10% have lower pole scarring, and 4% have lower pole VUR.[75] Both lower pole UPJO and rarely upper pole UPJO have been reported, as has upper pole ectopia. Long-term renal outcome is generally related to the presence of these associated conditions, although ureteral duplication itself is a risk factor for a slower rate of spontaneous reflux resolution.[76]

Ureteral Ectopia

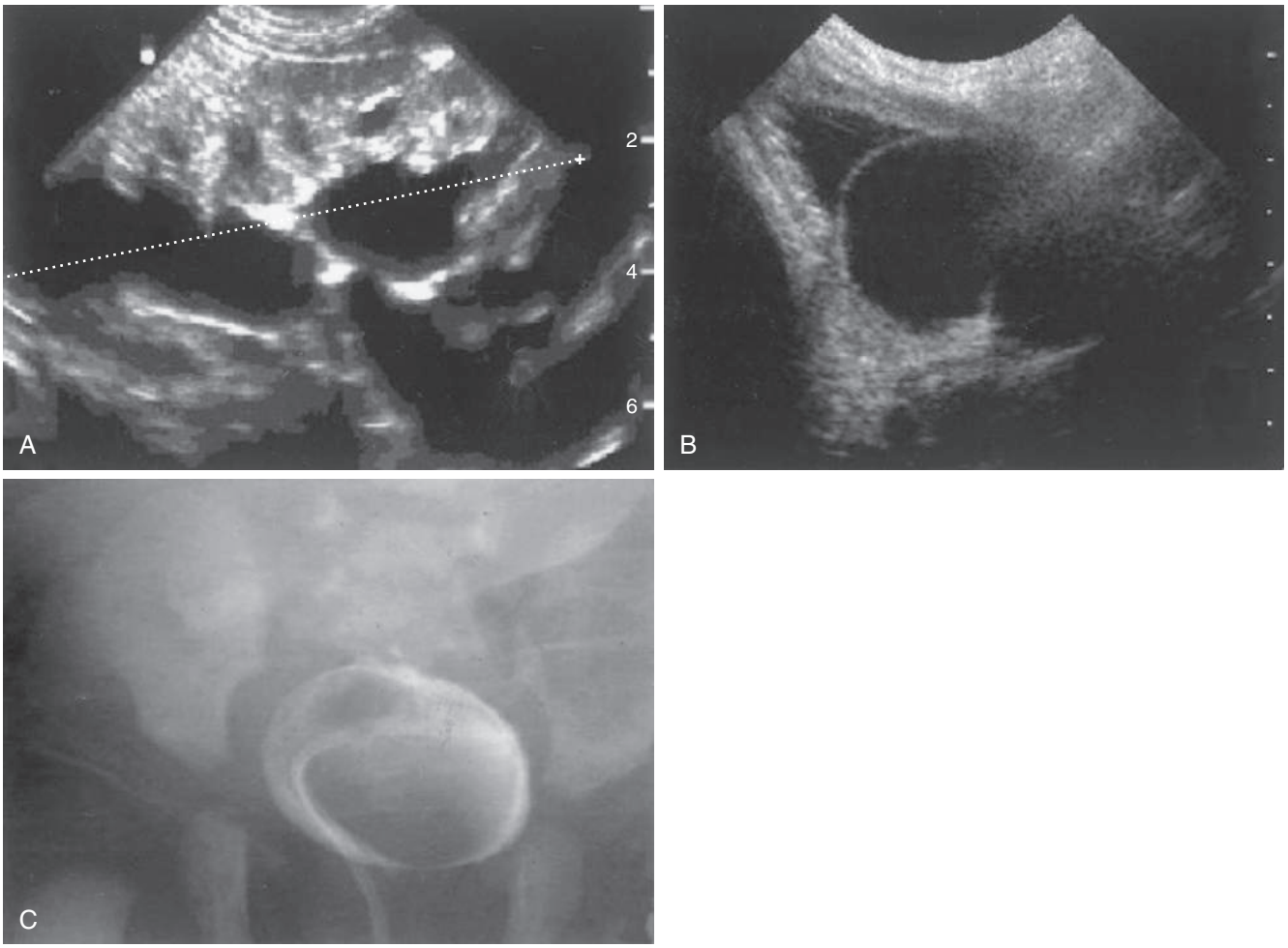
Ureteral ectopia occurs whenever the opening of the ureter is in a position other than the expected location in the bladder trigone. The incidence is 1 in 2000 to 4000 on autopsy study.[77] The cause is thought to be from ectopic location of the initial ureteric bud, and because of the predictable pathway of the bud during embryologic development, these ureters are generally found in predictable locations. The most common form is lateral migration, which is thought to cause vesicoureteral reflux. Medially located ureters are generally found along the path of the Wolffian duct. In males, an ectopic ureter can be found in the bladder neck, the posterior urethra, seminal vesicles, the vas deferens, or the epididymis. In females, an ectopic ureter can be found in the bladder neck, the urethra, the uterus, the proximal part of the vagina, or the Gartner duct.

In males, the ectopic ureter can present with dilatation or infection of the structure into which it inserts. In females, the ectopic position is more likely to be distal to the urethral sphincter, resulting in continuous incontinence, which is often discovered after failed attempts at toilet training. Ectopic ureters are generally from the upper pole of a duplex kidney. While single-system ureteral ectopia occurs, it is difficult to diagnose because of the association with poor renal function on that side. Single-system ureteral ectopia should be considered in females who present with continuous urinary incontinence and what appears to be a solitary or duplex kidney on conventional imaging.[78] Dimercaptosuccinic acid scanning and computed tomography are the most sensitive techniques for diagnosis, although ultrasound, MRI, and intravenous pyelogram can also detect a single-system ectopic ureter.[79]

Ureterocele

A ureterocele is a cystic dilatation of the distal submucosal or intravesical ureter that results in obstruction of urine flow. Ureteroceles occur within a single system or a duplex system. If the system is duplex, the ureterocele is associated with the ectopic upper pole. Ureteroceles occur in between 1 in 500 in autopsy series.[80] The cause is thought to be failure of apoptosis of a distal membrane at the ureteral orifice known as the *Chwalle membrane*. Because of familial occurrence a genetic cause is suspected.[81] Ultrasound of ureteroceles shows hydronephrosis associated with a cystic structure within the bladder (Fig. 76.3). Most ureteroceles are diagnosed prenatally and have been detected as early as 16 weeks.[82] Symptoms include infection, retention, or prolapse through the urethra in a female, which generally presents as a bulging vulvar mass.[83]

Multiple options have been described for management of ureteroceles, and there is no clear consensus.[84] If the child has no infections, minimal associated vesicoureteral reflux, and either no obstruction or an already nonfunctioning portion of the kidney, close observation without intervention can have good outcomes.[85] For patients who require treatment, transurethral incision or puncture provides decompression in a minimally invasive fashion, although it can result in secondary vesicoureteral reflux. It is most successful as a definitive operation in patients with intravesical single-system ureteroceles and can also be used as an urgent temporizing measure in septic, obstructed children.[86] Fetoscopic incision and puncture has been successful, as has bedside puncture without anesthesia.[87,88] More definitive surgical procedures include ureteroureterostomy, ureteropyelostomy, and ureterocele



• **Fig. 76.3** Images demonstrating upper pole hydronephrosis that is secondary to a dilated ureter (A), which empties into a larger ureterocele within the bladder, evident in (B). There is also secondary dilatation of the lower pole renal pelvis. The ureterocele is demonstrated as the cystic filling defect on bladder views from the ultrasound study (B) as well as the voiding cystourethrogram (C).

excision with reimplantation into the bladder for functioning renal units or nephrectomy or heminephrectomy for nonfunctioning renal units. Regardless of the management selected, most children do well in the long term, with low risk of major bladder dysfunction.[89]

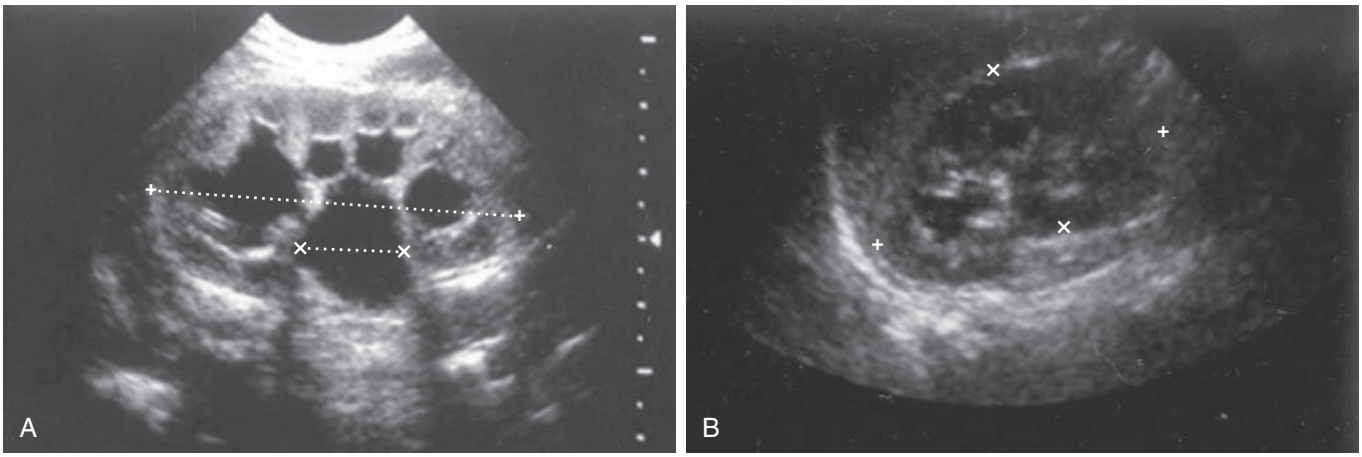
Ureteropelvic Junction Obstruction

Ureteropelvic junction obstruction (UPJO) refers to a blockage where the renal pelvis meets the ureter (Fig. 76.4). It occurs in an estimated 1 in 1500 live births and is the most common cause of prenatal hydronephrosis, accounting for 41% of cases.[90,91] UPJO in newborns and infants is generally caused by intrinsic narrowing of the area, while in childhood and adolescence it is generally caused by extrinsic compression by an accessory vessel to the lower pole of the kidney. Because of increased prenatal ultrasound screening, many cases are diagnosed prenatally. Delayed presentations can occur with flank pain, nausea, and emesis later in life. Diagnosis of functional obstruction is confirmed with a diuretic renogram or MR urography.

Between 23% and 51% of patients with UPJO will require intervention because of worsening hydronephrosis or decreasing renal function.[92,93] Surgery is still primarily performed in an open fashion, but laparoscopic or robotic surgery is at least as successful.[94,95] While surgical success rates are between 90% and 100%, patients are still at risk of later development of hypertension and proteinuria.[96] Half of patients with UPJO will have other urologic anomalies, including contralateral obstruction and VUR.[97] A routine VCUG is generally recommended if UPJO is present, although some pediatric urologists are questioning the benefit of this invasive test because of the high prevalence of clinically insignificant reflux.[98,99]

Ureterovesical Obstruction

Ureterovesical obstruction, also sometimes called an *obstructed megaureter*, refers to a blockage where the ureter meets the bladder. The cause is thought to be an adynamic segment of the distal ureter with insufficient peristalsis.[100] Megaureter accounts for 5% to 10% of prenatal hydronephrosis, and up to 80% of cases resolve without the need for intervention.[101,102] The true incidence of obstructed megaureter is not known. As with UPJO, ureterovesical obstruction is confirmed with the functional study of a diuretic renogram since not all megaureters are obstructed.



• **Fig. 76.4** An example of prenatal hydronephrosis consistent with a partial ureteropelvic junction obstruction (A) that spontaneously resolved over a 6-month period (B).

Surgical management can be with endoscopic treatment or open or robotic reconstruction.[103,104]

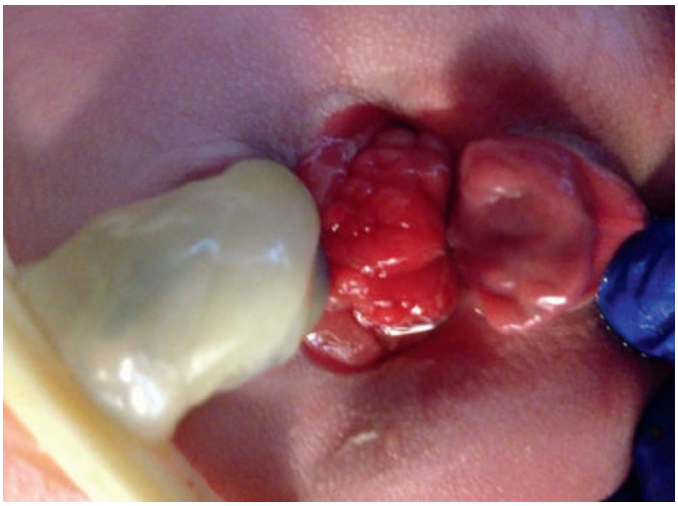
Vesicoureteral Reflux

Vesicoureteral reflux describes retrograde flow of urine from the bladder to the ureters. The normal ureterovesical junction is designed with a long intramural tunnel through which the ureter travels before reaching the bladder and prevents retrograde flow of urine. Primary VUR is thought to be due to an abnormal or immature formation of this area, allowing urine to reflux from the bladder into the ureters and kidneys.

As many as 17% of all children may have VUR, including 31% to 36% of those who develop a urinary tract infection.[105,106] The first sign of VUR is often hydronephrosis on the prenatal ultrasound, with 20% of these infants eventually found to have reflux.[107] Neonates with a history of unilateral prenatal hydronephrosis should undergo ultrasound again within the first week of life. If the hydronephrosis was bilateral or in a solitary kidney, the ultrasound should be done before discharge from the hospital.[101] Patients with high-grade hydronephrosis on late prenatal or postnatal imaging should be screened with a VCUG.[108]

VUR is graded after a VCUG from I to V. Lower-grade reflux is more likely to resolve spontaneously without the need for intervention, with early resolution rates of 13% yearly for grades I to III and 5% for grades IV and V.[109] Continuous antibiotic prophylaxis is recommended for infants with grade III to V VUR or any grade if the infant has a history of febrile urinary tract infection. There is evidence that prophylaxis prevents recurrent infections, but its benefit in terms of renal scarring is less clear.[110] Other options for reducing the risk of urinary tract infection are circumcision or early preputial retraction using a prescription steroid cream.[111,112] Breakthrough infections despite continuous administration of prophylactic antibiotics generally trigger a change in the antibiotic or surgical correction via an open or endoscopic approach.

About 27% of siblings and 36% of children of patients with VUR will have reflux. A screening ultrasound can be considered, with a VCUG pursued if there is evidence of renal scarring or a history of urinary tract infection.[113]



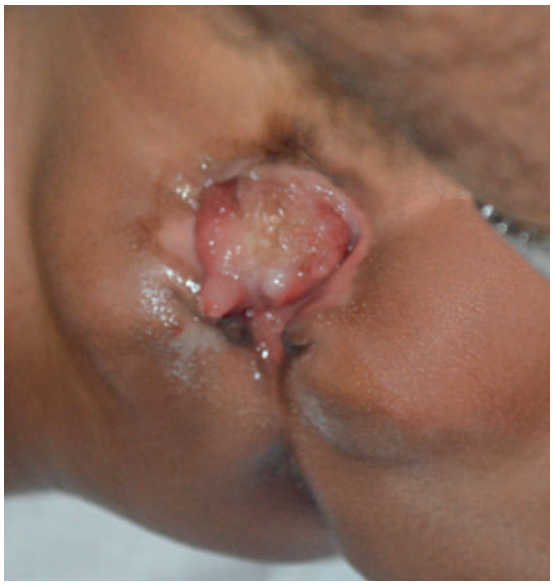
• **Fig. 76.5** Male patient with bladder exstrophy showing exstrophied bladder, low umbilicus, and penile and urethral defect.

Anomalies of the Bladder

Bladder Exstrophy

Bladder exstrophy and epispadias are congenital anomalies that have characteristic external physical manifestations. The anterior portion of the bladder and/or urethra and abdominal wall structures are deficient, and the symphysis pubis is widely separated from the midline (Figs. 76.5 and 76.6).

Bladder exstrophy has been described as "if one blade of a pair of scissors were passed through the urethra of a normal person; the other blade were used to cut through the skin, abdominal wall, anterior wall of the bladder and urethra, and the symphysis pubis; and the cut edges were then folded laterally as if the pages of a book were being opened."[114] Bladder exstrophy is usually found in isolation, with other organ systems, including the kidneys, rarely affected. The incidence of bladder exstrophy is an estimated 1 per 50,000 live births, and it is about twice as common in males than females.[115] The pathogenesis of exstrophy is thought to be caused



• **Fig. 76.6** Female patient with bladder exstrophy showing exstrophied bladder and bifid clitoris.

by persistence of the cloacal membrane during development. This, in return, prevents the mesoderm from fusing in the midline. The persistent cloacal membrane then ruptures to produce an exstrophic condition.[116]

Ultrasonography can reliably detect exstrophy with the following findings: (1) an absent or nonfilling bladder, (2) a mass protruding from the abdominal wall, (3) normal kidneys with a low-set umbilicus, (4) widened pubic diastasis, and (5) a smaller penis with anteriorly displaced scrotum.[117,118] Fetal MRI can also aid in diagnosis. Prenatal diagnosis allows better counseling of parents as the overall prognosis of these children is excellent if they are treated in specialized centers. Counseling of families by healthcare providers unfamiliar with the long-term outcomes is associated with an increase in the abortion rate for these fetuses.[119]

Management after delivery includes ligation of the umbilical cord with silk suture rather than plastic or metal clamp to prevent trauma to the exposed bladder. A transparent film, hydrated gel, or even plastic wrap placed on the exposed bladder protects it from superficial trauma. The dressing should be replaced daily, and the bladder irrigated with saline with each diaper change. A renal ultrasound and a pelvic radiograph should be performed to evaluate the kidneys and the pubic diathesis. If a sacral dimple is found, a spinal ultrasound should be performed to rule out spinal cord tethering.

Surgical management has evolved, with most centers today performing a complete primary repair of exstrophy, which includes closure of the bladder exstrophy, reconstruction of the bladder neck, repair of the dorsal chordee, repair of epispadias with urethroplasty, and bilateral iliac osteotomies. Some still advocate a staged repair with closure of the bladder exstrophy and bladder neck as the first stage and repair of the epispadias as a second stage.[120,121] Ureteral reimplantation is sometimes performed at the time of bladder closure; if not children develop VUR after surgery and are often given antibiotic prophylaxis for the first 18 months of life.[122]

The timing of repair has also undergone some recent changes. In the past these repairs were primarily performed at birth. Recently many have advocated delayed closure at around 3 months of age, and this was based on studies showing that delayed closure does not compromise the rate of bladder growth.[123–125] Delayed closure allows better coordination of care between the teams of urologists, orthopedic surgeons, anesthesiologists, and operating room staff. Moreover, it allows the child to bond with the parents and allows penile growth to occur because of the physiologic surge of testosterone.

The goal of reconstruction includes preservation of renal function, urinary continence with volitional voiding, and functional and cosmetically acceptable external genitalia. With the primary repair, volitional voiding can be achieved with a single operation in around 40% of children, with around 40% requiring further bladder neck reconstruction at age 4 to 5 years to achieve continence. A small percentage of patients with small bladders will require reconstruction, including bladder augmentation and use of intermittent catheterization.[126]

Cloacal Exstrophy

Patients with cloacal exstrophy have multiple organ systems affected, and as a result it is also referred to the *OIES complex* (omphalocele, exstrophy, imperforate anus, and spinal defects). Other organ systems affected may include the extremities, the upper urinary tract, and the cardiovascular, pulmonary, and craniofacial systems. In this condition the hindgut is also open to the abdominal wall and separates the hemibladder plates.

Before the 1960s cloacal exstrophy was considered incurable, with a very high mortality rate. In the past 2 decades, survival rates have increased to close to 100%.[127] The principles of management include neurologic evaluation for assessment of myelomeningocele, neonatal closure of omphalocele and intestinal diversion using the exstrophied hindgut, approximation of bladder halves, delayed bladder exstrophy closure with placement of the bladder deep in the pelvis, and iliac osteotomies.[128]

Patent Urachus

The urachus is a tubular structure that connects the urogenital sinus and the allantois. It begins to narrow at between 4- and 5-months' gestation, generally being obliterated before birth. A patent urachus is often diagnosed because of drainage of clear fluid from the umbilicus. While ultrasound will often show the anomaly, a VCUG or sinogram can be used to confirm the diagnosis.[129] Because the urachal remnant resolves spontaneously in up to 80% of cases, surgery is generally avoided in patients younger than 1 year.[130] While malignant transformation in adulthood is possible, it is so rare that these are generally only removed if symptomatic.[131] If a urachal anomaly is excised, this can be performed via the open or laparoscopic approach.

Posterior Urethral Valves

PUVs are a congenital obstruction of the posterior urethra. They are the most common cause of bladder outlet obstruction in newborn males, affecting approximately 1 in 4000 live births.[132] Importantly, PUVs occur only in males. PUVs have been classified into three types. Type 1 valves account for the majority of PUVs, with leaflets of tissue arising from the verumontanum and

fusing in the midline. Type 2 valves have not been described since the initial reports and likely do not exist. Type 3 valves are an annular ring thought to be due to persistence of the urogenital membrane.[133] This classification has been challenged, with some arguing that the different types are due to postnatal rupture or instrumentations.[134]

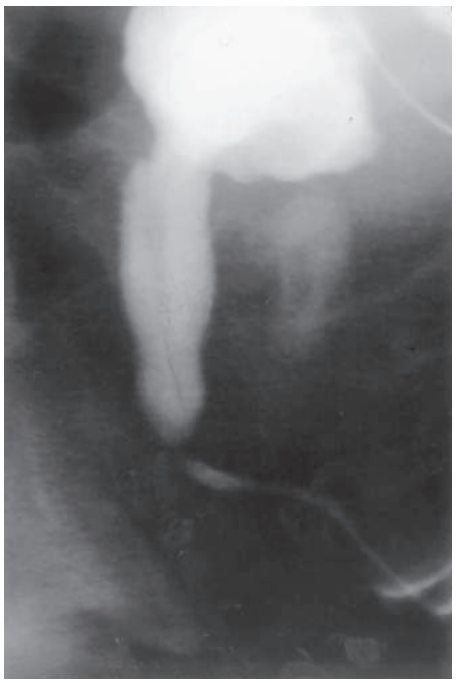
Prenatal ultrasound is often able to detect PUVs. The classic findings include a thickened, dilated bladder with bilateral hydroureter, especially in the presence of oligohydramnios and a dilatated posterior urethra with the "keyhole sign," although some of these anomalies may be present in urethral atresia as well.[135] A VCUG is used to confirm the diagnosis after birth and will clearly show the obstructing membranes in the urethra (Fig. 76.7).

PUVs have consequences for multiple organ systems. Pulmonary hypoplasia and Potter facies can be seen if the obstruction was severe enough to cause oligohydramnios. Renal dysplasia is common and can lead to progressive renal failure later in life, often associated with polyuria and nephrogenic diabetes insipidus. The bladder is generally small, with poor compliance that progresses to myogenic failure over time. The ureters often remain chronically dilated.[100]

Initial management of PUVs involves support in the neonatal intensive care unit and passage of a urinary catheter for drainage. There is some evidence that inflation of a catheter balloon can cause ureteral obstruction in the valve bladder, and so this should be avoided.[136] Once the infant is stable to undergo the procedure, the valves are ablated endoscopically. Vesicostomy is reserved for infants with urethras that are too small to accommodate the scope, though progressive urethral dilation via catheters has been described.[137]

Long-term renal outcomes with PUVs are poor, with 20% to 60% of boys progressing to end-stage renal disease. Nadir creatinine level is predictive of eventual renal outcome, with initial creatinine nadir level greater than 1.0 mg/dL generally used in modern series as the cutoff to predict a high risk of future renal damage.[138,139] The bladder decompensates to an overly large bladder with poor sensation and elevated postvoid residuals that often require overnight catheter drainage or clean intermittent catheterization.[140]

In the past decade several fetal interventions have been trialed for PUVs. These include vesicoamniotic shunting and fetoscopic valve ablation. While oligohydramnios abates and perinatal mortality is reduced, there is no evidence of a decrease in the percentage of patients who progress to end-stage renal disease.[141]



• **Fig. 76.7** This voiding cystourethrogram shows a classic posterior urethral valve, with narrowing of the urethra at the most distal end of the prostate. This area corresponds to a flap of tissue that serves as an obstructing valve leaflet.

Genital Abnormalities in Males

Cryptorchidism

Cryptorchidism, also known as *undescended testicle*, is one of the most common congenital anomalies. The incidence is variable and dependent on gestational age. The incidence ranges from 1% to 4.5% in full-term infants and from 30% to 45% in premature infants. During the first 3 to 6 months of life the testicle may spontaneously descend such that the incidence at 1 year of age is around 0.8% to 1.2%.[142] Diagnosis is made on physical exam. Ultrasound is not recommended given poor sensitivity and specificity.[143]

Cryptorchidism is commonly seen in healthy infants but can occur in conjunction with more than 400 syndromes.[144] Abnormal gubernacular attachment or migration is the theorized etiology, and the cause is thought to be multifactorial and includes familial predisposition and environmental exposure (phthalates, pesticides, flame retardants).[145] It has also been included in "testicular dysgenesis syndrome," a mechanism that interferes with normal fetal testicular development and includes undescended testicles and genitourinary disorders such as hypospadias, semen production abnormalities, and infertility.[146] A newborn who appears phenotypically male with bilateral nonpalpable gonads should be evaluated for possible difference of sexual differentiation (DSD) such as congenital adrenal hyperplasia (CAH). Patients with a unilateral nonpalpable testicle and hypospadias should also be evaluated for a DSD; namely, mixed gonadal dysgenesis or ovotesticular DSD.[147]

The most recent American Urological Association guidelines on the management of cryptorchidism state: "Providers should refer infants with a history of cryptorchidism (detected at birth) who do not have spontaneous testicular descent by six months (corrected for gestational age) to an appropriate surgical specialist for timely evaluation (Evidence Strength: Grade B)".[147] The main reasons for treatment are risk of impaired fertility potential, testicular cancer, and testicular torsion. The number of spermatogonia per tubule is adversely affected in boys with undescended testicles beyond 1 year of age, and therefore it is recommended that orchiopexy be performed early to minimize germ cell loss, ideally between 6 and 18 months of age.[148] Surgically bringing the testicles into the scrotum may reduce but does not prevent long-term sequelae such as reduced fertility and cancer.[147]

Testicular Tumors

There is a bimodal age distribution for the incidence of testis tumors, with one peak occurring during the first 2 years of life and a second, larger peak occurring in young adulthood. The incidence of pediatric testis tumors is 0.5 to 2.0 per 100,000 children, accounting for only 1% to 2% of all pediatric tumors.[149]

Teratomas are the most common benign tumors in prepubertal patients. Cases have been described from 6 days to 13 months.[150] Testis-sparing surgery is a consideration for prepubertal children, but frozen sections should be obtained to confirm the diagnosis. Orchiectomy is necessary if the entire testicle is involved.

Testicular Torsion

This is a rare event, and its management is controversial. The newborn presents with swelling of the hemiscrotum with or without discomfort. In this condition the testicle, epididymis, and tunica vaginalis twist around the spermatic cord (extravaginal torsion). This condition may be unilateral or bilateral, and the bilateral torsion can either be synchronous or asynchronous. The cause is unknown, but it is theorized that complicated pregnancies and vaginal delivery are predisposing factors.[151]

The presentation depends on when the torsion occurred. If torsion occurs several days before birth, the newborn will have a firm painless scrotal mass. If it occurred hours before birth, the newborn usually has a painful, enlarged, and high-riding testicle.[151] Diagnosis is usually made by physical examination and Doppler testicular ultrasonography showing no blood flow to the testicle.

Management of neonatal testicular torsion is controversial. Because of the low salvage rate and risk of surgical or anesthetic complications, some advocate conservative management.[152] Most pediatric urologists, however, advocate immediate surgical exploration. The rationale behind this recommendation is that viability of the testicle can be assessed only surgically. Moreover, asynchronous torsion has been described in 3% of cases, and fixing the normal testicle in place before this happens will prevent anorchia.[153]

Hydrocele

Hydroceles describe persistent fluid within the tunica vaginalis adjacent to the testicle. They occur in more than half of newborn male infants, and most at this age fluctuate in size due to a persistent communication of the processes vaginalis with the peritoneum.[154] In the absence of an associated hernia or undescended testicle, 90% of communicating hydroceles will resolve spontaneously by 12 months of age.[155] If surgery is needed, it can be performed open or laparoscopically.

Hypospadias and Chordee

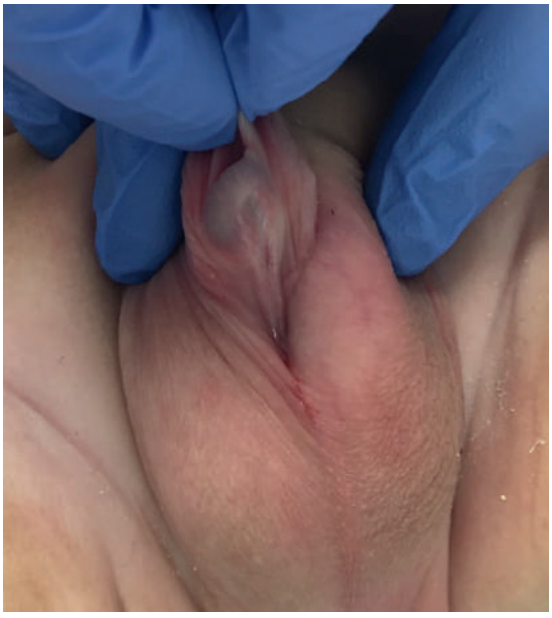
Hypospadias and chordee is one of the most common abnormalities of the penis and occurs in 1 in every 250 to 300 male births worldwide, or around 0.3% of all male births.[156] From an analysis of all boys born in Denmark between 1973 and 2005, it was found that the relative recurrence risk for a brother to have hypospadias was 13.4 (95% confidence interval [CI] 11 to 16.4) and 10.4 (95% CI 7.54 to 14.4) for offspring of an affected male. The risk in same-sex twins was 50.8 (95% CI 34.2 to 75.5).[157]

The term *hypospadias* describes a urethral opening more proximal than the tip of the glans. This difference is a spectrum that includes an abnormal ventral curvature (chordee) of varied degree, a dorsally hooded foreskin, and penoscrotal transposition. The spectrum ranges mild chordee without hypospadias to severe chordee and hypospadias with the meatus in a perineal location. A small group of patients will have a normally formed foreskin such that the hypospadias is discovered at the time of circumcision or preputial retraction. These children also have a wide urethral plate and thus are described as having megameatus intact prepuce variation of hypospadias.[158]

In most patients, hypospadias is the only atypical finding. But there are over 200 syndromes that include hypospadias, including the Wilms tumor, aniridia, genitourinary malformations, and intellectual disability (WAGR) and the Denys-Drash syndrome.[159] The most common associated anomaly is cryptorchidism, which is reported in around 3% of infants with distal hypospadias and in up to 10% of infants with proximal hypospadias.[160] In one study chromosomal anomalies were found in 22% of patients with hypospadias and cryptorchidism.[161] DSD such as CAH and mixed gonadal dysgenesis should be considered when hypospadias is found with either a unilateral or bilateral undescended testicles.[162,163]

Hypospadias is usually classified based on the location of the meatus: distal portion of the shaft, midshaft, proximal portion of the shaft; penoscrotal, scrotal, perineal. This classification, however, is not sufficient to explain the complexity of the defect. Because of this, a classification system called the *GMS score* (glans, meatus, shaft) has been used that includes a scale of 1 to 4 for each component (glans size and urethral plate quality, meatal location, and degree of shaft curvature), with the more unfavorable characteristics assigned higher scores. This new scoring system provides a means by which hypospadias severity can be standardized and surgical outcomes better classified.[164,165]

Hypospadias repair is undertaken to allow for straight erections and standing to void. Surgery is generally performed between 6 and 18 months of age, with the more extensive repairs sometimes postponed until after the age of 1 year.[166,167] A two-stage repair is advocated for those with severe hypospadias and chordee (Fig. 76.8).[168] If the phallus is small, testosterone injections may be given to increase phallic size and facilitate repair, but it is unclear whether this increases or decreases postoperative complications.[169] Poor response to testosterone injections may reveal children with androgen insensitivity.[170] Reported outcomes after hypospadias repair have historically been limited by short follow-up; modern series report complications requiring an unplanned additional



• **Fig. 76.8** Severe Scrotal Hypospadias with Chordee. The opening is in between the scrotal folds.

surgery in 12% to 17% for distal hypospadias and around 50% to 60% for proximal hypospadias.[168,171,172]

Phimosis

Phimosis is a condition in which tight foreskin cannot be pulled back over the head of the penis. In phimosis, the prepuce forms as a roll of epithelium that fuses ventrally at the frenulum. Once formed, the inner preputial surface fuses with the glans epithelium and may not separate from it until later in childhood. In the process of separation, cystic spaces between the two layers may form and fill with desquamated epithelium that form white beads or infantile smegma. These may be quite large and present as a mass under the foreskin. They eventually drain spontaneously. Incomplete formation of the prepuce is associated with other penile anomalies such as hypospadias, chordee, and epispadias.

Current recommendations for phimosis are to allow the foreskin to separate from the glans and to consider management if the phimosis does not resolve during childhood or by 5 to 7 years of age. Forcible retraction of the foreskin is contraindicated as this produces tears in the foreskin that will result in scarring that leads to pathologic phimosis, inflammation, and infection (posthitis).

The benefits of circumcision at birth have been the subject of debate and controversy. An American Academy of Pediatrics statement indicates that circumcision may have benefits in reducing the risk of urinary tract infections (UTIs) and sexually transmitted diseases.[173] On the other hand, the Canadian Pediatric Society concluded that since the benefits do not exceed the risks, circumcision should be performed only in boys in high-risk populations or circumstances.[174]

The most common complications of newborn circumcision include bleeding, infection, formation of penile adhesions between the skin and the glans, and incomplete circumcision. Other less common complications include iatrogenic amputation of the glans, loss of penile skin, and injury to the urethra.[175] When newborn circumcision is being discussed with the parents, it is important to discuss the pros and cons of circumcision, including the possible benefits, risks, and complications.

Other Penile Anomalies

Webbed Penis

Webbed penis, also called *penis palmatus* or *penoscrotal fusion*, is a congenital condition where the scrotal skin extends to the ventral penile shaft with deficient ventral penile shaft skin. The penile shaft may be buried in the scrotum. The urethral meatus and the penile shaft are generally not impacted.[176]

If a circumcision is desired, it should not be performed as a newborn as outcomes will be poor. These infants should be referred to a specialist for repair after 6 months of age, which will include use of the foreskin to cover the ventral penile shaft and recreation of the penoscrotal and penopubic junction.[177]

Buried Penis

Buried penis, also known as *hidden penis*, is a congenital condition where the penis is partially or completely hidden below the surface of the skin, generally due to excessive prepubic fat or a lack of anchoring of the skin to the base of the penis. Large scrotal masses such as hernias or hydroceles can also lead to this appearance. Buried penis can sometimes lead to ballooning of the foreskin during voiding. Families may worry about the size of the penis, but the true length of the penis can easily be palpated through the skin when the suprapubic fat is depressed.[178]

If a circumcision is desired, it should be performed by a specialist after 6 months of age. Otherwise, if there are no symptoms, then no treatment is needed as most children lose their excessive prepubic fat with growth and puberty.

Micropenis

This is a congenital *condition* defined as a phenotypical male with expected internal and external genitalia with a stretched penile length 2.5 standard deviations below the mean (Table 76.1). Stretched penile length is measured by pressing the suprapubic fat pad down with a firm tool like a ruler, gently stretching the penis, and measuring from the pubic ramus to the top of the glans.[179] The reported incidence of this condition is 1.5 in 10,000 male births.[180]

Aphallia

Agenesis of the phallus is very rare and occurs in 1 in 10 million to 1 in 30 million live births. This suggests an early embryologic failure in development of the genital tubercle. The urethra is located in the perineum or rectum. The karyotype is mostly 46XY, but females have been described with absence of corporal bodies.[181] More than half of patients with aphallia will have an associated anomaly, such as renal agenesis, horseshoe kidney, skeletal and neural disorders, and imperforate anus. Male children were previously raised as females, but this practice has been abandoned. These children have normal testicles, with normal testosterone production and normal spermatogenesis, and should be raised as males. With improvements in phallic reconstruction and in vitro fertilization, these patients can father children and have the potential of having a functional phallus.[182]

Epispadias

Epispadias is characterized by failure of the urethral plate to tubularize on the dorsum, with a defect ranging from the glandular to the phallopubic location. Male patients also have dorsal curvature or chordee where females have a bifid clitoris.[183] This condition is usually associated with bladder exstrophy but can appear as an isolated defect. The incidence is around 1 in 117,000 males and 1 in 150,00 to 1 in 300,000 females.[184]

Those with the more proximal defects (penopubic) are incontinent because of incompetence of the bladder neck, but urinary incontinence has also been reported in the more distal defects.[185]

TABLE 76.1 Stretched Penile Length by Age

Age	Mean (cm)	SD (cm)	Mean − 2.5 SD (cm)
Preterm newborns, 30 weeks	2.5	0.4	1.5
Preterm newborns, 34 weeks	3.0	0.4	2.0
Term newborn	3.5	0.4	2.5

SD, Standard deviation.

There are different techniques for repairing this defect, with the proximal defects also requiring a bladder neck reconstruction for continence.[186] The current recommendation is to delay repair of these defects until 3 to 6 months of age. The more proximal defects (penopubic) will also require a bladder neck reconstruction including pelvic osteotomies to place the bladder neck area in an anatomically correct location, deep in the pelvis.

Urethral Duplication

This is an uncommon anomaly and can present as a complete or partial duplication. It is more common in males than in females. The two urethras are oriented in an anteroposterior plane, with the ventral urethral meatus being the functional urethra and the dorsal urethra being stenotic.[187] In females, urethral duplication may accompany cloacal anomalies and be associated with duplication of the bladder.[188]

Disorders of Sexual Differentiation

Chapter 85 in this text provides a full review of disorders of sexual differentiation. These patients pose a difficult diagnostic and therapeutic challenge and require a multidisciplinary approach to evaluation and management, including genetics, urology, gynecology, endocrinology, social work, and pediatric psychiatry.

Any phenotypically looking male, even if completely masculinized, with bilateral nonpalpable gonads should be evaluated for CAH. Failure to do so may result in an Addisonian crisis due to salt-losing adrenogenital syndrome.

Urinary Tract Infections

UTIs are the most common serious bacterial infection in infants, with a higher prevalence in males than females.[189] Uncircumcised male infants appear to be at increased risk, with a 2.15% incidence of UTIs during the first year of life, while females have a 2.05% incidence and circumcised males a 0.22% incidence.[190] A retractable foreskin appears to reduce UTIs, and steroid cream can be used if physiologic phimosis is present.[112,191]

Newborns with culture-documented UTI should be evaluated with at least a renal ultrasound. Some advocate a VCUG or DMSA in addition.[192] If any findings are abnormal, a VCUG should be performed.

Myelodysplasia

Spina bifida and meningomyelocele occur when the neural tube fails to close due to a lack of folic acid during pregnancy. They are found in approximately 36 per 100,000 live births in the United States.[193] The urinary tract is involved in most of these children given the location of the bladder nerves. Increased bladder pressures and impaired bladder function due to the neurologic deficit eventually result in hydronephrosis and ultimately renal damage. The urologic goal of treating children with myelomeningocele is preventing upper tract deterioration.

In utero closure of myelomeningocele has resulted in reduced need for shunting and improved motor outcomes.[194] Open prenatal surgery has not significantly reduced the need for clean intermittent catheterization, but fetoscopic treatment has shown more impact on urodynamic function.[195,196]

All children should be evaluated with a renal ultrasound. Urodynamic studies are currently recommended early in life to risk-stratify children into those at high risk of renal damage (those with high-pressure bladder, high leak point pressures, dyssynergic voiding) and those at low risk (normal bladder compliance, low leak point pressure).[197]

An indwelling catheter is placed in the immediate postclosure period, and clean intermittent catheterization is implemented every 3 to 4 hours. Parents should be taught to perform clean intermittent catheterization, but continuing catheterization if volumes are low is controversial. Early initiation of clean intermittent catheterization may decrease the need for future reconstruction such as bladder augmentation.[198,199]

These children should be treated by a multidisciplinary team that includes neurologists, neurosurgeons, urologists, and orthopedic surgeons.[200]

Prune-Belly Syndrome

Prune-belly syndrome, also known as *Eagle–Barrett syndrome*, is a rare defect occurring in about 1 in 40,000 births, 95% of them male (Fig. 76.9). This condition is usually characterized by the triad of abdominal wall deficiency, cryptorchidism, and dilatation of the urinary tract, including severe hydroureteronephrosis, bladder distension, and urethral dilatation.[201]

Prognosis ranges from death in utero to near normal life expectancy. Management of the disorder depends on the severity of the symptoms. Patients with incomplete bladder emptying and recurrent UTIs may require a vesicostomy. All male children will have to undergo bilateral orchiopexy before the age of 1 year. Some children will require urinary tract reconstruction or reconstruction of the abdominal wall, and up to 40% will develop chronic kidney disease.[202]

VACTERL Association

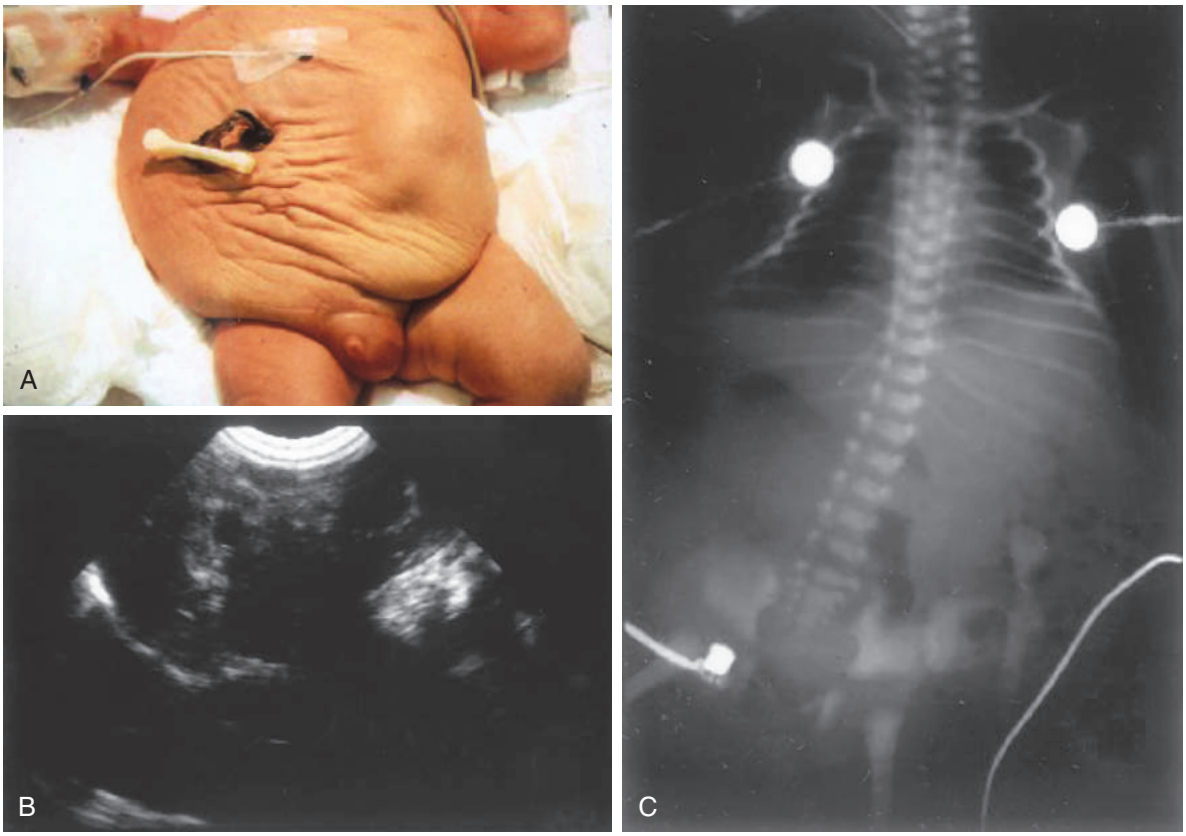
Since the 1970s, a co-occurrence of anomalies in multiple systems has been noted, and the heterogeneous group of these conditions is known as *VACTERL association* (vertebral, anorectal, cardiac, tracheal, esophageal, renal and/or radial, and limb).[203] Most clinicians agree that this condition is present if at least three systems are impacted. Urologic anomalies are frequently seen and can result in severe renal impairment if treated inadequately. The most common renal anomalies seen are hydronephrosis and VUR.[204] All children should undergo a renal and bladder ultrasound and, if the findings are abnormal, a VCUG. Sacral x-rays and ultrasound of the spine will further detect defects of the spinal cord that may also affect bladder function and cause renal impairment.

Female Genital Anomalies

Female Genital Tract Development

The female reproductive system is formed by the müllerian or paramesonephric ducts. The process is complex and involves many genes. Mostly because of the absence of müllerian inhibiting substance (produced by the testicle), there is involution of the wolffian ducts. The müllerian ducts form lateral to the wolffian duct at around 6 to 8 weeks' gestation. The müllerian ducts then migrate medially, fuse in the midline and are incorporated into the urogenital sinus to form the uterovaginal canal by the 10th week of gestation. The vagina forms from the fused müllerian ducts and the urogenital sinus. The upper four-fifths of the vagina is müllerian derived, and the lower fifth is of urogenital sinus in origin.

The external genitalia differentiate during the 12th to 16th weeks. The genital tubercle forms the clitoris, the urethral folds become the labia minora, and the genital swellings become the labia majora.[205]



• **Fig. 76.9** The classic wrinkled abdominal wall seen in prune-belly syndrome is accompanied by bilateral undescended testes (A). Affected patients will have marked hydronephrosis. In severe cases as illustrated here, these small kidneys may have a markedly dysmorphic sonographic appearance (B), and renal insufficiency may be present from the beginning. In cases with severe renal insufficiency, pulmonary development may be compromised, as evident on the radiograph (C); the patient required prolonged mechanical ventilation in the neonatal period.

Hydrocolpos and Hydrometrocolpos

Congenital hydrocolpos is an uncommon disorder characterized by vaginal distension with fluid. Hydrometrocolpos is associated with accumulation of fluid in both the vagina and the uterus. It is believed to be due to increased secretion by cervical mucous glands secondary to maternal hormone stimulation that expands and builds up into a pelvic mass because of vaginal outlet obstruction.[206] Hydrocolpos can be associated with genitourinary anomalies such as persistent urogenital sinus and cloacal anomalies (Fig. 76.10).[207] Vaginal atresia can be associated with several syndromes (e.g., McKusick-Kaufman syndrome and Bardet-Biedl syndrome).

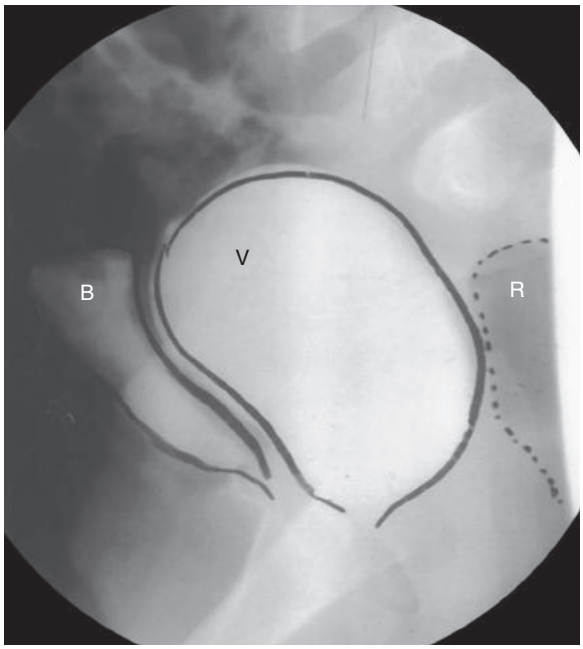
The most common complication of hydrocolpos is compression of the bladder, leading to urinary retention and hydronephrosis, which can ultimately cause kidney damage. This can be prevented by drainage of the accumulated fluid.[208] If the hydrocolpos is secondary to an imperforate hymen, incision with drainage is performed. If, on the other hand, the hydrocolpos is due to vaginal atresia or cloacal anomaly, drainage of the vagina can be accomplished either by clean intermitted catheterization or a transabdominal vaginostomy performed by an interventional radiologist under ultrasound guidance, which enables real-time evaluation without radiation exposure. The transabdominal drainage of hydrocolpos with an indwelling tube is more preferred than transvaginal drainage to prevent reaccumulation. In general, infants with hydrocolpos and urogenital sinus have increased risk of sepsis due to collection of urine in the vaginal vault. There have been reported deaths due to sepsis associated with hydrocolpos.

Vaginal Agenesis

Vaginal agenesis (Mayer-Rokitansky-Küster-Hauser syndrome) occurs in 1 in 5000 live female births and is due to failure in canalization of the vaginal plate.[209] Most of these patients present later in life with amenorrhea with normal secondary female sex features. The ovaries and external genitalia are normal, and the uterus may be rudimentary. Type I involves isolated ureterovaginal differences, while type II includes associated anomalies, including renal, skeletal, ear, or cardiac malformations. These have been termed *MURCS* (müllerian duct aplasia, renal aplasia, and cervicothoracic somite malformations).[210] Surgical correction is often delayed until the patient is mature enough to participate in postoperative care and individualized on the basis of the extent of atresia, development of the uterine and vaginal remnants, and goals of the patient.[211]

Cloacal Anomalies and Urogenital Sinus

A cloacal anomaly is defined when the urogenital sinus is combined with an anorectal malformation. A common urogenital



• **Fig. 76.10** This genitogram, performed by retrograde injection of contrast medium into a single sinus anterior to the patient's rectum, demonstrates the anteriorly placed bladder *(B)* and posteriorly placed vagina *(V)*. The vagina and urethra merged into a common sinus, which then traveled a distance of 2 cm before emerging on the perineal body. The rectum *(R)* was normally placed. The vagina distended as a result of urinary entrapment, and the patient presented with a lower abdominal mass.

sinus is a normal part of development of the fetus.[212] If müllerian duct development stops during the first trimester, a common urogenital sinus will persist at birth. The confluence of the vagina into the common channel varies and depends on when the development arrest occurred. The earlier the arrest, the higher the connection of the vagina will be. The anus will be normally located but sometimes is anteriorly displaced. On examination, only one opening is found in the introitus, and there is a second, anal opening.

Sometimes the vagina is distended, with urine causing compression of the ureters and hydronephrosis. This can be easily managed with initiation of intermittent catheterization, which will decompress the distended vagina.[208]

Evaluation includes an examination of the patient under anesthesia and determination of the length of the common channel and the length of the urethra from the bladder neck to the common channel. A cloacogram with three-dimensional CT reconstruction will also provide details of the anatomy, which aids in surgical planning.[213] Surgical repair is usually undertaken during the first year of life, and the approach for reconstruction depends on the location of the confluence of the vagina into the urogenital sinus.[214]

Infants with cloacal anomaly have only one opening into the perineum and confluence of the vagina, rectum, and urethra into a common channel.[215] The incidence is 1 in 35,000 to 50,000.[212] These children require a colostomy for stool divergence, and at the same time an examination under anesthesia can be performed to determine the extent of the common channel and the length of the urethra and vaginal channels. The reconstruction is usually performed at around 6 to 8 months of age or when the child is nutritionally stable. Multiple teams are involved in the reconstruction, including pediatric general surgery, pediatric urology, and pediatric gynecology teams.

Müllerian Duplication Anomalies

If fusion of the müllerian duct is incomplete, duplication anomalies occur. This can range from a septate vagina to vaginal duplication, where one or both vaginas are open to the perineum. These children may have a uterus that is partially fused (bicornuate uterus) to complete duplication with two cervices (uterus didelphys).[216]

Introital Masses in Children

These are seen on physical examination of the infant, and the differential diagnosis includes:

- Imperforate hymen
- Prolapsed ureterocele
- Urethra prolapse
- Skene or Gartner duct cysts
- Rhabdomyosarcoma[217]

Suggested Readings

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77

Acute Kidney Injury

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KEY POINTS

- Acute kidney injury (AKI) is common in critically ill neonates. AKI affects survival, hospital expenditures, and long-term outcomes, independent of the severity of illness and comorbidities.
- Kidney development continues until 34 weeks' gestation. Neonatal intensive care unit graduates, especially those with a history of AKI, those born prematurely, and those with intrauterine growth retardation, are at risk for long-term chronic kidney disease.
- New technological advancements are making the use of kidney replacement therapy (dialysis) more common, safer, and effective in neonates.

Acute Kidney Injury

Acute kidney injury (AKI) is characterized by a sudden impairment in kidney function, which may result in dysregulation of fluid balance, acid-base balance, electrolytes, and build-up of nitrogenous waste products. The term *injury* highlights the spectrum of organ injury and differentiates a damaged organ from an organ that has dysfunction to an organ that has failed. AKI is incrementally staged based on severity by oliguria or rise in serum creatinine (SCr).

The development and utilization of standardized definitions of AKI have created a commonality in defining AKI. Incremental degrees of AKI independently impact survival in critically ill neonates,[1] children,[2] and adults.[3] All three of the above multicenter cohort studies used the Kidney Disease: Improving Global Outcomes (KDIGO) AKI definition. In neonates, a modified version of this definition (Table 77.1) is the consensus, standard definition. This classification system utilizes the lowest SCr as a baseline and subsequent rise in SCr values to stage kidney injury. In April 2013, neonatologists and pediatric nephrologists participating in the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) workshop carefully scrutinized this definition and concluded that, although there are limitations inherent to utilizing SCr, the modified, neonatal KDIGO definition represents the current consensus definition of neonatal AKI for research purposes and clinical care.[4] This group also pointed out that this definition represents the first step in an iterative process to better define AKI, pointing to the need for neonatal-specific evidence to guide future definitions.

A critical component to understanding the shortcomings of the current AKI definitions is to recognize the limitations of SCr as a biomarker. Importantly, SCr is a marker of kidney function and detects damage that has occurred in the preceding 48 to 72 hours. Thus, the current AKI definitions utilizing SCr do not detect kidney damage; instead, they document changes in kidney function.[5] Table 77.2 details the limitations inherent to utilizing SCr.

Over the last decade, there has been a significant amount of work to identify urine and serum biomarkers of AKI. Ideally, novel AKI biomarkers will show acute damage hours after an insult, distinguish between different causes and locations of tissue injury, and prognosticate clinical outcomes. Potential biomarkers include cystatin C, urine and serum neutrophil gelatinase-associated lipocalin (NGAL), urine interleukin-18, kidney injury marker-1, copeptin, and liver fatty acid-binding protein among others.[6] Many of these were originally identified and studied in neonates undergoing cardiopulmonary bypass[7,8] and now are being studied in neonates in the neonatal intensive care unit (NICU) (Fig. 77.1). These serum and urine biomarkers notably vary based on gestational age (GA), day of life, and gender[9,10] but show promise in their ability to predict AKI in preterm infants,[11–13] very low birth weight (VLBW, i.e., birth weight <1,500 g) infants,[14,15] near-term/term neonates,[16,17] and neonates with perinatal asphyxia.[18,19] Future work is needed to determine how best these biomarkers can be used at the bedside.

Epidemiology

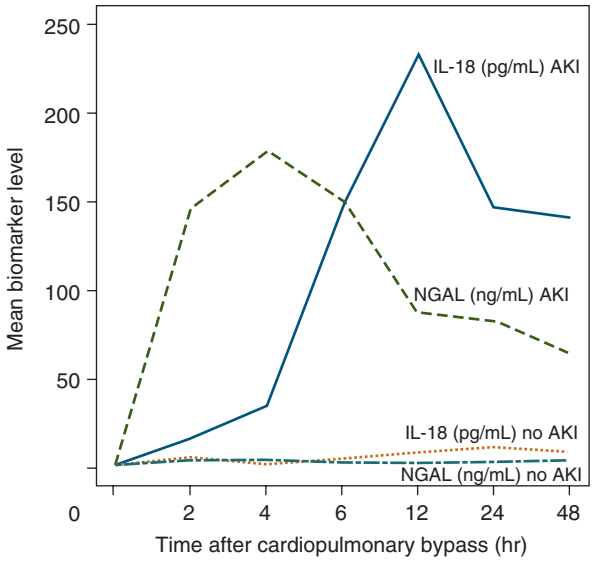
Over the past decade, there has been a significant amount of research utilizing modern staged definitions of AKI to evaluate the incidence and impact of AKI in the NICU. In the largest epidemiologic study to date, the Assessment of Worldwide Acute Kidney Injury Epidemiology in Neonates (AWAKEN) study, more than 2000 infants from 26 NICUs and 4 countries were enrolled allowing investigators to compare AKI incidences and associated outcomes across the GA spectrum in a multicenter, multinational cohort for the first time.[1] Using this general NICU cohort, AWAKEN investigators demonstrated that 30% of neonates admitted to the NICU develop AKI and that the incidence of AKI varied across GA groups. Importantly, they also demonstrated that neonatal AKI was independently associated with both morbidity and mortality.

In the NICU there are a number of high-risk patient sub-populations that warrant separate discussion, including neonates with perinatal asphyxia, those undergoing cardiac surgery or receiving extracorporeal membrane oxygen (ECMO), and VLBW and extremely low birth weight (ELBW, i.e., birth weight <1,000 g) infants (Table 77.3).

TABLE 77.1 Modified Neonatal, Kidney Disease: Improving Global Outcomes Definition and Classification of Neonatal Acute Kidney Injury

Stage	Serum Creatinine	Urine Output
0	No change in SCr *or* rise < 0.3 mg/dL	≥0.5 mL/kg/h
1	SCr rise ≥0.3 mg/dL rise within 48 hrs *or* SCr rise ≥1.5–1.9 × baseline SCr[a]	<0.5 mL/kg/h for 6–12 hr
2	SCr rise ≥2.0–2.9 × baseline SCr	<0.5 mL/kg/h for ≥12 hr
3	SCr rise ≥3 × baseline SCra *or* SCr ≥2.5 mg/dL[b] *or* Receipt of dialysis	≤0.3 mL/kg/h for ≥24 hr *or* Anuria for ≥12 hr

[a]Baseline SCr defined as lowest previous SCr value.
[b]SCr value of 2.5 mg/dL represents glomerular filtration rate of <10 mL/min/1.73 m^2.
SCr, Serum creatinine.
Adapted from Kidney Disease: Improving Global Outcomes (KDIGO) Acute Kidney Injury Workgroup. KDIGO clinical practice guideline for acute kidney injury. *Kidney Int Suppl.* 2012;2:1–138.



• **Fig. 77.1** Mean values of urine interleukin-18 (pg/mL) and neutrophil gelatinase-associated lipocalin (ng/mL) over the first hours after cardiopulmonary bypass in infants who develop acute kidney injury (50% increase in serum creatinine) compared with those who did not develop acute kidney injury. *AKI*, Acute kidney injury; *IL-18*, interleukin-18; *NGAL*, neutrophil gelatinase-associated lipocalin.

TABLE 77.2 Acute Kidney Injury Definitions: Limitations Inherent to Serum Creatinine

- Up to 50% kidney function is lost prior to SCr change
- It may take 48–72 hr for SCr to rise after an insult
- At a low GFR, SCr will overestimate renal function[a]
- Different measurement methods (Jaffee reaction vs. enzymatic) produce different SCr values
- Bilirubin can alter SCr Jaffee-based measurement
- SCr varies by muscle mass, diet, hydration, sex, age, gender
- Nephrogenesis continues until 34 weeks of gestation
- Dependent on the degree of prematurity, GFR improves steadily after birth:
  - To 10–20 mL/min/1.73 m^2 during the first week of life
  - To 30–40 mL/min/1.73 m^2 by 2 weeks of life
- Neonatal SCr values in the first days of life reflect maternal SCr values (Fig. 77.3)
- Unique, absolute SCr rise threshold values for different gestational ages improve the prediction of mortality.

[a]Due to tubular secretion.
AKI, Acute kidney injury; *GFR*, glomerular filtration rate; *SCr*, serum creatinine.
From Brion LP, Fleischman AR, McCarton C, et al. A simple estimate of glomerular filtration rate in low birth weight infants during the first year of life: noninvasive assessment of body composition and growth. *J Pediatr.* 1986;109(4):698–707; Rajs G, Mayer M. Oxidation markedly reduces bilirubin interference in the Jaffe creatinine assay. *Clin Chem.* 1992;38(12):2411–2413; Lolekha PH, Jaruthunyaluck S, Srisawasdi P. Deproteinization of serum: another best approach to eliminate all forms of bilirubin interference on serum creatinine by the kinetic Jaffe reaction. *J Clin Lab Anal.* 2001;15(3):116–121; Mian AN, Schwartz GJ. Measurement and estimation of glomerular filtration rate in children. *Adv Chronic Kidney Dis.* 2017;24(6):348–356; Abrahamson DR. Glomerulogenesis in the developing kidney. *Semin Nephrol.* 1991;11(4):375–389; Gallini F, Maggio L, Romagnoli C, et al. Progression of renal function in preterm neonates with gestational age < or = 32 weeks. *Pediatr Nephrol.* 2000;15(1–2):119–124; Askenazi D, Abitbol C, Boohaker L, et al. Optimizing the AKI definition during first postnatal week using Assessment of Worldwide Acute Kidney Injury Epidemiology in Neonates (AWAKEN) cohort. *Pediatr Res.* 2019;85:329–338.

Neonates With Perinatal Asphyxia

Infants with perinatal asphyxia represent a population at high risk for the development of AKI. Selewski et al. (2013) evaluated 96 newborns undergoing therapeutic hypothermia for perinatal asphyxia and found that 38% developed AKI.[20] In this cohort, AKI was associated with adverse outcomes, including prolonged mechanical ventilation by a mean of 4 days ($P < .01$) and prolonged hospitalization by 3.4 days ($P < .03$). In the same cohort, those with AKI were more likely to have abnormal brain magnetic resonance imaging (MRI) findings at 7 to 10 days of life, implicating AKI as a potential marker and/or mediator of poor neurologic outcomes.[21] Recently, in a randomized controlled trial (RCT) of 120 term neonates with perinatal asphyxia, those randomized to therapeutic hypothermia had lower rates of AKI (32% vs. 60%, $P < .05$), suggesting therapeutic hypothermia may protect against the development of AKI.[22]

When examining infants greater than 34 weeks of gestation with perinatal asphyxia within the AWAKEN cohort, Kirkley et al. (2019) found that 42% (47/113) developed AKI and that those with perinatal asphyxia and AKI remained in the hospital with an average of 8.5 days longer than those with asphyxia but no AKI.[23] More recently, Cavallin et al. (2020) demonstrated that AKI was associated with an increased likelihood of unfavorable neurodevelopmental outcomes at 24 months (100% vs. 59% in neonates without AKI; $P = .01$).[24]

Neonates Undergoing Cardiac Pulmonary Bypass Surgery

Several factors contribute to the risk of postoperative AKI in neonates undergoing cardiac pulmonary bypass (CPB) surgery, including prematurity, CPB characteristics and duration, surgical complexity, perioperative morbidities, hypotension, deep hypothermic circulatory arrest, and hypoxia.[25] Reported rates of post-CPB AKI in neonates range from 45% to 64%.[25–28] In this population, AKI is consistently found to be independently

TABLE 77.3 Neonatal Acute Kidney Injury Studies

Population	Study	Definition	Incidence of AKI	Findings
General NICU Population	Jetton et al. 2017 ($n = 2162$)	Neonatal modified KDIGO criteria	29.9%	AKI varies by gestational age • 22 to <29 WGA: 48% • 29 to <36 WGA: 18% • ≥36 WGA: 37% AKI is associated with higher mortality and longer length of hospital stay.
Perinatal Asphyxia/HIE	Selewski et al. 2013 ($n = 96$)	Neonatal modified KDIGO criteria	38%	AKI predicted prolonged mechanical ventilation, length of stay, and abnormal brain MRI findings at 7–10 days of life.
Cardiopulmonary Bypass Surgery	Alabbas et al. 2013 ($n = 122$)	AKIN criteria	62%	Severe AKI (stage III) was associated with increased mortality and length of stay after adjusting for severity of illness.
ECMO	Murphy et al. 2021 ($n = 446$)	KDIGO criteria	51%	AKI most common in those with cardiac disease but varies by diagnostic cause for ECMO. Risk of mortality differed by diagnostic category in the presence or absence of AKI; without AKI, CDH independently predicts mortality.
	Zwiers et al. 2013 ($n = 242$)	RIFLE criteria	64%	Increased risk of mortality at highest level of AKI (failure).
VLBW Infants	Rhone et al. 2014 ($n = 107$)	Neonatal modified KDIGO criteria	26.2%	AKI is associated with nephrotoxic medication exposure.
	Koralkar et al. 2011 (n = 229)	Neonatal modified KDIGO criteria	18%	Adjusting for severity of illness AKI was associated with increased mortality.
	Askenazi et al. 2009 ($n = 195$)	AKIN criteria	Matched case-control study	AKI is associated with increased mortality after adjustment for confounders.

AKI, Acute kidney injury; *AKIN*, acute kidney injury network; *CDH*, congenital diaphragmatic hernia; *ECMO*, extracorporeal membrane oxygenation; *HIE*, hypoxic-ischemic encephalopathy; *KDIGO*, Kidney Disease Improving Global Outcomes; *MRI*, magnetic resonance imaging; *NICU*, neonatal intensive care unit; *RIFLE*, risk, injury, failure, loss, end-stage; *VLBW*, very low birth weight; *WGA*, weeks' gestational age.

associated with morbidity, including prolonged length of intubation, postoperative ventilation days, ICU stay, and hospitalization,[26–28] as well as with the increased risk of mortality.[25,26]

Neonates Requiring Extracorporeal Membrane Oxygenation

Neonates on ECMO are predisposed to AKI for a number of reasons, including those inherent to their underlying critical illness (sepsis, ischemia, respiratory failure, cardiac failure, hypotension, nephrotoxic medications) and elements associated with ECMO (hemodynamic fluctuations, hemolysis, systemic inflammation). Several early studies of infants and children who received ECMO suggest AKI is associated with mortality.[29–33] In a retrospective cohort study of 7941 neonates in the Extracorporeal Life Support Organization (ELSO) registry, where AKI was defined as infants who had an SCr greater than 1.5 mg/dL or an ICD-9 code for acute renal failure, neonatal mortality was 2175/7941 (27.4%).[34] Nonsurvivors experienced more AKI than survivors (413/2175 [19.0%] vs. 225/5766 [3.9%]; $P < .01$), and more received renal replacement therapy (RRT, also known as kidney support therapy [KST]) (863/2175 [39.7%] vs. 923/5766 [16.0%]; $P < .01$). After adjusting for confounding variables, the adjusted odds ratio (OR) for mortality was 3.2 ($P < .01$) following AKI. Zwiers et al. (2013) evaluated AKI in 242 neonates on ECMO, reporting an AKI incidence of 64% and a mortality of 65% when AKI progressed to the highest stage.[35]

More recently, the multidisciplinary, international Kidney Intervention during Extracorporeal Membrane Oxygenation (KIDMO) study group has produced several manuscripts investigating the epidemiology and impact of AKI, fluid overload, and RRT utilization in neonates and pediatric patients receiving ECMO. Utilizing the KDIGO criteria, KIDMO investigators found that 66% of neonates receiving ECMO develop AKI.[36] In their cohort, AKI occurred by 48 hours of ECMO support in 93% of cases and was independently associated with increased duration of ECMO and increased odds of in-hospital mortality. When examining only neonatal patients within the cohort, they found that neonates receiving ECMO for cardiac diagnoses experienced higher rates of AKI than those cannulated for respiratory indications or congenital diaphragmatic hernia (CDH; cardiac 68% vs. respiratory 33% vs. CDH 38%; $P < .01$), and an interaction suggested that risk of mortality differed by diagnosis in the presence or absence of AKI in neonates receiving ECMO; in the absence of AKI, CDH independently predicted mortality while fluid overload and RRT receipt both independently predicted mortality regardless of the underlying diagnosis.[37] These findings suggest that physiologically distinct ECMO diagnoses warrant individualized treatment strategies.

Very Low Birth Weight and Extremely Low Birth Weight Neonates

There are now multiple single-center studies describing the epidemiology of AKI in VLBW and extremely low birth weight (ELBW) neonates with particular attention being paid to premature neonates. Rates of AKI in this population range from 12.5% to 56% and AKI is independently associated with increased morbidity and mortality.[38–43] In a recent 2020 study of an overlapping population, extremely low gestational age neonates (ELGANs), Askenazi et al. reported AKI occurred at least once in 38% of their cohort, and severe AKI, defined as KDIGO stage 2 or higher AKI, occurred at least once in 18.2%.[44]

Several studies examining the association between common morbidities of prematurity, including bronchopulmonary dysplasia (BPD) and necrotizing enterocolitis (NEC), and AKI have recently been published. One of the most common morbidities of prematurity is BPD, affecting 10% and 40% of surviving VLBW and ELBW infants, respectively.[45] In 2015, Askenazi et al. showed an association between AKI and BPD in premature infants; neonatal AKI was independently associated with a higher risk of oxygen requirement/death at 28 days old (RR 1.45, 95% CI 1.07 to 1.97; $P < .02$).[46] In the AWAKEN cohort, neonates born between 29 and 32 weeks of gestation who developed AKI had a higher likelihood of moderate or severe BPD/death than those without AKI after controlling for multiple factors (adjusted OR 4.21, 95% CI 2.07 to 8.61, $P < .01$).[47]

An association between AKI and NEC has also been reported. NEC is an inflammatory disease of the intestines that typically affects VLBW and ELBW infants and significantly increases the risk of mortality.[48] Bakhoum et al. (2018) studied a population of premature infants with NEC and found that AKI occurred in 42.9% of infants (Stage 1 NEC: 18.2%, Stage 2 NEC: 13.0%, Stage 3 NEC: 11.7%) and was independently associated with mortality (adjusted hazard ratio (HR) 20.3, 95% CI 2.5 to 162.8; $P < .01$).[49] Criss et al. (2018) found AKI in 54% (98/181) of neonates with NEC and reported that those with AKI experienced higher mortality (44% vs. 25.6%, $P < .01$) and a higher chance of death (HR 2.4, CI 1.2 to 4.8, $P < .01$).[50] When examining risk factors for and outcomes of severe AKI (i.e., stage 2 and 3 AKI), Garg et al. (2021) found severe AKI in 33% (66/202) neonates after NEC and in 58.7% (61/104) neonates after surgical NEC.[51] They also reported severe AKI was associated with significantly longer hospitalization (124 days [IQR 88 to 187] vs. 82 days [IQR 42 to 125]; $P < .01$).

Pathophysiology

Prerenal Azotemia

Prerenal azotemia occurs in response to decreased kidney blood flow (RBF). Causes of prerenal azotemia in neonates include loss of effective circulating blood volume, dehydration, capillary leak, increased abdominal pressures, and decreased cardiac output.[52] Nonsteroidal anti-inflammatory drugs (NSAIDs) such as indomethacin and angiotensin-converting enzyme inhibitors (ACE-Is) can also decrease RBF (Table 77.4).

TABLE 77.4 Causes of Acute Kidney Injury in the Newborn

Prenatal Azotemia	Intrinsic Acute Kidney Injury	Postrenal (Obstructive) Renal Failure

• Loss of effective blood volume
  • Perinatal blood loss
  • Hemorrhage
  • Dehydration
  • Diarrhea
  • Transepidermal free water losses
  • Poor intake
  • Gastric losses
  • Chest tube losses
• Capillary leak
  • Hydrops fetalis
  • Infection
  • Hypoalbuminemia
• Increased abdominal pressures leading to renal hypoperfusion
  • NEC
  • Repair or reduction of gastroschisis
  • Omphalocele
  • CDH
  • Ascites
• Decreased cardiac output
  • Sepsis
  • Cardiac surgery
  • CHF
  • ECMO
• Pharmacologic agents
  • NSAIDs such as indomethacin
  • ACE inhibitors
	• ATN
	• Severe renal ischemia
	• Nephrotoxic medications
	  • NSAIDs
	  • Amphotericin B
	  • Aminoglycosides
	  • Acyclovir
	  • Radiocontrast dyes
	• Sepsis and infections
	  • Congenital infections
	  • Pyelonephritis
	  • Bacterial endocarditis
	• Renal vascular causes
	  • RVT
	  • RAT
	• Intrarenal obstruction
	  • Uric acid nephropathy
	  • Hemoglobinuria
	  • Myoglobinuria
	• Congenital malformations
	  • Bilateral renal agenesis
	  • Renal dysplasia
	  • Polycystic kidneys
		• Congenital malformations
		  • Imperforate prepuce
		  • Urethral stricture
		  • Eagle-Barrett syndrome (i.e., prune belly syndrome)
		  • PUV
		  • Urethral diverticulum
		  • Ureterocele
		  • Megaureter
		  • UPJ obstruction
		• Neurogenic bladder
		• Extrinsic compression
		  • Hematocolpos
		  • Sacrococcygeal teratoma
		• Intrinsic obstruction
		  • Renal calculi
		  • Fungus balls

ACE, Angiotensin-converting enzyme; *ATN*, acute tubular necrosis; *CDH*, congenital diaphragmatic hernia; *CHF*, congestive heart failure; *ECMO*, extracorporeal membrane oxygenation; *NEC*, necrotizing enterocolitis; *NSAID*, nonsteroidal anti-inflammatory drug; *PUV*, posterior urethral valves; *RAT*, renal artery thrombosis; *RVT*, renal vein thrombosis; *UPJ*, ureteropelvic junction.

When low RBF occurs, kidney autoregulation preserves glomerular filtration rate (GFR) by increasing kidney sympathetic tone, activating the renin-angiotensin–aldosterone system, and increasing activation of hormones such as vasopressin and endothelin. An increase in filtration fraction (GFR/RBF × 100) increases peritubular oncotic pressure, resulting in enhanced proximal tubular sodium and water reabsorption.[53] These hemodynamic changes lead to a decrease in water and sodium losses which helps to maintain systemic volume expansion. In some newborns, anticipated oliguria does not develop because of poor vasopressin secretion and/or weak kidney responsiveness to vasopressin,[54] immature/poor tubular function, or prolonged/severe hypoperfusion. In prolonged states of kidney hypoperfusion, parenchymal damage occurs, and a pre-renal state may transition to kidney tubular cell damage (acute tubular necrosis, ATN).

Intrinsic Acute Kidney Injury

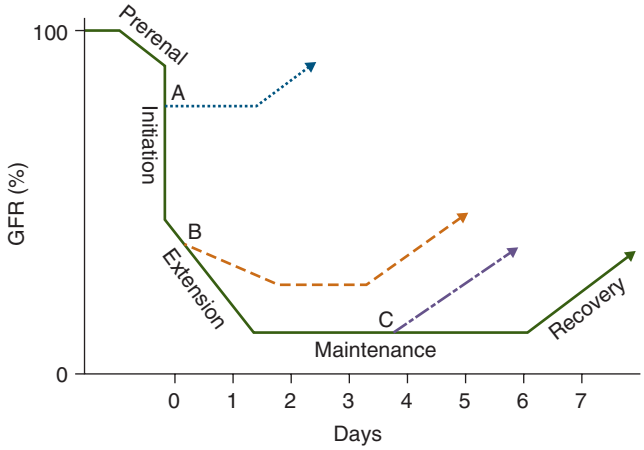
Prolonged or severe hypoperfusion leading to ischemic AKI and ATN is the most common cause of intrinsic AKI (see below). Other causes of intrinsic AKI in neonates include nephrotoxic medications and sepsis. Other rare causes of AKI include renal vein thrombosis (RVT), renal artery thrombosis (RAT), uric acid nephropathy, hemoglobinuria, myoglobinuria, and congenital malformations (Table 77.4).

Ischemic Acute Kidney Injury

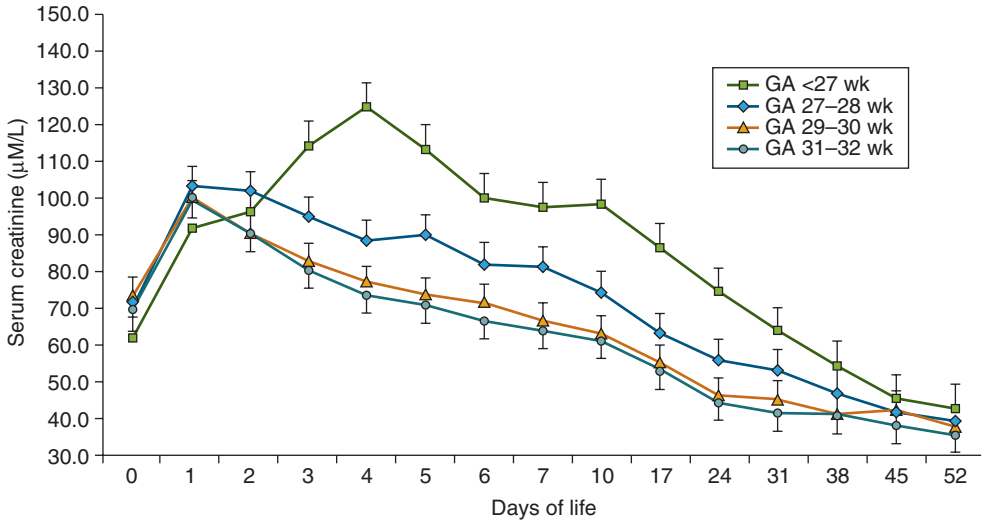
The presentation and course of the kidney damage after hypoxic-ischemic injury depends on the severity and duration of the insult. In contrast to prerenal azotemia, kidney function abnormalities in ischemic AKI are not immediately reversible. In states of prolonged hypoperfusion, kidney parenchymal damage develops, particularly to the tubular epithelium of the terminal medullary portion of the proximal tubule (S3 segment) and of the medullary portion of the thick ascending limb of the loop of Henle. The severity of intrinsic AKI ranges from mild tubular dysfunction to ATN, to kidney infarction and corticomedullary necrosis with irreversible kidney damage.[53]

The course of ischemic AKI may be subdivided into the prerenal, initiation, extension, maintenance, and recovery phases (Fig. 77.2).[55] If, during prerenal azotemia, restoration of RBF occurs, GFR can normalize. The initiation phase includes the original insult and the associated events resulting in a drop in GFR. The extension phase includes alterations in kidney perfusion, continued hypoxia, and inflammatory insults to the kidney's microvasculature. Tubular dysfunction with low GFR represents the maintenance phase. The duration of the maintenance phase depends, at least in part, on the severity and duration of the initial insult. The recovery phase is characterized by the gradual restoration of GFR and tubular functions, which may take months to occur. During the maintenance and recovery phases of ischemic AKI, the kidney is susceptible to further damage from additional insults. Recognition of the different phases of intrinsic, ischemic AKI is helpful in the diagnosis, clinical management, and prognostication of the disorder.

The histologic hallmark of severe ischemic AKI is damage to epithelial tubular cells with characteristic bleb formation, loss of brush border in the apical portion of the cell cytoskeleton, and loss of tight junctions between cells. If an injury is severe enough, apoptosis and necrosis will occur with the resultant desquamation of cells, which lead to tubular obstruction. Not only are tubular epithelial cells critical in the pathophysiology of ischemic AKI, but damage to the innermost lining of the kidney vascular system, the endothelial cells, has a critical role in the initiation, extension, maintenance, and recovery phases of ischemic AKI.[56]



• **Fig. 77.2** Stages of progression in acute kidney injury. *GFR*, Glomerular filtration rate.



• **Fig. 77.3** Serum creatinine concentrations (μM/L) during the first days of life, with values given as means and standard error for infants born at different gestational ages. *GA*, Gestational age; *wk*, weeks.

When endothelial cell damage occurs, activation of vasoconstriction, impaired vasodilation, and impaired leukocyte adhesion result in capillary obstruction and distorted peritubular capillary morphology and a cycle of increasing ischemia and vascular inflammation. The loss of endothelial cell function may represent an important therapeutic target in which vascular support and/or endothelial regeneration by progenitor cells may impact the short- and long-term consequences of AKI.[57]

Damaged endothelial and tubular cells not only lead to dysfunction within the kidney, but they produce a systemic inflammatory response. The inflammatory dysregulation is due (at least in part) to dysfunctional immune, inflammatory, and soluble mediator metabolism. AKI also has been shown to directly affect the brain, lung, heart, liver, bone marrow, and gastrointestinal tract.[58]

Nephrotoxic Acute Kidney Injury

Exposure to nephrotoxic medications is a potentially modifiable risk factor for intrinsic AKI. Table 77.5 lists commonly used nephrotoxic medications in the NICU and their mechanisms of nephrotoxicity. Until recently the epidemiology and burden of nephrotoxic medication exposure in neonates were unknown. Rhone et al. (2014) evaluated the cumulative nephrotoxic medication exposure of a cohort of 107 VLBW neonates.[59] In this study, 87% of the cohort was exposed to at least one nephrotoxic medication, and on average these neonates were exposed to over 14 days of nephrotoxic medications. In 2017, Barhight et al. reported that 84% of VLBW neonates in their single-center cohort received nephrotoxic medications, and antibiotics were the most common of these (80%).[60] In their regression modeling, these investigators found birthweight (OR 0.995, 95% CI: 0.991 to 0.998; $P = .004$) and number of nephrotoxic medication exposures (OR 1.83, 95% CI 1.33 to 2.53; $P = .0002$) were predictive of AKI.

TABLE 77.5 Nephrotoxic Medications and Mechanisms

Drug	Mechanism
Acyclovir	Urinary precipitation, especially with low flow and hypovolemia, with renal tubular obstruction and damage and decreased GFR • May cause direct tubular toxicity (metabolites)
Angiotensin-converting enzyme (ACE) inhibitors	Decreased angiotensin II production inhibiting compensatory constriction of the efferent arteriole to maintain GFR
Aminoglycosides	Toxic to the proximal tubules (transport in the tubule, accumulate in lysosome, intracellular rise in reactive oxygen species and phospholipidosis, cell death); intrarenal vasoconstriction and local glomerular/mesangial cell contraction
Amphotericin B	Distal tubular toxicity, vasoconstriction, and decreased GFR
NSAIDs	Decreased afferent arteriole dilatation as a result of inhibiting prostaglandin production resulting in reduced GFR
Radiocontrast agents	Renal tubular toxicity secondary to increase in reactive oxygen species; intrarenal vasoconstriction may play a role
Vancomycin	Mechanism of AKI unclear, possible mechanism includes proximal tubular injury with generation of reactive oxygen species

AKI, Acute kidney injury; *GFR*, glomerular filtration rate; *NSAID*, nonsteroidal antiinflammatory drug.

In the NICU, nephrotoxins can cause AKI by decreasing kidney perfusion, direct tubular injury, interstitial nephritis, and tubular obstruction. Although not a comprehensive review, some of the most common nephrotoxic medications in neonates are described below.

Indomethacin, a prostaglandin inhibitor used to treat patent ductus arteriosus (PDA) in premature infants, is a commonly used medication in the NICU. The potentiation of the vasoconstrictive and sodium- and water-retaining effects of angiotensin II, norepinephrine, and vasopressin by the indomethacin-induced inhibition of kidney prostaglandin production is the primary mechanism of the kidney actions of the drug which is accentuated by the dependence of immature neonatal regulatory mechanisms of RBF on prostaglandins. Ibuprofen, another prostaglandin inhibitor used to treat PDA in the NICU, has been shown to be equally efficacious in achieving PDA closure as indomethacin but with a reduced risk of kidney injury.[61]

Amphotericin B alters kidney function by directly affecting tubular function, resulting in renal tubular acidosis and increased urinary potassium excretion. Although these nephrotoxic effects are somewhat rare and most often reversible, cases of fatal neonatal AKI caused by amphotericin B toxicity have been reported.[62] A liposomal formulation of amphotericin B is available which has a higher affinity to fungal rather than mammalian cellular membranes and therefore is likely less nephrotoxic.[63,64]

Aminoglycosides inhibit lysosomal phospholipases, leading to primary proximal tubule cell damage,[65] although changes in the ultrastructure of the glomerulus also occur.[66] Aminoglycosides should be used with caution in any person with kidney dysfunction, concomitant nephrotoxic medication use, or poor kidney perfusion. Unfortunately, aminoglycosides are used concurrently with other nephrotoxins such as NSAIDs frequently in the NICU. In a single center study, neonates receiving concurrent aminoglycosides and NSAIDs experienced more AKI than infants receiving aminoglycosides alone (14.8% vs. 9.1%; RR 1.6, 95% CI 1.0 to 2.6), and the attributable risk of NSAID use was 5.7% (95% CI 0.5 to 11.0).[67] In those with kidney dysfunction, serial monitoring to assure proper clearance of the medication is needed to prevent AKI. Because aminoglycoside toxicity is usually nonoliguric, serial monitoring of SCr values is necessary, especially during prolonged administration of these antibiotics, to detect their potential nephrotoxicity in the newborn.

Acyclovir is used to treat neonatal herpes simplex virus infections. Acyclovir is an antiviral agent that is eliminated rapidly in the urine through glomerular filtration and tubular secretion. It is nearly insoluble in the urine and may precipitate, particularly in the distal tubular lumen. Intravenous high-dose acyclovir treatment may lead to intratubular crystal precipitation and AKI. Acyclovir-related nephrotoxicity can be limited by maneuvers to prevent crystallization including avoiding use in those with AKI or intravascular volume depletion, prolonged infusion (over several hours), and by maintaining a high urinary flow rate.[68]

Postrenal Acute Kidney Injury

The most common causes of postrenal or obstructive kidney dysfunction in the newborn are congenital malformations, including imperforate prepuce, urethral stricture, prune belly syndrome,

and posterior urethral valves. Other causes of acute obstruction include neurogenic bladder, extrinsic compression (e.g., hematocolpos, sacrococcygeal teratoma), and intrinsic obstruction from kidney calculi or fungal balls. Depending on the cause and associated damage to the kidneys, relief of the obstruction will markedly improve kidney function.

Evaluation and Management

There are three main goals in the care of neonates with AKI. The first is to understand the cause of the problem. The second is to take steps to intervene to prevent further deterioration in kidney function. The third is to manage the homeostatic functions that the kidney plays as it relates to electrolytes, fluids, acid/base balance, and waste product removal. If medical interventions and adjustments to nutritional contents do not achieve the proper homeostasis, artificial (dialysis) kidney support may be indicated.

Step 1: Understand the Cause of Acute Kidney Injury

The pregnancy history, timing, vital signs, changes in weight, physical examination, interventions, and medications prescribed provide important clues about the cause of neonatal AKI. SCr often does not rise until days after an injury, thus monitoring these values for several days after the inciting event is necessary to determine if AKI occurred. If possible, a urinalysis, urine culture, and a spot urine sample for sodium, creatinine, and osmolality should be collected as they can assist diagnostically.

One of the major goals in the initial evaluation of neonatal AKI is to determine if the kidney is hypoperfused. Several laboratory, clinical, and therapeutic interventions can help delineate prerenal azotemia from intrinsic AKI (Table 77.6). A decrease in body weight, tachycardia, dry mucous membranes, poor skin turgor, flattened anterior fontanel, and elevation of serum sodium can be seen in those with low intravascular volumes.

When the kidney is hypoperfused, the kidney will avidly retain sodium and water to preserve intravascular volume. Laboratory markers of prerenal azotemia include low urinary sodium excretion, low fractional excretion of sodium (FENa), low kidney/renal failure index, and high blood urea nitrogen: SCr ratio. However, it is important to recognize that the preservation of urine sodium and water is dependent on intact tubular function; therefore, disturbances of tubular function (diuretic use, tubular injury, or primary tubular diseases) can affect the results of urine electrolyte values. As premature infants have poor tubular function, these studies have important limitations. Normal FENa in preterm infants born at less than 32 weeks of gestation is usually higher than 3%.[69] Additionally, because of the developmentally regulated limitation of their concentrating capacity and the effects of low protein intake and urea excretion on urine osmolality, the urine-to-plasma creatinine ratio instead of the urine-to-plasma osmolar ratio should be used in newborns to evaluate their kidney tubular reabsorptive capacity.[53]

If the suspicion of kidney hypoperfusion is high, an appropriate fluid challenge with 10 to 20 mL/kg of isotonic fluids should be given. Close observation of vital signs and urine output (UOP) may serve to delineate if intravascular hypoperfusion is present. Several boluses may be necessary with careful prescription of fluid volume for the next 24 hours. Care to avoid fluid challenges is advised in those with suspected urinary outlet obstruction, lung pathology such as BPD, or congestive heart failure. In situations where there is low oncotic pressure (serum albumin levels <2.5 mg/dL), consideration for 20% to 25% albumin infusions should be considered. Medications to ensure adequate cardiac output is present to perfuse the kidneys are sometimes required.

TABLE 77.6 Diagnostic Indices Suggestive of Prerenal Azotemia Versus Intrinsic Acute Kidney Injury in the Newborn

Diagnostic Indices	Prerenal Azotemia	Intrinsic Acute Kidney Injury
Urine flow rate (mL/kg/h)	Variable	Variable
Urine osmolality (mOsm/L)	>400	≤400
Urine-to-plasma osmolar ratio	>1.3	≤1.0
Urine-to-plasma creatinine ratio (mean ± standard deviation)	29.2 ± 1.6	9.7 ± 3.6
Urine [Na^+] (mEq/L)	10–50	30–90
FENa[a] (%, mean ± standard deviation)	<0.3 (0.9 ± 0.6)	>3.0 (4.3 ± 2.2)
Renal failure index[b] (mean ± standard deviation)	<3.0 (1.3 ± 0.8)	>3.0 (11.6 ± 9.5)
Response to fluid challenge	• Improved tachycardia • Increased UOP	No effect on tachycardia or UOP

[a]Fractional excretion of sodium (FENa) = (urine [Na^+]/serum [Na^+])/(urine [Cr]/serum [Cr]) × 100.
[b]Renal failure index = urine [Na^+]/(urine [Cr]/serum [Cr]).
Cr, Creatinine; *UOP*, urine output.
Data from Feld LG, Springate JE, Fildes RD. Acute renal failure. I. Pathophysiology and diagnosis. *J Pediatr*. 1986;109(3):401–408; Karlowicz MG, Adelman RD. Acute renal failure in the neonate. *Clin Perinatol*. 1992;19(1):139–158; Mathew OP, Jones AS, James E, et al. Neonatal renal failure: usefulness of diagnostic indices. *Pediatrics*. 1980;65(1):57–60. See text for details.

Another important part of the evaluation of AKI is to rule out obstruction to urine flow. A kidney and bladder ultrasound should be performed without delay if an obstructive process is suspected and to determine if congenital kidney abnormalities are present. If hematuria or hypertension (or both) are present, the possibility of kidney/renal vascular disease should also be considered. Doppler ultrasound of the kidney vessels can be performed if renal vascular thrombosis is suspected.

Step 2: Intervene to Preserve or Prevent Further Acute Kidney Injury

For all neonates with AKI, the provision of fluid boluses (if appropriate) as part of the evaluation of prerenal azotemia also serves as the initial management of AKI. Careful evaluation of any potentially nephrotoxic medications to determine if they are necessary, and/or if alternatives are available, is crucial. The clinician can reverse or prevent further damage by maintaining adequate perfusion to the kidney, relieving abdominal compartment syndrome if present, assuring adequate oncotic pressure (keeping a serum albumin of 2.5 mg/dL or higher), and providing interventions to eliminate

any obstruction to urinary flow (if discovered). If systemic hypotension develops despite adequate volume administration, early initiation of blood pressure support often establishes appropriate kidney perfusion.[70,71] Other management goals include the maintenance of blood oxygen content, provision of blood products (as indicated), limiting severe acidosis, and maintenance of normal serum albuminemia (at least 2.5 mg/dL preferably).

Several therapies are commonly employed in AKI and consideration of these options should be given; however, little, if any, data are available to support the use of low-dose dopamine, fenoldopam, or diuretics for the treatment or prevention of AKI in neonates. Low-dose dopamine increases kidney perfusion in sick infants, however, well-powered RCTs have failed to demonstrate an improvement in survival, length of hospitalization, or dialysis receipt in adult patients.[71–76] Fenoldopam, a selective dopamine-1 receptor agonist, leads to kidney and splanchnic vasodilation with subsequent increased RBF and GFR, but results of small, single-center studies in neonates have conflicting results[77–79]; the single prospective, placebo-controlled trial of fenoldopam in infants failed to show kidney-related benefits.[79] Diuretics are commonly used to induce diuresis in critically ill neonates; however, studies in neonates, children, and adults have failed to demonstrate that diuretics are effective in preventing AKI or improving outcomes once AKI occurs.[74] Although diuretics have failed to improve outcomes, a time-limited trial of diuretics is reasonable as multiple studies demonstrate associations between fluid overload and morbidity.[80]

In neonates with perinatal asphyxia and hypoxic ischemic encephalopathy (HIE), several RCTs have suggested a single dose of theophylline after birth may protect against AKI (Table 77.7).[81–85] During episodes of acute kidney hypoxemia and ischemia, increased kidney adenosine content leads to afferent arteriole and intra-renal vasoconstriction with subsequent decreased RBF and GFR.[86–88] These changes suggest adenosine may play a role in the pathogenesis of hypoxia-related AKI, leading investigators to hypothesize that theophylline, an adenosine receptor antagonist, may block this vasoconstriction and preserve kidney function. Based on these studies, the KDIGO AKI guidelines state "we suggest that a single dose of theophylline may be given in neonates with severe perinatal asphyxia, who are at high risk of AKI."[89] Further, large multicenter RCTs are warranted before widespread adoption of prophylactic theophylline for neonatal asphyxia and AKI protection, particularly given none of the infants in the studies to date received therapeutic hypothermia.

Similarly, emerging evidence suggests adenosine receptor antagonists, including theophylline as well as caffeine, may prevent AKI in premature infants. In a retrospective, single-center study, Carmody et al. (2016) found that VLBW infants who received caffeine were less likely to develop neonatal AKI.[90] These findings persisted after controlling for confounders such as BW, GA, illness severity, and receipt of nephrotoxic medications (aOR 0.22; 95% CI 0.07 to 0.75; $P = .02$). Even more recently, a secondary analysis of the AWAKEN cohort, showed that AKI occurred less frequently among neonates who received caffeine (50/447 (11.2%) vs. 72/228 (31.6%); $P < .01$).[91] Again, after multivariable adjustment, caffeine receipt remained associated with reduced odds of developing AKI (aOR 0.20; 95% CI 0.11 to 0.34) suggesting that for every 4.3 neonates exposed to caffeine, 1 case of AKI was prevented. Caffeine receipt was also associated with a decreased likelihood of developing severe, KDIGO stage 2 or 3 AKI (aOR 0.20; 95% CI 01.2 to 0.34). Further research is needed to better understand how best to utilize caffeine in those neonates at the highest risk to develop AKI.

Given the lack of treatment options for neonatal AKI, significant efforts have been given to establishing protocols and guidelines to help prevent AKI. Quality improvement programs designed to prevent harm from nephrotoxic mediations called NINJA (Negating Injury from Nephrotoxins with Just-in-time Action) and Baby NINJA have been reported.[92,93] Stoops et al. (2019) reported a reduction in nephrotoxic medication exposures (16.4 to 9.6 per 1000 patient days; $P = .03$), a reduction in the percentage of nephrotoxin-associated AKI (30.9% to 11.0%; $P < .001$), and a reduction in AKI intensity (9.1 to 2.9 per 100 suspectable patient days; $P < .001$).[94] These authors estimated Baby NINJA prevented 100 AKI episodes during the 18-month sustainability era of their project.

Step 3: Manage Consequences of Kidney Failure

The clinician has an important role in helping to achieve homeostasis during episodes of AKI, and careful attention to the fluids and electrolytes being delivered to the patient is critical. Managing fluids in the critically ill neonate with AKI can be very difficult and requires close attention. Once adequate intravascular volume has been restored, prevention of severe fluid overload is paramount. Severe fluid restriction, limiting intake to insensible, gastrointestinal, and kidney losses, is sometimes required to prevent or treat fluid overload but is performed at the heavy price of inadequate nutrition. Trending of cumulative fluid overload on daily rounds should be reported. Decisions on the placement of dialysis access should be considered early in those with severe fluid overload as fluid overload is associated with adverse outcomes across critically ill patient populations.[37,95–98]

Although diuretics do not impact the course of AKI, they can be used to assist in maintaining fluid homeostasis. If an adequate dose of a loop diuretic (i.e., 1 mg/kg of furosemide intravenously) does not improve UOP, it is unlikely that higher doses, changing diuretic, and/or changing to continuous dosing will benefit the infant, and their use could have negative side effects. The KDIGO Clinical Practice Guidelines for AKI suggest "not using diuretics to treat AKI, except in the management of volume overload."[89]

Electrolyte abnormalities can vary depending on the cause of AKI. For example, aminoglycoside toxicity is commonly non-oliguric with ongoing potassium and magnesium losses. Alternatively, ischemic AKI causes oliguria/anuria, hyponatremia, hyperkalemia, hyperphosphatemia, and hypocalcemia. Polyuria with electrolyte (especially bicarbonate) losses may occur following the relief of a urinary obstruction. Management of electrolyte disorders can typically be managed by attention to electrolyte intake during the initial course of AKI, frequent evaluation, and specific therapies.

Most cases of hyponatremia are due to water overload and, less commonly, low total sodium body composition. Attention to fluid status is critical to determine the cause and proper treatment of hyponatremia. In cases of non-symptomatic hypervolemic hyponatremia (serum sodium concentrations usually between 120 and 130 milliequivalent [mEq]/L), restriction of free water intake is recommended. If hyponatremia at this level results in clinical signs and symptoms (lethargy, seizures) or serum sodium concentration falls below 120 mEq/L, the use of 3% sodium chloride over 2 hours should be considered, but caution should be used to assure that the sodium is not corrected too quickly. Possible complications of hypertonic saline administration include congestive heart failure, pulmonary edema, hypertension, intraventricular hemorrhage, and periventricular leukomalacia. Care should be taken not to increase serum sodium concentration more rapidly than 0.5 mEq/h.

TABLE 77.7 Randomized Controlled Trials of Theophylline in Neonatal Hypoxic Ischemic Encephalopathy

Study	Population and Study Design	Dose and Timing of Theophylline (vs. Placebo)	Findings and Kidney-Related Outcomes
Raina et al. 2016	Single center ($n = 159$ severely asphyxiated term newborns)	Theophylline 5 mg/kg IV × 1 during first hour of life	• Theophylline group had lower SCr levels[a] (0.83 ± 0.35 vs. 1.47 ± 0.61; $P = .00$) • Theophylline group had higher endogenous CrCl (32.16 ± 16.34 vs. 17.73 ± 7.92; $P = .00$) • More AKI in placebo group[b] (117 [48%] vs. 36 [15%]; $P < .01$)

Eslami et al. 2009	Single center ($n = 36$ severely asphyxiated term and post-term newborns, >2.5 kg at birth)	Theophylline 5 mg/kg IV × 1 during first hour of life	• At 24 h of life, fluid balance was more positive in infants in placebo group[b]
			• On DOL 3 and 5, significantly higher SCr noted in placebo group[b] ($P < .05$ all)
			  • DOL 3: 1.06 ± 0.47 vs. 0.63 ± 0.22
			  • DOL 5: 0.73 ± 0.14 vs. 0.56 ± 0.14
			• Severe kidney dysfunction more prevalent in placebo group[b] (8 [42.1%] vs. 2 [11.7%])
			• On DOL 3, GFR[c] increased in theophylline group[a] (42.4 ± 19.1 vs. 27.5 ± 10.7; $P < .05$)

Bhat et al. 2006	Single center ($n = 70$ severely asphyxiated term newborns)	Theophylline 8 mg/kg IV × 1 during first hour of life	• Plasma Cr (mg/dL) was higher on DOL 2–5 in control group[b] ($P < .01$)
			  • DOL 2: 1.56 ± 0.92 vs. 0.92 ± 0.65
			  • DOL 3: 1.59 ± 0.92 vs. 0.95 ± 0.50
			  • DOL 4: 1.62 ± 1.03 vs. 0.94 ± 0.45
			  • DOL 5: 1.57 ± 0.90 vs. 0.82 ± 0.47
			• From DOL 2–3, endogenous CrCl[c] higher in theophylline group[a] (20.54 ± 7.96 vs. 7.36 ± 3.52; $P < .01$)
			• Excretion of β2 M (mg/L) was lower in theophylline group[a] (6.7 ± 2.4 vs. 15.2 ± 5.6; $P < .001$)
			• The output/input ratio was higher in theophylline group[a] ($P < .001$ all)
			  • DOL 1: 0.34 ± 0.18 vs. 0.32 ± 0.16
			  • DOL 2: 0.55 ± 0.32 vs. 0.39 ± 0.21
			  • DOL 3: 0.63 ± 0.29 vs. 0.44 ± 0.22
			  • DOL 4: 0.81 ± 0.19 vs. 0.49 ± 0.24
			  • DOL 5: 0.88 ± 0.15 vs. 0.52 ± 0.22
			• Mean weight (grams) was higher in control group[b] ($P < .01$ all)
			  • DOL 1: 2900 ± 250 vs. 2730 ± 410
			  • DOL 2: 2870 ± 240 vs. 2680 ± 420
			  • DOL 3: 2830 ± 230 vs. 2640 ± 410
			  • DOL 4: 2800 ± 250 vs. 2610 ± 410
			  • DOL 5: 2740 ± 210 vs. 2560 ± 430
			• Urine output (mL/kg/h) on DOL 2–5 is higher in theophylline group[a]

Bakr 2005	Single center ($n = 49$ severely asphyxiated term and postterm newborns)	Theophylline 5 mg/kg IV × 1 during first hour of life	• SCr (g/dL) lower in theophylline group on days 2–5 of life[a] ($P < .05$ all)
			  • Day 2: 1.22 ± 0.24 vs. 1.41 ± 0.29
			  • Day 3: 1.24 ± 0.39 vs. 1.57 ± 0.61
			  • Day 4: 1.31 ± 0.38 vs. 1.59 ± 0.44
			  • Day 5: 1.29 ± 0.27 vs. 1.47 ± 0.41
			• CrCl higher in theophylline group on days 2–4 of life[a] ($P < .05$ all)
			  • Day 2: 1.51 ± 0.09 vs. 0.81 ± 0.8
			  • Day 3: 1.62 ± 0.46 vs. 0.92 ± 0.6
			  • Day 4: 1.68 ± 0.51 vs. 1.1 ± 0.54
			• GFR[c] higher in theophylline group on days 2–5 of life[a] ($P < .05$ all)
			  • Day 2: 25.13 ± 9.10 vs. 15.32 ± 7.10
			  • Day 3: 26.02 ± 10.80 vs. 12.39 ± 4.60
			  • Day 4: 27.48 ± 10.20 vs. 14.35 ± 6.80
			  • Day 5: 29.31 ± 9.90 vs. 19.10 ± 8.70
			• β2 M excretion (mg/L) lower in theophylline group on days 1–5[a] ($P < .05$ all)
			  • Day 1: 7.38±2.40 vs. 10.72±6.90
			  • Day 2: 7.21 ± 2.20 vs. 12.83 ± 7.30
			  • Day 3: 5.45 ± 1.90 vs. 11.51 ± 5.90
			  • Day 4: 6.12 ± 2.10 vs. 12.56 ± 7.10
			  • Day 5: 6.34 ± 2.20 vs. 11.93 ± 6.60

Continued

TABLE 77.7 Randomized Controlled Trials of Theophylline in Neonatal Hypoxic Ischemic Encephalopathy—Cont'd

Study	Population and Study Design	Dose and Timing of Theophylline (vs. Placebo)	Findings and Kidney-Related Outcomes
Jenik et al. 2000	Single center ($n = 51$ severely asphyxiated term newborns)	Theophylline 8 mg/kg IV × 1 during first 60 minutes of life	• Fluid balance more positive in placebo group • Fewer infants with severe renal dysfunction in theophylline group[a] (4/24 [17%] vs. 15/27 [55%]; RR 0.30, 95% CI 0.12–0.78) • Higher mean CrCl in theophylline group[a] (21.84 ± 7.96 vs. 6.42 ± 4.16) • Urinary β2 M concentrations were reduced in theophylline group[a] (5.01 ± 2.3 mg/L vs. 11.5 ± 7.1 mg/L) • Fewer infants had urinary β2 M above normal limit in theophylline group[a] (9 [33%] vs. 20 [63%]; $P < .02$)

[a]Compared to placebo group.
[b]Compared to theophylline group.
[c]mL/min/1.72 m^2.
95% CI, 95% Confidence interval; *AKI*, acute kidney injury; *β2M*, β-2 microglobulin; *Cr*, creatinine; *CrCl*, creatinine clearance; *DOL*, day of life; *GFR*, glomerular filtration rate; *IV*, intravenous; *RR*, relative risk; *SCr*, serum creatinine.

TABLE 77.8 Medical Management of Hyperkalemia in the Newborn

Medication	Dose	Onset of Action	Duration of Action
Calcium gluconate (10%)	0.5–1 mL/kg (IV over 10 min)	1–5 min	15–60 min
Sodium bicarbonate (3.75% solution)	1–2 mEq/kg (IV over 10 min)	5–10 min	2–6 h
Insulin	1 IU/5 g glucose (IV bolus or continuous infusion)	15–30 min	4–6 h
Glucose	≤14 mg/kg/min (IV bolus or continuous infusion)	15–30 min	4–6 h
Furosemide	1 mg/kg dose or as continuous infusion	5–10 min	2–3 h
Sodium polystyrene sulfonate[a]	1 g/kg dose q 6 h as needed (orally/rectally)	1–2 h[b]	4–6 h
Dialysis	As per nephrology	Immediate	Duration of therapy

[a]Risk of gastrointestinal complications, specifically necrotizing enterocolitis, requiring cautious use.
[b]Onset of action may take up to 6 h, and the drug may be ineffective in preterm infants born at less than 29 weeks of gestation. See text for details.
IU, International unit; *IV*, intravenous.

Severe hyperkalemia is a life-threatening medical emergency. Signs of progressive hyperkalemia on the electrocardiogram, in order of severity, consist of tall, peaked T waves, heart block with widened QRS complexes, U wave formation, the development of sine waves, and finally cardiac arrest. Medications used to manage hyperkalemia, with their dose, onset, and duration of action, are listed in Table 77.8. Measures to remove potassium from the body include oral or rectal sodium polystyrene powder (Kayexalate), loop diuretics (if not anuric), and dialysis. Notably, sodium polystyrene powder should be used with caution given a known association with NEC, particularly when the suspension contains sorbitol.[99–103] Sodium polystyrene power can also be used to pretreat infant formula or expressed breastmilk to decrease the potassium load and decrease potential gastrointestinal complications.[104] Several methods to move potassium from the extracellular to the intracellular compartment are available including albuterol inhalation, sodium bicarbonate, and insulin with glucose. Adequate ionized calcium levels for cardio protection should be sought in the context of hyperkalemia.

Hyperphosphatemia is common in AKI and should be treated with low phosphorus intake. Breast milk and Similac 60/40 both contain low phosphorous and low potassium in comparison to other infant formulas. Significant elevations in serum phosphate pose a risk for the development of extra-skeletal calcifications of the heart, blood vessels, and kidneys in the newborn, especially when the calcium-phosphorus product exceeds 70.[29] Calcium carbonate may be used as a phosphate-binding agent in those whose phosphorous intake exceeds excretion. Although rarely an indication for dialysis without fluid overload or hyperkalemia, severe hyperphosphatemia can be treated with dialysis.

Hypocalcemia is possible in neonates with severe and prolonged AKI, especially those who develop an inability to convert 25-hydroxy-vitamin D to 1-25-hydroxy-vitamin D. Ionized calcium should be measured in those with low total calcium levels as concurrent hypoalbuminemia can affect total calcium levels. If ionized calcium is decreased and the newborn is symptomatic, 100 to 200 mg/kg of calcium gluconate should be infused over 10 to 20 minutes and repeated every 4 to 8 hours as necessary. Oral or intravenous calcitriol may be administered to increase intestinal reabsorption of calcium.

Normal acid-base homeostasis depends on the kidney's ability to reabsorb bicarbonate. Thus, infants with AKI commonly have a non-anion gap metabolic acidosis. Replacement with bicarbonate or acetate is indicated in those with AKI to avoid or treat metabolic acidosis. In infants with severe respiratory failure, large doses of bicarbonate should be avoided as this can result in respiratory acidosis.

Nutritional goals in infants with AKI are similar to those of infants without AKI. Commonly, parental nutrition and/or feeds will need to be concentrated to avoid excessive fluid gains. If

nutritional goals are unable to be achieved due to oliguria and ongoing fluid overload, the potential risks of dialysis therapy versus the potential risks associated with inadequate caloric and protein needs should be discussed. If a neonate is on continuous peritoneal dialysis (PD) or hemodialysis (HD), an additional 1 g/kg/day of protein is needed to supplement the protein losses that occur.[105,106]

In a neonate with AKI, careful assessment of medication dosing is imperative. Because many drugs are excreted in the urine, impaired metabolism or clearance from the kidneys can cause drug accumulation and adverse side effects. In those on dialysis, pharmacokinetic properties (volume of distribution, protein binding, size, charge) of drugs, dialysis modality (PD vs. HD), and interval of dialysis (intermittent vs. continuous) will affect drug availability.[107] Consultation with pharmacists and nephrologists familiar with drug dosing in kidney failure is invaluable for neonates with AKI.

Kidney Support Therapy With Dialysis

Kidney support therapy (KST; also known as renal support therapy, renal replacement therapy, or dialysis), either through the use of the peritoneal membrane or with extracorporeal blood systems, does not prevent or treat kidney failure; instead, it is used solely to support the infant who lacks the ability to maintain electrolyte, fluid, or acid/base homeostasis despite maximal medical management. KST can be provided via peritoneal access (i.e., PD) or intravenous access (i.e., HD) and can be provided continuously or intermittently. The decision to initiate KST (especially in those infants with severe congenital malformations of the kidney and urinary tract) is complex and requires a multidisciplinary approach (see later on decisions to initiate KST). Access placement and technical challenges make neonatal KST more difficult than KST in older children, but this therapy is feasible in experienced programs with dedicated pediatric nephrologists, neonatologists, dialysis nurses, and surgeons.

Indications for Dialysis Initiation

Absolute indications to initiate KST/dialysis include severe electrolyte abnormalities that are not correctable with medical interventions, life-threatening intoxications of medications that can be cleared with dialysis, inborn errors of metabolism that lead to hyperammonemia, fluid overload that leads to pulmonary edema or other organ dysfunction, and inability to provide adequate nutritional requirements because of oliguria and uremia. If kidney dysfunction and/or fluid overload occurs, discussions about dialysis initiation should occur early because prolonged fluid overload/uremia can worsen pulmonary edema and cardiopulmonary instability, making placement of access for dialysis very difficult.

The timing of initiation of dialysis for those with AKI is controversial. Most pediatric and neonatal AKI occurs due to impaired fluid homeostasis. Pediatricians have been at the forefront of identifying the degree of fluid overload at the initiation of continuous kidney replacement/support therapy as an independent risk factor for survival in critically ill children.[108–111] Further studies evaluating the impact of fluid overload on outcomes in neonates need to be performed. Advocates for early initiation of kidney support argue that critically ill patients benefit from early dialysis because they can remove excess fluid sooner, gain metabolic control faster, and provide maximal nutrition without progressive fluid overload. As technical access and machine advances have made neonatal dialysis safer and technically possible, early initiation of dialysis may improve outcomes in critically ill neonates with AKI. Further studies are needed before recommendations on the timing of dialysis can be made.

Access

The limiting factor in performing dialysis in the smallest of babies is access to the peritoneal space or the vascular space. The ideal acute PD access is a non-cuffed or single-cuffed coiled catheter specifically designed for neonatal PD. If this is not available, the use of a catheter that is typically used for chest tube drainage, or other catheters that may be available, can be lifesaving. The advantage of the straight uncuffed catheter is that it can be placed at the bedside and used soon after insertion. However, these catheters may be more likely to become infected and/or develop leakage of fluids around the insertion site. For the patient who requires chronic dialysis, a catheter with two subcutaneous cuffs and the use of a downfacing exit site away from the diaper area and away from a gastrostomy tube (if present) are recommended.[112] As with all pediatric surgery procedures, the exact type of catheter and the timing and location of catheter insertion need to be tailored to the individual patient.[33]

Vascular access for HD requires a rigid, large double lumen catheter (ideally ≥6.5 French [F]) placed in the femoral or internal jugular vein. Standard intravenous catheters are too flexible and too small to maintain patency with high blood flows; two 4 F or 5 F catheters in different sites can be lifesaving. If dialysis is anticipated to last more than one week, a cuffed catheter is preferred to decrease the likelihood of surgical revision. The length of the catheter should be chosen so that the tip of the catheter resides in the right atrium for internal jugular catheters and in the inferior vena cava (IVC) for femoral catheters. Unless no other choices are available, the subclavian artery should be avoided in infants who are likely to require long-term KST because a future forearm fistula of the ipsilateral arm can fail with "mild stenosis" of the subclavian vein.

Peritoneal Dialysis

Once PD access is placed and the decision to start dialysis has been made, small volume continuous cycles (10 mL/kg) are performed. The dialysate solution is left dwelling in the peritoneal cavity, during which time solute and fluid removal take place, and the solution is then drained. Continuous cycles are performed with each cycle lasting about an hour. The dextrose concentration in the dialysate fluid will determine the amount of net water loss (ultrafiltration). Complications associated with PD include peritonitis, leakage around the catheter exit site, tunnel infection, catheter malfunction, and catheter obstruction by the omentum.[113] Leakage of fluid into other compartments (including the chest in patients without an intact diaphragm) can occur, and, if suspected, the fluid composition of fluid removed from that compartment will reveal high glucose levels if a leak is indeed present. Absolute or relative contraindications to PD include NEC, abdominal wall defects, and the presence of an intraabdominal foreign body, such as a ventriculoperitoneal shunt or diaphragmatic patch.

Hemodialysis and Continuous Renal Replacement Therapy

Once reliable access to the vascular space is achieved, the HD procedure can be performed. The two types of HD, intermittent HD and continuous HD (i.e., continuous renal replacement therapy [CRRT]), differ mainly by the duration of the procedure.

Intermittent HD is significantly more efficient at solute removal than CRRT but technically challenging and often impossible in small neonates. The blood flow and time on therapy are the limiting factors for solute clearance on HD. Even with the smallest dialyzers and neonatal tubing, most infants need blood priming of the extracorporeal circuit for the therapy. Skilled pediatric HD nurses are required at the bedside during the entire procedure, which typically lasts 3 to 4 hours. Achieving the adequate fluid removal necessary for the entire day can be difficult to achieve in the few hours on dialysis, especially in hemodynamically unstable infants. This technique usually requires systemic heparinization, with activated clotting time usually kept at 180 to 200 seconds, rendering the technique risky in preterm newborns and others at high risk for intracranial bleeding.

The main advantage of a continuous modality is that lower blood flow and fluid removal rates can be used to accomplish the desired ultrafiltration and clearance goals. CRRT requires a double-lumen venous catheter whereby the CRRT pump pulls blood from one port of the catheter and returns the blood via the other side of the catheter. CRRT is slowly becoming a more common procedure in the pediatric and neonatal ICU.

Anticoagulation with CRRT is achieved with either systemic heparin or regional citrate anticoagulation. The advantage of regional citrate anticoagulation is that the patient is not systemically anticoagulated; however, this approach has the added risk of hypocalcemia caused by citrate excess (especially in those with impaired liver metabolism) and metabolic alkalosis.[114] Because citrate is metabolized by the liver, caution must be exercised when dialyzing premature infants or newborns with multiorgan failure who may have impaired liver function.

Outcome data in neonates who require CRRT are scarce. Symons et. al. (2003) reported a survival rate of 37.6% (32/85) in neonates who received CRRT in five large children's hospitals in the United States.[115] Between 2001 and 2007, the prospective pediatric CRRT group, a multicenter registry of 14 pediatric CRRT programs, reported outcomes for neonates in whom CRRT was initiated before 1 month of age.[110] About 8% (35 neonates) in the registry were dialyzed in the first month of life. In this group, the median age was 8 days old; the median weight was 3.2 kg, with the smallest infant weighing 1.3 kg. Of the 35 infants in the registry, 24 were dialyzed for either fluid overload, electrolyte imbalance, or both, and 11/35 were dialyzed for inborn errors of metabolism. Overall survival was 43%. Infants dialyzed for inborn errors had a better survival rate (73%) than the others (30%).

Several technical issues specific to infants arise when using CRRT for dialysis. The extracorporeal volume can incorporate greater than 50% of the infant's blood volume. For example, a 2 kg baby has a blood volume of ~80 mL/kg (160 mL); the smallest circuit volume available on the most commonly used machine in the United States has an extracorporeal volume of around 60 mL, about 37.5% of this example infant's blood volume. More recently, low extracorporeal volume CRRT machines have been developed for use in neonates.

The CARPEDIEM (Bellco Medtronic, Mirandola, Italy) is a machine designed for use in neonates.[116] It has been used across Europe and was US FDA approved for use in the United States in the Fall of 2020. The Nidus (Allmed, London, England) is currently being tested in five pediatric hospitals in England.[117] The Aquadex (CHF Solutions, Eden Prairie, MN) has been adapted for neonatal CRT in the United States.[118] With a circuit volume less than half of all other CRRT circuits available, these devices dramatically decrease the hemodynamic instability often experienced during circuit initiation. With less risk and fewer complications have come improved outcomes and broader acceptance of CRRT for neonates.

Priming the CRRT circuit with blood will minimize the risks of hypotension during circuit initiation, but additional complications (acidosis, hypocalcemia, thrombocytopenia, and dilution of coagulation factors) make initiation of CRRT challenging. The risk for the bradykinin reaction that can occur at the initiation of CRRT with AN69 dialyzer (Baxter, Chicago, IL) membranes can be reduced using several techniques that minimize exposure of acidotic blood to the membrane including blood buffering, transfusion of blood postfilter in a saline-primed circuit, and zero balance ultrafiltration.[119,120]

Hyperammonemia, due to an inborn error of metabolism such as a urea cycle enzyme defect, can rapidly lead to brain damage in a newborn and requires high index of suspicion to diagnose. Treatment involves both decreasing the production of ammonia and avoidance of protein but also removal of toxic metabolites via dialysis. An ammonia level exceeding 560 µg/dl (400 ummol/L) is an indication for dialysis.[121]

Acute Kidney Injury as a Cause of Long-Term Chronic Kidney Disease

Total GFR is determined by the filtration rate of single nephrons and the number of nephrons present. When the number of nephrons is diminished, single nephron GFR increases as the kidney works to compensate for low nephron numbers. This compensatory hypertrophy causes glomeruli to function under increased intracapillary hydraulic pressure, which, over time, causes damage to capillary walls. This abnormal process leads to progressive glomerulosclerosis, proteinuria, hypertension, and chronic kidney disease (CKD).[122] This hyperfiltration hypothesis has been applied and confirmed in autopsy data of hypertensive patients[123,124] and has been written about at length regarding infants with intrauterine growth restriction.[125–129] A systematic review and meta-analysis in 2009 concluded that low BW babies (≤5.5 lb) were 70% more likely to develop CKD later in life compared with individuals with normal birthweight.[129]

Nephrogenesis continues through 34 weeks' gestation. Premature infants are therefore born with low nephron numbers compared with term infants. Autopsy studies suggest that AKI in premature neonates impairs nephrogenesis.[130–133] Follow-up single-center studies in term and premature newborns suggest that AKI may lead to CKD.[134–139] For example, the Follow-Up of Acute Kidney Injury in Neonates during Childhood Years (FANCY) study evaluated 34 premature infants (20 with AKI and 14 without AKI) at an average of 5 years of age.[139] Those without AKI had higher rates of CKD (compared to those without AKI 13/20 [65%] vs. 2/14 [14%]; $P < .05$).

Renal Vascular Disease in the Newborn

Thromboembolic events in neonates usually result from an imbalance of the delicate homeostasis between bleeding and thrombosis. Some may be of genetic origin, some may relate to underlying stresses during pathologic processes, and some may relate to treatments for the pathologic processes. Both RAT and RVT occur in the neonate and pose a significant threat to short and long-term kidney function.

Renal Arterial Thrombus

The major risk factor for RAT is umbilical artery catheterization. However, shock, coagulopathy, and congestive heart failure all pose risks. The detection, and thus incidence rates of RAT vary depending upon the diagnostic test chosen. Doppler ultrasonography estimates the incidence of umbilical artery-related thromboembolism from 14% to 35%, whereas studies using angiography document incidences up to 64%; however, clinical symptoms of umbilical artery-related thromboembolism occur in only 1% to 3% of infants.[140] RAT in the neonate is far less common than RVT.

Clinical presentation varies with the extent and severity of thrombosis. Thrombosis of the abdominal aorta or renal arteries can manifest in any of the following ways: signs of congestive heart failure, hypertension, oliguria, kidney failure, decreased femoral pulses with lower limb ischemia, or bowel ischemia/frank NEC secondary to superior or inferior mesenteric artery thrombosis. Symptoms of RAT manifest within the first few postnatal days in a term neonate, compared with a median age of 8 days in a preterm neonate. Laboratory findings associated with RAT are thrombocytopenia, hypofibrinogenemia, elevated fibrin split products, variable prothrombin and thromboplastin times, conjugated hyperbilirubinemia, elevated blood urea nitrogen and creatinine, hyperreninemia, and hematuria.

Doppler ultrasonography is used as the first line of imaging for diagnosing RAT, although it usually fails to detect smaller intra-arterial thrombi.[141] If ultrasonography is inconclusive, radionuclide imaging can be used. Angiography is the standard diagnostic modality and should be performed through the umbilical artery line if surgical intervention or fibrinolytic therapy is being considered.

For asymptomatic or minimally symptomatic newborns, only supportive care is recommended, such as removal of the umbilical artery catheter and close ultrasonographic monitoring. Most of these thrombi resolve spontaneously. In newborns with mild signs of organ dysfunction and stable thrombosis, management of hypertension, transient kidney insufficiency/AKI, and mild congestive heart failure is recommended. Systemic heparin is given for anticoagulation. Close laboratory monitoring is done to avoid excessive heparinization, and clinical response is monitored by Doppler ultrasonography.

In case of potentially life-threatening complications of aortic or renal thrombosis, fibrinolytic therapy (systemic or intrathrombotic) along with supportive care is indicated. There are limited data on the efficacy, dose, and safety of fibrinolytic agents in infants.[142,143] The intrathrombotic infusion of a fibrinolytic agent, typically tissue plasminogen activator (tPA) reduces the cumulative dose and possible systemic adverse effects. Close monitoring by ultrasonography or angiography should be done to evaluate the response to this therapy.

The overall mortality rate with aortic thromboses and RAT is between 9% and 20%, with mortality being higher with major aortic thromboses and RAT.[144] Renovascular hypertension is the most common long-term complication. In most cases, these infants eventually are weaned from antihypertensive medications and remain normotensive.

Renal Vein Thrombosis

RVT is the most common thrombosis in infancy and occurs primarily in the newborn period with an incidence of 2.2 cases per 100,000 live births.[145] RVT has a male predominance of approximately 67%; it is unilateral in more than 70% of patients and more prevalent on the left side (approximately 63%). The thrombus also involved the IVC in approximately 43% of the cases, and it was associated with adrenal hemorrhage in approximately 15%.[146,147]

The cause of RVT is unknown, although a number of factors are associated with this disorder including maternal diabetes, traumatic delivery, prematurity, hyperviscosity, hypovolemia, hemoconcentration, sepsis, birth asphyxia, cyanotic congenital cardiac disease, congenital renal vein defects, and an indwelling umbilical venous catheter.[144,145,147,148] Prothrombotic conditions have a significant role in the pathogenesis of neonatal RVT.[147,149,150]

There are three cardinal signs of RVT: macroscopic hematuria, palpable abdominal mass, and thrombocytopenia; these signs have been found in approximately 56%, 45%, and 47% of cases, respectively.[147] Other signs and laboratory findings associated with RVT are oliguria or anuria, hemolytic anemia, metabolic acidosis, azotemia, and variable prothrombin and partial thromboplastin times.

Kidney/renal ultrasonography (RUS) is used to diagnose RVT, typically demonstrating unilaterally or bilaterally enlarged and echogenic kidneys with attenuation or loss of corticomedullary differentiation and little blood flow. In many cases, calcification and thrombus may be seen extending into the IVC.[148] Doppler studies are useful for detecting resistance or absence of flow in renal venous branches and collateral vessels. Although RUS is most commonly used, contrast angiography is considered the gold standard. MRI has also been reported to give excellent diagnostic findings in RVT, although it should be reserved for those cases in which Doppler findings are inconclusive.[151]

Treatment of RVT remains controversial as literature is lacking to compare therapies which include anticoagulation, fibrinolysis, or both. Supportive therapy should be provided to all affected infants. Hypertonic solutions, nephrotoxic medications, hyperosmotic radiographic contrast agents, and unnecessary use of diuretics should be avoided. In the case of a spontaneous, unilateral RVT without kidney impairment/AKI or extension, current guidelines recommend radiologic monitoring for thrombus extension or therapeutically dosed anticoagulation (unfractionated heparin [UFH] or low molecular weight heparin [LMWH][143]). Therapeutically dosed UFH or LMWH are recommended for spontaneous, unilateral RVT with renal impairment/AKI or RVT without renal impairment/AKI but with extension into the IVC. In the case of bilateral spontaneous RVT with renal impairment/AKI, anticoagulation with UFH or LMWH or initial thrombolytic therapy with TPA followed by anticoagulation with UFH or LMWH is recommended. Monitoring and continued therapy until resolution for a total duration of 6 weeks to 3 months is included in the guidelines. Surgical interventions such as thrombectomy or nephrectomy have not shown any benefit.

Affected neonates must be followed closely for kidney complications. Kidney scarring and atrophy are well-recognized complications of RVT. Approximately 19% of patients have a persistent elevation of blood pressure, which has been shown to be slightly higher—at 21% for those with bilateral RVT. The mortality rate for neonates with RVT is approximately 3%.[147] Because more than 80% of neonates with RVT have shown persistent abnormalities on kidney imaging and there are not enough data on long-term outcomes of such neonates, continued follow-up is strongly recommended.

Suggested Readings

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The complete reference list is available at Elsevier eBooks+.

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74 Renal Development

IRENE MCALEER AND KAI-WEN CHIANG

KEY POINTS

- Renal organogenesis is a complex interaction between gene stimulation of planned growth and complementary apoptosis, allowing appropriate development of the functioning renal organ system, vascular bed, and intertwined genital system.
- The renin-angiotensin system (RAS) is critical to normal renal development through delicate interaction between the maternal–placental RAS and the developing fetal RAS, which has central hormone-specific and sex-specific configurations that help the developing vascular and renal organogenesis.
- Preterm birth (<37 weeks gestation), as well as low birth weight, are associated with chronic kidney disease (CKD), and the improved survival with treatment warrants more research into long-term health optimization of these patients.

The human kidney, specifically the metanephric kidney, is an extremely complex organ with more than 25 functionally and morphologically different distinct cell types performing vitally important functions for the developing human. These actions include filtering waste products, maintaining electrolyte and fluid homeostasis, facilitating bone mineralization, regulating blood pressure, and blood composition.[1] The nephron of the kidney performs these functions. The number of human nephrons range from 200,000 to 2,000,000 for each kidney with peak growth during the third trimester and complete nephrogenesis by 36 weeks' gestation.[2] Any further growth of the kidney is due to an increase in the size of the individual nephrons rather than an increase in nephron number.

Renal organogenesis is a complex and incompletely understood process. Factors affecting maternal and fetal health, including environmental factors in utero, can lead to failure of normal morphogenesis, resulting in congenital anomalies of the kidney and urinary tract. If the complement of nephrons is decreased by prematurity, low birth weight (LBW), or exposure to environmental agents causing damage to the developing renal system, adult renal disease and hypertension may occur.[2] Many steps in the development of the human kidney are unknown but are extrapolated from the study of animal models. This chapter will review known (and postulated) molecular and cellular mechanisms of renal development, the impact of environmental factors on the developing fetal kidney, and the long-term risks of CKD and other associated adult health outcomes.[2–4]

Factors Influencing Organogenesis

Organogenesis, or organ formation, begins with early patterning of cell groups through gene expression with transcription factors that act to determine cell fates specific to each given organ.[5]

Transcription factors, generally ribonucleic acids (RNAs) directed by DNA information, are incorporated into larger molecular networks and ultimately, direct downstream signaling pathways. Transcription factors may have different purposes in each developing organ system. Similarly, signaling pathways are used repeatedly in each different organ's development, and these pathways may also be used in multiple stages of differentiation in the same organ.

Signaling pathways establish epithelial morphogenesis by allowing cell polarity, bending, and folding to shape developing tissues and to allow these tissues to interact with the loose mesenchyme surrounding the epithelia through signaling. This reciprocal induction between epithelia and mesenchyme is well illustrated in kidney development, where expression of the transcription factor Wilms tumor 1 (WT1) in the metanephric mesenchyme leads to glial cell–derived neurotropic factor (GDNF) expression critical for the outgrowth of the developing epithelial ureteral bud. Reciprocally, the ureteral bud then signals back to the metanephric mesenchyme to induce formation of the renal nephron units. There is a delicate balance between the interplay between the ureteral bud and the developing mesenchymal metanephros to allow tissue differentiation into the final renal unit.

Enhanced imaging, ex vivo organ culture, and computational strategies have advanced our understanding of the physical forces inside and outside the cells in organ development.[5] Study of different species has led to better understanding of the role of extracellular matrix in regulating the mechanical properties of the developing tissues. The extracellular matrix also provides substrata for cell migration, rotation, and elongation necessary for organ development. Most of this research is in nonhuman tissue at present, but basic understanding of generalized organogenesis is important in determining the steps critical in human organ development, with potential implications for regenerative medicine as well.

Development of the Renal Vascular Bed

The vascular systems of all vertebrates are highly organized branched networks of arteries, veins, and capillaries. The circulatory system in combination with the hematologic system is the

first functioning physiologic system to develop in embryogenesis.[6] Development of the cardiovascular and hematologic systems must occur in tandem because simple diffusion of oxygen and nutrients would be insufficient for organ development as the embryo enlarges. Formation of the vascular system is therefore crucial for proper tissue growth and differentiation, delivery of oxygen and nutrients to the developing organism, and removal of waste.

Vasculogenesis and angiogenesis play a primary role in determining patterning of the developing embryo through the paracrine action of the endothelial cells. Blood vessels are critical for organ development, differentiation, and postnatal remodeling. There is a reciprocal relationship, with the developing organ providing signals to the endothelial cells of the developing vasculature, while the endothelial cells signal patterning instructions for organ formation. Vascular development begins shortly after gastrulation when the blood islets form in the yolk sac and angioblast precursors form in the head mesenchyme and the posterior lateral plate mesoderm. Vascular precursor cells are present in the metanephric blastema. Local environmental cues initiate differentiation of the endothelial layer in the developing kidney by forming the renal vascular tree after transplant of different extrarenal endothelial cells to the developing renal bed as studied in developing mouse kidneys. Angiogenesis generally occurs later in embryogenesis by increasing the previously laid vascular bed by sprouting, bridging, and intussusceptive growth. Ultimately, the primitive vessels branch, prune, and specialize to accommodate the probable function of each respective organ that they feed. Oxygen tension and hemodynamic forces are critical for developing the delicate patterns specific to the local vasculature. Angiogenic growth factors are necessary for vasculature development as well as for organ patterning. The most important factors responsible for angioblast differentiation and tube formation are vascular endothelial growth factors (VEGFs). VEGFs are found early in blood vessel patterning and later help modulate endothelial maintenance in normal tissues before and after birth. The endothelial cells themselves are the source of these proteins. VEGF also helps mobilize blood elements from the bone marrow through interactions with hematopoietic stem cells there.[6] The development of the area-specific branching pattern is likely due to local factors and different local progenitor cell populations. The podocyte epithelial cells in the developing glomerular region secrete large amounts of VEGF, stimulating increased branching of the vessels forming in this area (Fig. 74.1). Fibroblast growth factors (FGFs) are heparin-binding growth factors that interact with endothelial cell surface receptors. Their primary purpose is to help control branching morphogenesis of the vascular tree during organogenesis. The post-glomerular vascular bed is closely aligned to the vasa recta in the renal tubule structures and is likely regulated by angiogenic factors such as VEGF secreted by the tubules themselves. Postnatal development of the vasa recta has also been found to be mediated by VEGF. Renal vasculature development occurs through simultaneous vasculogenic and angiogenic processes. VEGF is critical in the regulation of blood vessel formation in the kidney and is also responsible for the coordinated development of the glomerulus and renal tubular development simultaneous to its regulating vasculogenesis. Animal model studies suggest that postnatal branching may be mediated by the renin-angiotensin system (RAS).

The renal vascular bed is unique compared with other organ systems. The kidneys compose less than 0.8% of the human body weight but receive 20% of the cardiac output at any given time.[7] The volume of blood sent to the kidney is critical for clearing the body of metabolic waste, as well as maintaining fluid and ion homeostasis in the bloodstream. The renal artery enters the kidney near the central portion of the kidney or hilum, which is close to the medulla of the kidney. Although the renal artery is proximal to the medulla of the kidney, 90% of the blood it delivers is sent directly to the glomerular capillary bed in the periphery of the kidney, and only 10% is delivered to the medulla.

The arterial structure of the kidney is critical for directing blood primarily to the glomerulus. The mechanisms for this patterning during development are not well understood, but as development proceeds, the medullary portion of the arterial tree has very little branching, whereas the cortical arteries have extensive branching, especially the afferent arterioles surrounding the glomeruli.

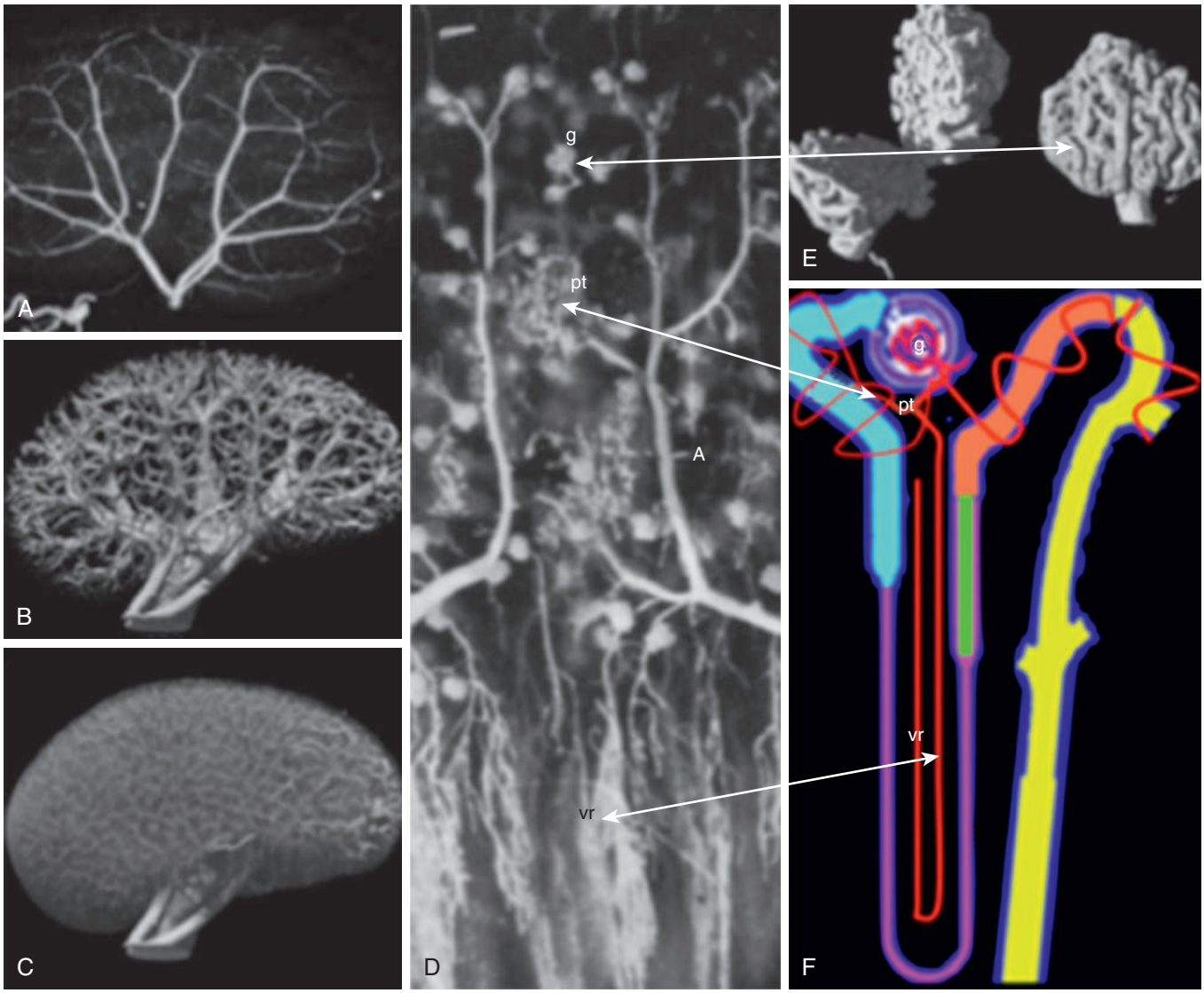
For appropriate renal function, the glomerulus endothelium is very permeable to fluids and low molecular weight solutes. The resistance of the renal vasculature surrounding the glomerulus is very high, to allow the ultrafiltrate produced to flow through the urinary space at 125 mL/min. These high-resistance arterioles allow the amount of fluid emanating from glomerular capillaries to be about 50 times greater than the fluid outflow of other capillary systems in the body. In contrast, the peritubular capillaries have high oncotic pressure, allowing them to reabsorb solutes and fluids that would otherwise be lost by this high glomerular filtration rate (GFR). There is a complementary interplay between the renal tubules reabsorbing fluid, electrolytes, and solutes from the glomerular filtrate and the peritubular capillaries returning these substances to the systemic circulation because of this high oncotic pressure pull.

The vasa recta is an additional renal capillary bed adjacent to the renal tubules in the medulla. These vessels act by delivering oxygen and nutrients to the medulla and return electrolytes and solutes reabsorbed by the medullary renal tubules. The long-looped arrangement of the vasa recta close to the loop of Henle is critical for urine concentration by conservation of water through increased osmolarity in this region in the renal medulla.[7] The blood is ultimately collected in the venous system at the cortical medullary region of the kidney and flows out of the kidney through the renal vein.

Renal Morphogenesis

The mammalian urogenital system develops from the intermediate mesoderm. Paired epithelial nephric ducts arise dorsally and elongate caudally on the right and left sides of the embryo until they induce development of the pronephric and mesonephric duct formation from intermediate mesenchymal mesoderm. These ducts elongate until they reach and fuse with the cloaca, which is the bladder and urethral precursor. Primitive tubules develop as the pronephros and mesonephros and are transient with minimal function, and only a portion remains of the mesonephric tubules as part of the male reproductive system.[1]

The metanephric kidney arises from the posterior nephric duct through reciprocal signaling induction between the metanephric mesenchyme and nephric duct causing the ureteral bud to form. Human metanephric differentiation starts at about 5 weeks' gestation, and the first functioning nephrons are formed at about week 8. The ureteral bud then invades the metanephric mesenchyme to initiate the mesenchymal to epithelial differentiation giving rise to the glomerulus–nephron–collecting duct system of the mature kidney. The requirement for intermediate mesoderm appears to

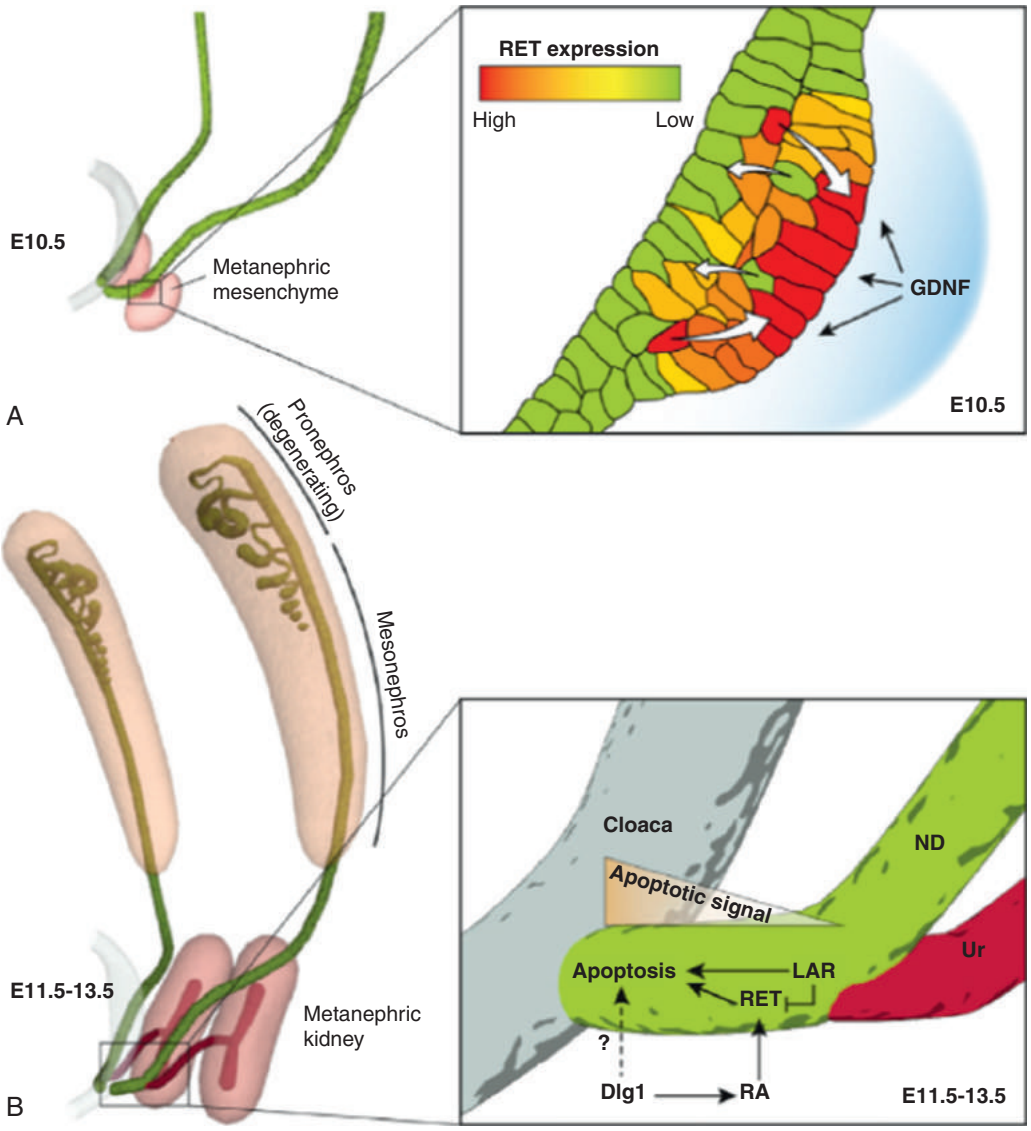


• **Fig. 74.1** Anatomy of the Renal Vascular Bed. Reconstructed scan of an entire mouse kidney corrosion cast by nano computed tomography (A–C). Thresholding for large vessels (A) illustrates the stereotypic architecture of the renal artery and its major branches. As thresholding is adjusted to visualize smaller vessels, the cortical arterial tree up to the level of the intralobular arteries is detected (B). Finer thresholding allows the visualization of the complex architecture of the cortical microvasculature (C). (D) Corrosion cast of rat kidney images by scanning electron microscopy. The glomeruli (*g*), peritubular capillaries (*pt*), and vasa recta (*vr*) are easily visualized. (E) A corrosion cast of an isolated glomerulus imaged by nano computed tomography illustrating the complexity of the capillary loops. (F) A single nephron and its associated glomerulus (*g*), peritubular capillaries (*pt*), and vasa recta (*vr*). (D, E courtesy Dr. Wilhelm Kriz; F from Herzlinger D, Hurtado R. Patterning the renal vascular bed. *Semin Cell Dev Biol*. 2014;36:50–56.)

be regulated by bone morphogenetic protein (BMP) signaling, which is also required for maintenance of the pronephric duct gene expression to allow survival and differentiation of the developing nephric duct.

Paired box genes (*PAX2* and *PAX8*) appear to be necessary to drive nephric duct development, elongation, and maintenance.[8] The final stage in early nephric development is the separation of the genital tract from the urinary tract. Once the ureteral bud extends from the nephric duct, the urinary tract separates the ureter from the common nephric duct through apoptosis to form the new ureterovesical junction into the cloacal structure that will be the urinary bladder. This apoptosis is most active at the caudal region and less so at the ureteral bud branching point. The apoptosis occurs through downregulation of RET (receptor tyrosine kinase) activity. RET is still needed for ureteral remodeling but at an expression level lower than that needed for generation of the ureteral bud branching off the nephric duct. Ultimate elimination of the common nephric duct is necessary for normal urinary tract function. Absence or delay in ureteral remodeling may result in ureteropelvic junction obstruction. Overactivation of apoptosis of the common nephric duct may result in vesicoureteral reflux (Fig. 74.2).[8]

Development of the ureteral bud requires an active RET–glial cell line-derived neurotrophic factor (GDNF) pathway. GDNF

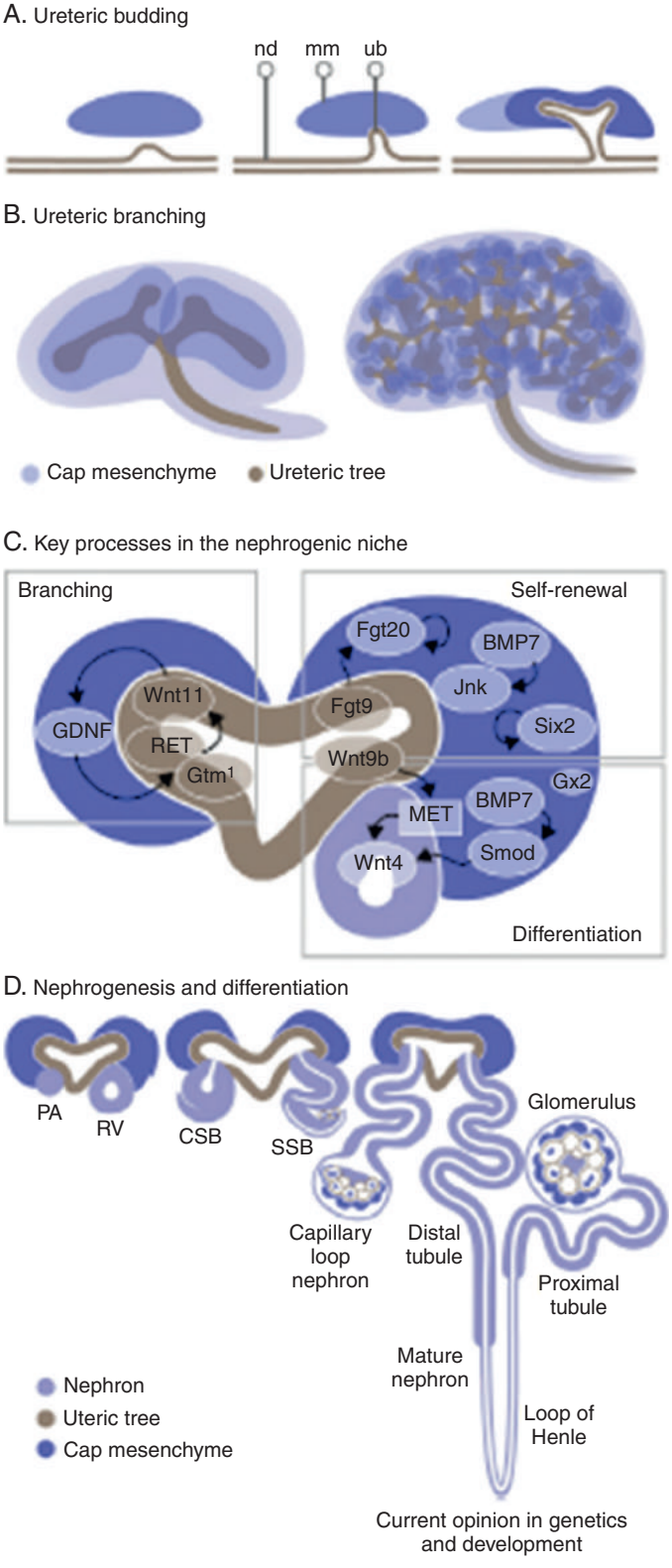


• **Fig. 74.2** Interaction between mesenchymal and nephric duct epithelial tissues drives cell sorting to form the ureteric bud and programmed cell death during ureter maturation. (A) Ureteric bud evagination involves RET tyrosine kinase–dependent cell sorting *(white arrows)* to assemble the highest RET-responsive cells toward glial cell–derived neurotropic factor (*GDNF*) secreted from the metanephric mesenchyme. (B) A graduated apoptotic signal drives elimination of the common nephric duct. Apoptotic cell death involves Dlg1 and retinoic acid (*RA*) signaling from the mesenchymal compartment, which appear to act through RET within the common nephric duct. Apoptosis additionally requires the expression of LAR-family receptor protein tyrosine phosphatases in the common nephric duct, which act partially by down-regulating RET prosurvival signaling. *E*, Embryonic day; *ND*, nephric duct; *Ur*, ureter. (From Stewart K, Bouchard M. Coordinated cell behaviours in early urogenital system morphogenesis. *Semin Cell Dev Biol*. 2014;36:13–20.)

is located in the metanephric mesenchyme adjacent to the nephric duct and activates ureteral bud development through interaction with RET located along the nephric duct. If RET is absent, ureteric induction fails, but the ureteral bud forms ectopically in studies where GDNF is cultured next to the nephric duct.[9] Wnt (Wingless/integrated) pathway factors, particularly factor 11, and FGFs upregulate GDNF to help with branching of the developing ureteral nephron tree.

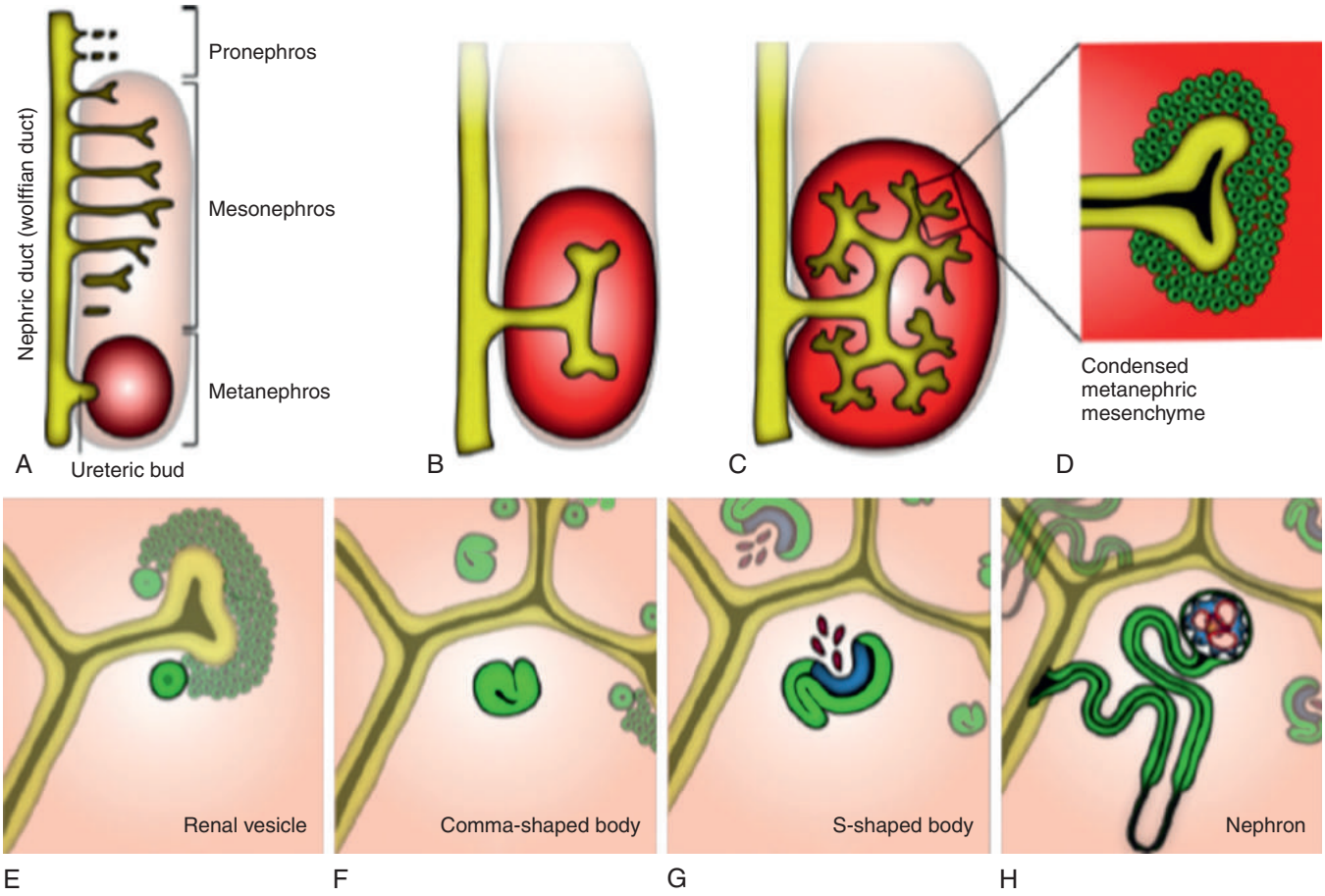
Once the ureteral bud has been stimulated to extend out to the developing metanephric mesenchyme, it begins to branch, forming the ureteric tree, which occurs only at the tips of ureteric tips with RET–GDNF reciprocal signaling, which upregulates RET–Wnt11, further upregulating GDNF secretion. This ureteral signal to release RET is localized to the cap mesenchyme. With continued proliferation, elongation, patterning, and segmentation, the specific functioning regions of the nephron form the recognizable adult nephron (glomerulus, proximal tubule, loop of Henle, distal tubule), which all connect back to the collecting duct (Fig. 74.3).

Once the ureteral bud epithelium invades the metanephric mesenchyme at the cap mesenchyme region, this mesenchyme condenses around the ureteral bud tips under the influence of



• **Fig. 74.3** Key inductive events in mammalian kidney morphogenesis. (A) Ureteric budding. The formation of the ureteric bud (*ub*) as a swelling of the nephric duct (*nd*), which grows toward the metanephric mesenchyme (*mm*) before undergoing initial bifurcation. (B) Ureteric branching. The branching ureteric epithelium of the developing mouse kidney from 11.5 days after conception (*left*) to 15.5 days after conception (*right*) showing the ureteric tree and surrounding cap mesenchyme. (C) Key processes in the nephrogenic niche. A nephrogenic niche illustrating the signaling pathways critical for branching (*left*) versus cap mesenchyme self-renewal (*top right*) and differentiation (*bottom right*). (D) Nephrogenesis and differentiation. The stages of nephron maturation from pretubular aggregate (*PA*) through renal vesicle (*RV*), comma-shaped body (*CSB*), S-shaped body (*SSB*), capillary loop nephron, and mature nephron. The RV represents the point of transition from mesenchyme to a polarized epithelial state. The formation of a connection between the forming nephron and the lumen of the adjacent ureteric epithelium occurs at the late RV stage and is shown here at the CSB stage. *BMP7*, Bone morphogenetic protein 7; *GDNF*, glial cell–derived neurotropic factor. (From Little MH. Improving our resolution of kidney morphogenesis across time and space. *Curr Opin Genet Dev*. 2015;32:135–143.)

Wnt genes.[10] The renal vesicle is the first epithelial structure that will ultimately become the future nephron and is activated by the developing ureteral bud. The next phase is the development of the comma-shaped bodies as the first condensation of the renal vesicle metanephric mesenchyme. Formation of the cleft in the comma-shaped bodies denotes the development of the S-shaped bodies, which ultimately initiate renal nephron development (Fig. 74.4). The S-shaped bodies are derived from the cap mesenchyme and become the glomerular tuft once endothelial cells infiltrate this area of the developing nephron. The



• **Fig. 74.4** Nephron Development. (A) In mammals, the kidney develops from the metanephric mesenchyme on invasion of the ureteric bud from the nephric duct. (B, C) The ureteric bud starts branching within the growing metanephric mesenchyme. (D) The mesenchyme condenses around the ureteric bud tips, forming the Six2-positive cap mesenchyme. (E) Renal vesicles form from the condensed cap mesenchyme. (F) A cleft develops in the comma-shaped bodies. (G) Podocyte progenitors start to attract angioblasts in the S-shaped body. (H) The developing nephron connects with the collecting duct. (From Schell C, Wanner N, Huber TB. Glomerular development—shaping the multi-cellular filtration unit. *Semin Cell Dev Biol*. 2014;36:39–49.)

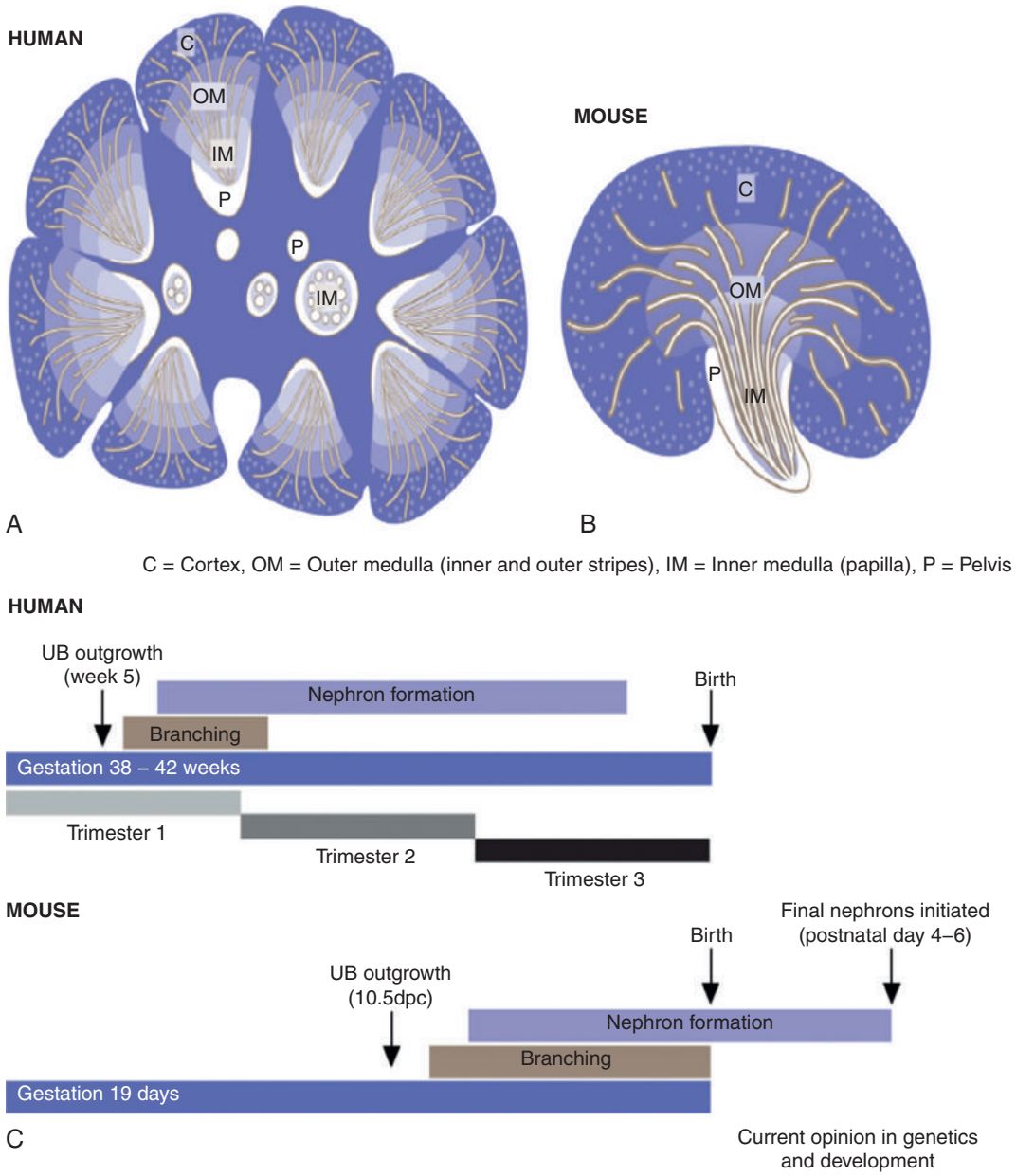
axis of the developing nephron is determined by the S-shaped body after it fuses to the ureteral tip. The proximal end of the S-shaped body will be the glomerulus, while the distal end will fuse to the ureteral bud branching system as the collecting duct. The S-shaped phase of development is when the nephrogenic and vasculogenic processes connect because of secretion of VEGF from the podocytes in the S-shaped body and attract migrating vascular endothelial cells to the S-shaped body cleft (Fig. 74.5).[11]

With time and continued development, the ureteral tip and cap mesenchyme decrease in size. Ureteric branching ceases in the human at around week 14 to 15 of gestation. The human kidney is multipapillate, with about 8 to 15 lobes, each having about 15 generations of collecting duct branching. The extensive patterning and segmentation that occur in nephron development are driven by anchor genes that have absolute specificity for each cell compartment where they are located in the developing embryo. The kidney alone has more than 15 distinct anatomic compartments. To date, 37 anchor genes have been defined for only six sub-compartments in the kidney. The actions of these genes are not yet well known, but the anchor genes may be responsible for initiating differentiation of the various compartments of the nephron by releasing promoters and tissue factors to the local mesenchyme to initiate formation of the functioning renal unit.[12]

Human nephron development is compared with mouse development to extrapolate similar or speculated steps in development that may be the same (see Fig. 74.5). The human nephron complement may differ between individuals, particularly with lower total nephron number with variability in number based on prematurity, extreme prematurity (<28 weeks gestation), low birth weight (<2500 g), and other gestation events such as in utero exposure to maternal factors (preeclampsia, other hypertension, smoking, malnutrition, diabetes, and other maternal conditions).[3]

Glomerular Development

Glomerular formation occurs in stages and is dependent on specific cell type signaling within the glomerular capillary. Malformation of any component of glomerular development may result in decreased nephron filtration ability, typically characterized by proteinuria.



• **Fig. 74.5** Comparative timeline of kidney development between human and mouse. The human (A) and mouse (B) kidney illustrating the anatomic differences. The human kidney consists of 8–15 lobes, each with a branching ureteric tree and inner medulla (*IM*; papilla), while the mouse kidney is unipapillate. (C) Comparative developmental timeline of human and mouse nephrogenesis identifying the duration of gestation, timing of initial ureteral bud (*UB*) outgrowth, period of ureteric branching, and period of nephron formation. Note the prolonged period of nephron formation in the human after the end of branching in comparison with the mouse. Note also that the final nephron formation in the mouse occurs in the immediate postnatal period. *C*, Cortex; *dpc*, date post conception; *OM*, outer medulla, *P*, pelvis. (From Little MH. Improving our resolution of kidney morphogenesis across time and space. *Curr Opin Genet Dev*. 2015;32:135–143.)

Development of the glomerulus is intricately linked with the development of the renal tubule. Glomerular formation begins at the S-shaped body stage of nephron development. Glomerular endothelial progenitors are found adjacent to the nephron-developing podocytes at the cleft of the S-shaped structure during the early stage of nephron development. An initial capillary loop will ultimately mature into six to eight capillary loops representing the mature glomerular capillary tuft. Migration of glomerular endothelia progenitors is likely due to VEGF secretion by the local podocytes. The amount of VEGF released by the podocytes controls capillary development through proangiogenic and antiangiogenic signaling.[7] It is critical that these podocyte foot processes connect tightly or the filtrate from the afferent arteriole will allow leakage of larger molecules such as protein and larger solutes,

causing loss of important proteins, fluid, and solutes that should be returned to the systemic vasculature. These foot processes are integral to the glomerular filtration barrier.

Vasculogenesis starts during the S-shaped body stage of glomerular development when expression of several VEGFs from the glomerular podocytes occurs, inducing migration of endothelial cells to the cleft of the S-shaped body. VEGF-A secretion by the podocytes of the S-shaped body causes fenestration of the podocytes near the glomerular basement membrane in close proximity to the glomerular endothelial cells, possibly allowing limited transmission of fluid from the vascular tuft into the glomerulus, but the function and mechanism of these fenestrations are not well understood.

WT1 is a zinc finger transcription factor, with four different isoforms isolated to date. It is thought to be a transcription factor that binds to DNA as well as messenger RNA as either a repressor or an activator in different organs during fetal development. Studies in mice showed that WT1 is important for genitourinary development. In humans, WAGR syndrome (Wilms' tumor, aniridia, genitourinary abnormalities, and mental retardation) is caused by interstitial deletion of the 11p13 locus, where the *WT1* and *PAX6* genes are located. Similarly, *WT1* mutations are thought to contribute to the abnormalities seen in Denys-Drash syndrome (mesangial sclerosis, Wilms' tumor, and gonadal dysgenesis). Studies of WT1 absence or mutations have been done extensively in mice, showing that WT1 is essential for podocyte development and possibly for podocyte maintenance. Further study in humans is needed to better understand WT1 interactions in normal genitourinary development as well as what aberration of WT1 expression does in disease states.

Ureteral Growth and Development

The ureters are muscular tubes conveying urine from each renal pelvis to the urinary bladder. The upper ureter, leaving the kidney at the renal pelvis, is a thin-walled, funnel-shaped tube that thickens as it passes through the abdomen and enters the bladder obliquely to terminate at the bladder trigone. There are two tissue components to the ureter: the specialized endothelial-lined lumen and the outer mesenchymal coat consisting of the lamina propria, multilayered smooth muscle, and the outer tunica adventitia. This outer tunica adventitia also contains ascending and descending blood vessels, nerves, and lymphatics. The ureter does not passively allow the urine to drain from the kidney to the bladder but directs the urine to the bladder with unidirectional peristaltic contractions propelling the urine to the bladder. The peristaltic waves, triggered by pacemaker cells, cause depolarizing contractions in the smooth muscle cells located in the ureteropelvic junction.

Abnormalities in the smooth muscle function, particularly in the ureteropelvic or ureterovesical junctions, prevent efficient drainage of urine from the renal pelvis to the bladder and can cause either urinary efflux or urinary reflux. Obstruction at either the upper ureter or the lower ureter, with dilation of the ureter (hydroureter) or renal pelvis (hydronephrosis), can cause damage to the renal parenchyma, with marked decrease or even absence of function if obstruction is severe. These conditions comprise a large subset of congenital anomalies of the kidney and urinary tract, and are frequent manifestations of underlying genetic defects. Diagnosis of such defects is now frequently made before birth with prenatal ultrasound imaging.[13]

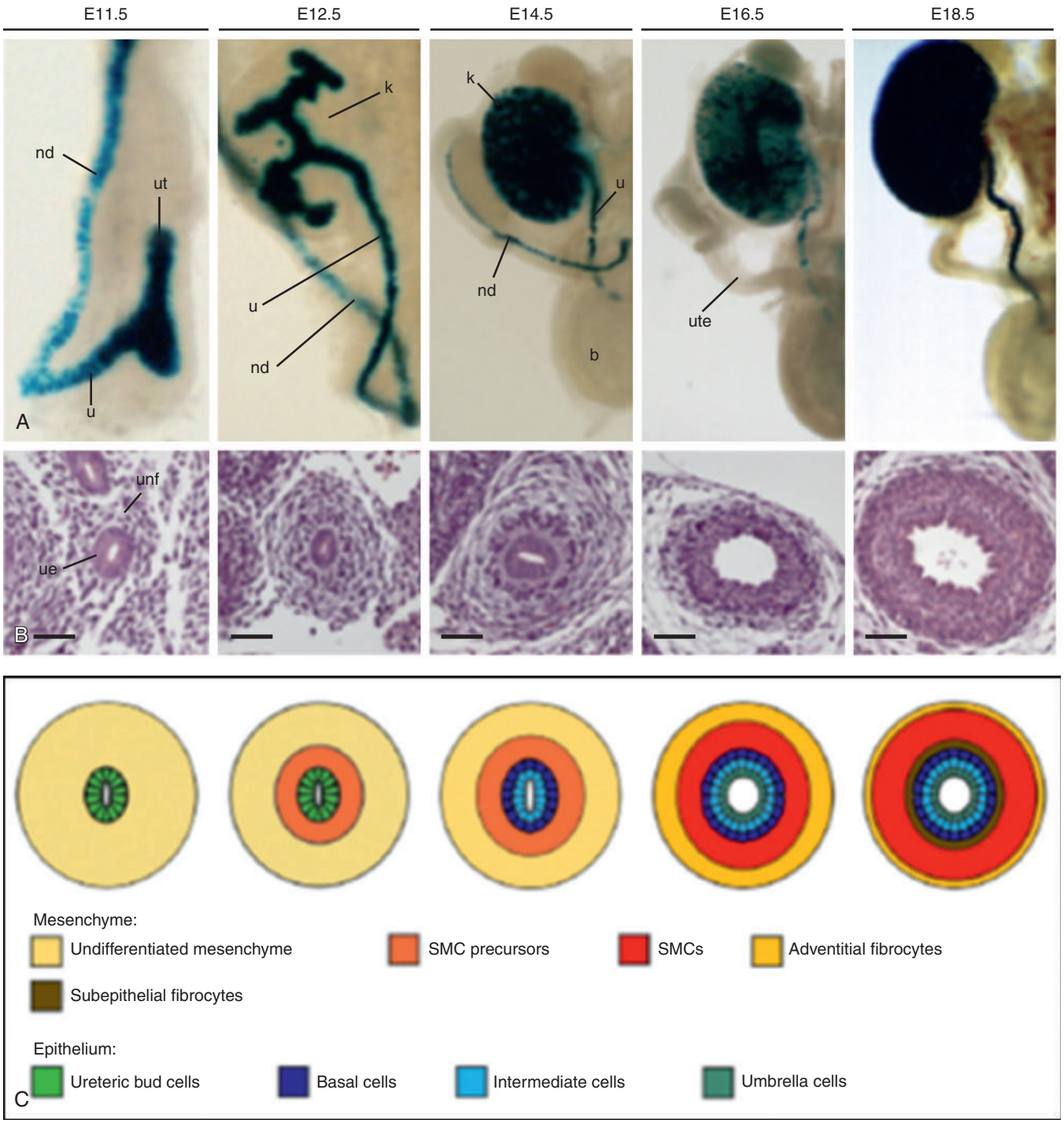
The ureters arise from the nephric duct, as do the metanephric kidneys. The ureteral bud evaginates from the nephric duct and becomes the ducting system in the kidneys ending as the collecting duets through extensive branching and elongation, and the stalk portion of the ureteral bud becomes the epithelial portion of the ureter. In the human, starting on embryonic day E12.5, the distal end of the stalk separates from the nephric duct to integrate into the urinary bladder and separate from the genital tube structures. Urine production starts around E16.5, by which time the epithelium around the ureteral stalk differentiates into urothelium. The development of the smooth muscle layer starts around E13.5 to E15.5.

The development of the ureter depends on ureteral bud development and on GDNF binding to RET, which activates the formation of the endothelium from the tissue near the nephric duct. If the GDNF signaling is anterior or posterior to the normal placement of the ureteral bud, the ureters may develop ectopically in the bladder or urethra.[13] The primordial ureters that arose from the nephric duct will differentiate into the vas deferens in the male, while they degenerate in the female.

To remain patent, the ureter must translocate from the nephric duct into the developing bladder wall in the cloaca. The first step occurs when the nephric duct fuses to the cloaca. This may occur from mutual signaling between the nephric duct to the cloaca. The precise mechanisms on this translocation are poorly understood. In the second step, the distal part of the ureter is incorporated into the bladder wall, where it undergoes apoptosis until the ureter is placed in its final position on the bladder trigone. If there is aberrant distal ureteral maturation, the ureter will end blindly inside or outside the bladder as a ureterocele or an ectopically located ureter in the bladder, urethra, or other ectopic location along the genitourinary tract following the path of the involuting nephric duct. These ureters are hydronephrotic.

Further formation of the proximal, mid, and distal parts of the ureter depends on the local epithelia and mesenchyme tissue in the region of that portion of the developing ureter. The mesenchyme near the distal ureteral stalk will elongate, while the same mesenchyme transplanted to the proximal ureteral region will branch and induce nephrogenic aggregates that will lead to nephron-like structures. The signaling molecule for distal ureteral development through mesenchymal specialization is likely bone morphogenetic protein 4 (BMP4). Absence of or a decrease in the level of BMP4 leads to ectopic budding of the nephric duct and the distal ureteral stalk. In the human, by E12, experiments on explant metanephric cell cultures have shown that the ureteral mesenchyme is radially arrayed with connective tissue and smooth muscle cells that depend on the adjacent epithelium to develop as the distal ureter. Signaling by sonic hedgehog (Shh) expressed by the ureteral epithelium at E11.5 to E18.5 also stimulates development of the ureter by increasing mesenchymal growth of the ureter. BMP4 appears to stimulate Shh to help differentiate the smooth muscle cells into the developing ureter (Fig. 74.6).

At E16.5, terminally differentiated smooth muscle cells with abundant contractile filaments are located all along the developing ureter. The ureteral smooth muscle differentiation occurs from the distal ureter near the ureterovesical junction and ascends to the upper ureter to the ureteropelvic junction.[14] These muscle fibers are tightly packed and interconnected with gap junctions, causing smooth muscle contractions to be propagated in proximal to distal waves. The waves are coordinated and are preceded by spontaneous electrical activity in the renal pelvis. This is independent of nerve function or stimulation.[13] The smooth muscle cells in this region show spontaneous oscillations of a membrane potential



• **Fig. 74.6** Ureter morphogenesis and differentiation. (A) Visualization of the ureteric epithelium by β-galactosidase activity from a *Pax2*(8.5)-*lacZ* transgenic line. (B) Hematoxylin and eosin stainings on transverse 5-μm proximal ureter sections. *Scale bars* represent 50 μm. (C) Mesenchymal and epithelial differentiation in ureter development. Stages are as indicated. *b*, Bladder; *E*, embryonic day; *k*, kidney; *nd*, nephric duct; *SMC*, smooth muscle cell; *u*, ureter; *ue*, ureteric epithelium; *unf*, ureteric mesenchyme; *ut*, ureter tip; *ute*, uterus. (From Bohnenpoll T, Kispert A. Ureter growth and differentiation. *Semin Cell Dev Biol*. 2014;36:21–30.)

that spreads to the other smooth muscle cells and generates the wave down the ureter to the distal part of the ureter.

The urothelium itself is highly specialized stratified epithelium, which is the inner lining of the urinary drainage system from the renal pelvis to the proximal part of the urethra. Molecular signals from the ureteral epithelial cells requires close interaction with the ureteral mesenchymal cells that ultimately allow the primitive ureteral epithelial cells to mature into multilayer urothelial cells that are impermeable to urine through uroplakin proteins in urothelial plaques in the final mature urothelial layer.[14] The derivation of

the epithelium in the bladder is endoderm in nature, while the ureteral epithelium is mesodermal in origin.

Renin–Angiotensin System Interaction for Programming Fetal Development

The RAS is a systemic hormonal process mediating sodium reabsorption, vasoconstriction, aldosterone production, and vasopressin secretion. Angiotensinogen is produced by the liver and activated by renin (from the kidney) to form angiotensin I. Angiotensin I is cleaved by angiotensin-converting enzyme (ACE) to create angiotensin II, which is the most biologically active peptide. Angiotensin II acts on the angiotensin type 1 (AT1) and angiotensin type 2 (AT2) receptors.

Beyond the circulating RAS, there is also a local RAS in many other organs which acts as an autocrine or a paracrine agent.[15] The RAS plays a critical role in the growth, development, and functioning of many organs, including the kidney and the placenta. During renal angiogenesis, angiotensin II, acting on AT1 receptors, mediates renal tubular growth and branching. AT2 receptors in the fetal kidney act as a growth mediator to prevent uncontrolled renal tubule growth through apoptosis. The uteroplacental circulation local aids RAS-assisted placental angiogenesis and modulates placental cytokine, growth factor, and vasoactive substance production, which will all affect fetal growth and development. Angiotensin II–stimulated AT1 receptor activity promotes trophoblast invasion, angiogenesis, growth, and branching of the placenta, and also regulates vasoconstriction in the uterine spiral arteries. AT1 receptors have also been found in the chorionic villi, regulating function of fetal blood flow within the placenta.

Studies have shown that sex hormones differentially regulate the RAS pathway: testosterone increases the expression of renin and AT1 receptor with upregulation of the vasopressor arm, and estrogens increase the upregulation of the vasodepressor arm (ACE2 and AT2 receptor).[15] The renal RAS and the placental RAS may be differentially expressed in male and female tissues, may respond differently to adverse stimuli, and may result in differences between adult males and females.

Prenatal exposure of the fetus to excessive glucocorticoids may have adverse effects. Glucocorticoids are potent regulators of fetal growth and development. Short course use during pregnancy for women at risk for preterm birth has improved neonatal survival but repeated doses has been associated with adverse fetal growth. There has also been increased evidence that antenatal glucocorticoid treatments have long-term adverse metabolic, cardiovascular and renal consequences including reduced GFR and increased insulin resistance in young adults who had prenatal glucocorticoid exposure.[4]

Renal Ascent

Renal ascent from the sacral region to the final renal position near the first lumbar vertebra has been thought to be due to the straightening of the body due to lumbosacral vertebral growth and decreased flexion of the lumbar spine. The changing vascular supply to the kidneys during ascent has been thought to be due to segmental vessels attaching to the migrating kidney that arise and disappear until the final most superior vessel that attaches to the kidney becomes the renal artery.

Renal ascent has also been associated with a change in orientation of the renal hilum from a ventral orientation to a 90-degree rotation, to a medially orientated renal hilum.

Recent studies have brought these theories into question. In recent studies, the final renal artery did not appear until after the kidney migrated to the first lumbar area and the segmental vessels disappeared as the kidney was migrating.[16]

Studies on human 5- to 7-week gestation embryos found that the developing metanephros, composed of several oval shaped pelvises, was surrounded by a belt of mesenchymal tissue, with the superior pole of the kidney adjacent to this belt of tissue. This band extended superiorly to developing adrenal cortex and is composed of densely developing nerve twigs connecting to major splanchnic nerves and is thought to be the origins of the celiac ganglia.

In the embryos studied, the kidneys were long and extended along the lengths of second or third to the fifth lumbar vertebrae and always adjacent to the umbilical artery. The mesonephros was also identified on the specimens caudal to the metanephros. The dense band connecting the adrenal cortex, the likely celiac ganglia, and the metanephros formed a solid band and allowed ascent of all the organ structures at the same time regardless of the change in curvature of the developing lumbosacral vertebrae. The researchers found that the kidney had reached its final first lumbar location while there was still ventral spinal curvature. They speculated that the migration of the kidney depended on renal growth itself and not on spinal growth and body straightening. Once the kidney reached its final location, the dense band connecting the adrenal cortex and developing celiac ganglia dissipated, and the kidney was then separated from the developing adrenal gland and celiac ganglia by an investing adipose capsule (Fig. 74.7).

They also found that the ladder of arteries thought to allow blood flow to the developing kidney were attached to the mesonephros and not adjacent to the migrating metanephros. This suggests that the proximity of the metanephros to the umbilical artery may be the source of blood flow to the developing kidney until it reaches its final vascular supply in the developing renal artery.

As nephrogenesis continues to occur in the metanephros after it reaches its final position, the glomerulus may not need direct blood flow while the kidney is ascending to the first lumbar region (Fig. 74.8).

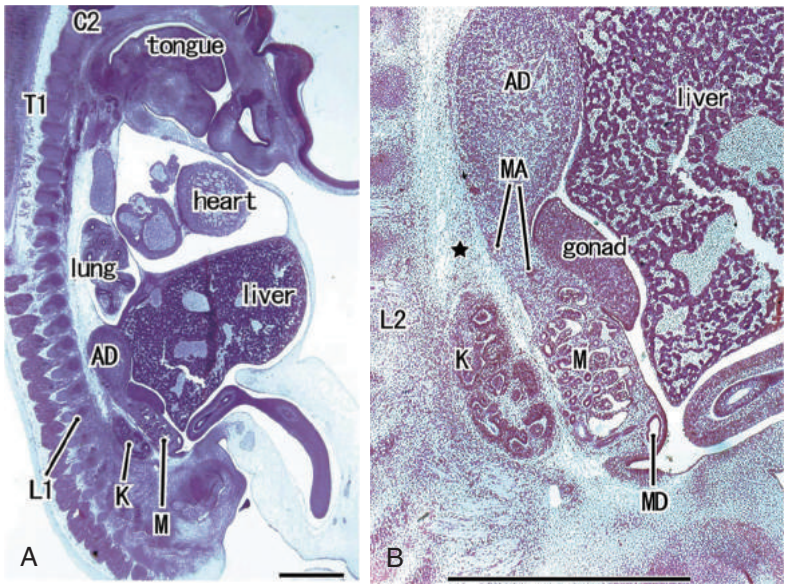
Fetal Programing of Renal Function and the Perinatal Environmental Factor's Influence

Development of Renal Function and Adult Renal Disease

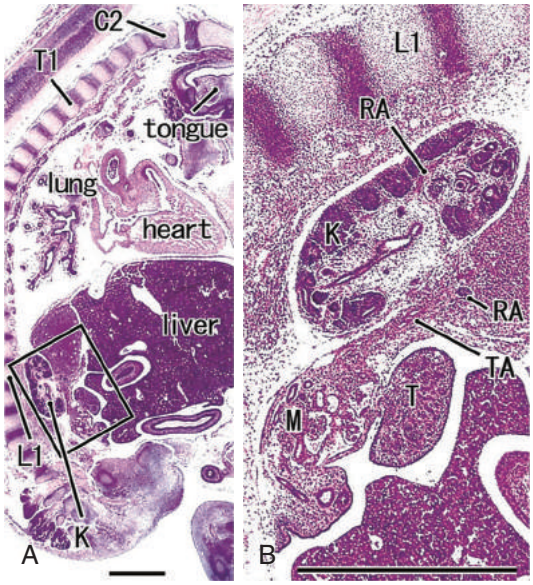
Genetic and environmental factors are important determinants of the development and function of the major organ systems of the body. It is becoming more and more evident that prenatal programing can affect subsequent organ function and some adult diseases.

Preterm birth (gestational age <37 weeks) has become more prevalent over the past few decades with incidence of prematurity up to 14% of live births worldwide. Over 95% of those born prematurely, including extreme premature infants (gestation <28 weeks), have survived to adulthood (>18 years).[3]

Although most of these preterm patients have low risks for early mortality, there are increased risks of chronic diseases involving



• **Fig. 74.7** Ascending right kidney in two embryos at 5 weeks' gestation. (A) A kidney (K) between the levels of the second to fourth lumbar vertebrae. (B) A higher magnification view of the kidney shows band-like tissue that connects the embryonic adrenal cortex and the kidney (star). *AD*, adrenal; *C2*, second cervical vertebra; *K*, kidney; *L1*, first lumbar vertebra; *L2*, second lumbar vertebra; *M*, mesonephros; *MA*, mesonephric artery; *MD*, mesonephric duct; *T1*, first thoracic vertebra. (Adapted from Fukuoka K, Wilting J, Rodríguez-Vázquez JF, et al. The embryonic ascent of the kidney revisited. *Anat Rec*. 2019;302:278–287.)



• **Fig. 74.8** Right kidney in male fetus in its final position at 7 weeks' gestation. Sagittal sections of an embryo with crown rump length (CRL) of 24 mm. (A) The rightmost plane. (B) Higher magnification view of the kidney and embryonic adrenal cortex. *C2*, Second cervical vertebra; *K*, kidney; *L1*, first lumbar vertebra; *M*, mesonephros; *RA*, definite renal artery; *T*, testis; *T1*, first thoracic vertebra; *TA*, testicular artery. (From Fukuoka K, Wilting J, Rodríguez-Vázquez JF, et al. The embryonic ascent of the kidney revisited. *Anat Rec*. 2019;302:278–287.)

the cardiovascular, renal, endocrinologic, metabolic, respiratory, and neurodevelopmental systems. These chronic conditions may be responsible for moderately increased mortality risks (30% to 50%) from early to mid-adulthood when compared to patients born at or close to term.[3] Preterm birth increases the likelihood of the infant being born before nephrogenesis can be completed. Nephrogenesis is most active during the third trimester of pregnancy where more than 60% of the functioning nephrons are formed.[3] Interrupting this process will cause a lower lifelong complement of nephrons increasing the likelihood that the patient will develop chronic kidney disease (CKD) and hypertension later in life. Although the nephrons continue to form after premature birth, nephrogenesis ceases with premature birth.

Males and females are similarly and equally affected by this risk of chronic kidney disease. Low birth weight infants (<2500 g) have also been found to have an increased risk of developing CKD regardless of gestational age. Extremely premature infants and small for gestational age (SGA) infants additionally are at higher risk for cardiovascular and renal diseases.[2,4]

The risk of intrauterine growth restriction (IUGR) and low birth weight (LBW) is twice as high in African Americans as in white populations. Risk factors for IUGR and LBW are consistently found more often in certain populations. Fetuses have a higher risk of IUGR and LBW when associated with maternal hypertension, maternal smoking, inadequate maternal weight gain or malnutrition, shorter maternal height, poor prenatal care, advanced maternal age, and lower socioeconomic status. Congenital anomalies, maternal obesity (BMI ≥30) and preeclampsia has also been found to have a higher risk of CKD in prematurely born infants.

Low birth weight and premature birth is associated with nephron deficit in infants. Nephrogenesis is completed around 34 to 36 weeks' gestation, with 60% of the nephrons being formed in the third trimester, especially between 28 and 34 gestational weeks. Brenner hypothesized, because of the lower overall nephron number in these infants, that each nephron available has to increase its filtration rate to meet the body's excretory demands and, in order to meet necessary renal sodium excretion, there must

be a commensurate increased blood pressure increasing likelihood of eventual development of essential hypertension.[4]

Human studies have found an inverse relationship between LBW and higher blood pressures in infancy through adulthood. Although not all children with LBW have hypertension, the blood pressure measurements in LBW infants, children, and young adults tend to be higher than those of normal birthweight individuals. High birthweight (>4000 g) has also been associated with adverse renal outcomes in later life. It is uncertain in humans if there is any relationship with abnormal nephron number. LBW infants also have an increased adult risk of coronary heart disease, insulin sensitivity, and hypertension. Females have approximately 12% fewer glomeruli then males, although, generally, the risks for CKD due to prematurity affect males and females equally. There are some conflicting data as to specific gender risk for CKD after premature birth. A Swedish study found prematurely born women were more likely to develop CKD than similarly prematurely born men, while another study found a 1.3-fold increased risk for CKD in premature males as compared to premature females.[2]

Additionally, IUGR is associated with about 30% to 35% nephron deficit, and these infants may develop secondary glomerular hypertension and nonnephrotic focal segmental glomerulosclerosis (FSGS).[4]

The numbers of nephrons are determined before birth, but the kidney can increase filtration capacity by hypertrophy of the glomerulus. Mean glomerular volumes correlate inversely with glomerular numbers but directly with current body size. Hypertensive individuals have a 133% higher mean glomerular volume, but a 46.6% reduction in glomerular numbers, compared with nonhypertensive controls.

Perinatal programing controls nephrogenesis from early fetal development until 34 to 36 weeks' gestation, at which time the full nephron development is complete. If an infant is born before 36 weeks' gestation, nephrogenesis is still ongoing, but the number of nephrons produced may be reduced because of intrauterine stress or prenatal/perinatal stressors on development. Although most of the risk for CKD is in infants born with extreme prematurity (<28 weeks) and premature birth (<37 weeks), there still is a significantly increased risk of CKD in infants born at early term (37 to 38 weeks). This increased risk appeared to be due to the direct effects of preterm birth and not due to shared genetic or environmental factors when co-siblings of prematurely born infants were also studied for CKD risk.[2]

Premature infants have their in utero environment abruptly terminated before full fetal development with immature organ development and need to adapt to extrauterine function and the stressful environment of an early birth. Early postnatal life has a different physiology from the in utero conditions that the fetus is accustomed to experiencing, and the premature infant is generally exposed to intensive care management, including various drug exposures, suboptimal nutrition, and possible life support measures.

Short course antenatal glucocorticoids are used frequently in women at risk for preterm birth to help with fetal lung maturation and to reduce neonatal morbidity and mortality seen with preterm birth. Although there may some benefits with repeated doses, there is a decreased GFR and increased insulin resistance in young adults born prematurely who were exposed to even one short course of prenatal glucocorticoids.[4]

Initial suboptimal nutrition is common in premature infants who have lost their in utero nutrition prior to normal development of feeding and the gastrointestinal organs where many premature infants have feeding issues and may suffer from extrauterine growth restriction (EUGR). EUGR was found to effect early postnatal growth with associated poor outcomes early in life and had adverse long-term cardiovascular and renal outcomes. To prevent EUGR, neonatal intensive care units have shifted to increased protein and energy formulas for infants postdischarge to help with catch-up growth. Catch-up growth may manifest as asymmetric overgrowth and rapid weight gain up to 3 months after hospital discharge and has been associated with high fat deposition and altered insulin sensitivity.

These high protein formulas may have adverse short- and long-term effects on renal and cardiac function due to the increased protein load exposure in the premature heart and kidneys.

These high protein formulas are both seen as problems of EUGR in premature infants who are relatively calorie and protein deficient without the rich in utero nutrition source, and then have later adverse effects from high protein diets with subsequent cardiac, metabolic, and renal damage, while used trying to overcome EUGR.

A randomized control trial that breastfed premature infants had lower blood pressure and better insulin sensitivity as adolescents than those premature infants who were formula fed. Further study is needed to better determine if there are any long-term adverse effects of high protein formula feeds as these premature infants become adults.[4]

Adverse environmental prenatal conditions such as malnutrition of the mother through low protein intake during intrauterine and neonatal life may have an adverse effect on renal function. Vitamin A has been found to determine fetal renal programming in rats through modulation of nephron number and vascular supply to the developing kidney. Vitamin A and other retinoids help regulate cell proliferation, differentiation, immune function, and apoptosis. Low circulating levels of vitamin A are common in women who are smokers, abuse alcohol, or have poor perinatal nutrition, which may cause low or absent vitamin A levels in the developing fetus and is associated with IUGR in infants.

Maternal intake of nephrotoxic drugs during pregnancy has also been associated with neonatal development of acute renal failure, especially in preterm or LBW infants. It is postulated that maternal exposure to non-steroidal anti-inflammatory drugs (NSAIDs) may cause hypoperfusion of the kidneys and fetal nephrotoxicity from vasomotor nephropathy due to decreased levels of prostaglandin E_2. There appears to be less damage to the newborn kidney with NSAID exposure, suggesting that the risk of damage is highest during nephrogenesis itself. Other fetoplacental factors causing a decrease in the necessary nutrients by restricting their transfer include maternal smoking, hypertension, poor nutrition, and socioeconomic factors affecting nutrition.

Perinatal obstructive uropathy may cause reduced nephron numbers in affected infants. The diminution of nephrons with obstruction may be due to mechanical stretching of the tubules, which in turn activates ion channels that increase intracellular calcium levels and, subsequently, cellular apoptosis. In animal models with unilateral ureteral obstruction, the major damage seen after relief of the obstruction was tubular apoptosis and atrophy, causing subsequent impaired growth of the previously obstructed kidney, reduced glomerular numbers, and decreased GFR. The uninvolved kidney showed compensatory growth, but both kidneys showed glomerular sclerosis, tubular atrophy, macrophage infiltration, and interstitial fibrosis. Urinary obstruction in adults does not appear to cause reduction in the nephron number as

is seen in congenital or perinatal obstruction. There may be an increased vulnerability of the nephrons to damage at this early phase of renal development with obstruction occurring during or just after completion of nephrogenesis. High-grade vesicoureteral reflux diagnosed shortly after birth may also be associated with poor renal outcome. Early postnatal diagnosis and treatment does not improve the renal outcomes in this group of patients.

Although low nephron number is associated with renal disease and hypertension, not all renal programing models show this; some studies have found associated hypotension. Some ethnic groups, for example, African Americans, with decreased nephron numbers have not exhibited associated hypertension. Some other as yet undetermined factors may be required to induce hypertension and renal disease. Some factors that may or may not have an association with low nephron number and hypertension/renal disease may include individual genetics, including the sex of the individual, with less hypertension generally seen in premenopausal females with decreased nephron numbers. Sex differences in arterial pressure may be due to sex hormones, as well as sex chromosomal complement. Female fetuses also appear in some studies to be less vulnerable to adverse in utero environmental factors than male fetuses.

New research into the mechanisms affecting organ system development due to premature birth and neonatal and perinatal conditions is focusing on epigenetics.

Epigenetics look at gene modifications due to environmental factors causing changes in gene expression while not changing the genetic code itself. There is increasing evidence that early epigenetic imprinting tracks early gene interaction with the environment and converts that imprinting into changes in gene expression allowing cellular differentiation based on which genes are activated and which are not. Arrested or altered development due to premature birth and delivery, where different postnatal stimuli due to stress, extra-uterine nutrition and toxicant exposures, changes gene regulation and may cause lifelong effects. Early studies are targeting epigenetic modifications of the angiogenic capacity of preterm infant progenitor cells and are the basis of studies into epigenetic expression in different organ systems, like the renal and cardiovascular systems. These studies show epigenetic alterations of DNA methylation, histone modifications, and non-coding RNAs affecting changes in gene expression in development and changes seen due to premature birth. Antenatal glucocorticoid exposure has been shown in animal models to alter gene methylation and elevate blood pressure, leading to concern about similar epigenetic manifestations in fetuses exposed to steroids before birth.[4]

Although fetal renal programming may put the LBW, IUGR, and individuals with in utero environmental exposure to renal damaging factors at risk of hypertension and poor renal function as an adult, there appears to be a significant ability of the kidney to compensate for these prenatal insults to normal renal development. Controlling secondary risk factors, such a diet and stress in the adult, may possibly reduce the adverse outcomes of hypertension and renal insufficiency that may arise from abnormal renal development.

Conclusion

Renal organogenesis is a complex process that is not yet fully understood but has complementary interactions stimulated by anchor genes, activator molecules, growth factors, and hormone effects. Epigenetic factors in the maternal-placental-fetal environmental interface are being further studied with implications in adverse adult health outcomes. Recent studies on adults who were born prematurely or were LBW infants show that they have a high subjective quality of life similar to age-matched full term or normal birth weight adults. Current survival of over 50% of premature birth adults without major comorbidities reflects well on advancements in early treatment interventions and in the resilience of this patient group in trying to maintain as good health as possible.

Suggested Readings

Andrew DJ, Yelon D. Editorial overview: developmental mechanisms, patterning and organogenesis. *Curr Opin Genet Dev*. 2015;32:v–viii.

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78

Chronic Kidney Disease

LAUREL WILLIG AND BRADLEY A. WARADY

KEY POINTS

- Kidney development continues until 34 weeks' gestation. Neonatal intensive care unit graduates, especially those with a history of acute kidney injury (AKI), premature infants, and those with intrauterine growth retardation, are at risk for long-term chronic kidney disease (CKD).
- Clinical sequelae of CKD include anemia, acidosis, electrolyte abnormality, growth restriction, renal osteodystrophy, fluid overload, hypertension, and uremia. Attention to these complications is critical to optimizing long-term outcomes.
- Long-term survival of neonates with end-stage kidney disease (ESKD) appears to be approaching that of older infants and young children, but they continue to have higher morbidity and mortality due to infectious and cardiovascular complications.

Chronic Kidney Disease

Neonatal chronic kidney disease (CKD) is diagnosed when sustained derangements of glomerular filtration or tubular function occur with minimal to no resolution over time. According to guidelines published by the Kidney Disease Outcomes Quality Initiative (KDOQI), CKD is present if there is evidence of kidney damage for more than 3 months, as defined by structural or functional abnormalities, with or without decreased glomerular filtration rate (GFR), or a GFR less than 60 mL/min/1.73 m^2 for more than 3 months in children older than 2 years with or without kidney damage.[1] These guidelines do not apply to infants less than 2 years of age as a result of ongoing maturation of the kidney and improvement in GFR over the first 2 years of life. In turn, the Kidney Disease: Improving Global Outcomes (KDIGO) guidelines from 2012 recommend that for the classification of CKD in neonates and infants, available normative values and conventionally accepted equations should be used to classify neonatal CKD into one of three categories: normal (GFR <1 standard deviation [SD] below the mean); moderately reduced (GFR >1 SD to ≤2 SD below the mean), or severely reduced (GFR >2 SD below the mean).[2]

Currently, none of the commonly used pediatric GFR estimating equations is validated for neonates with CKD. Studies in healthy neonates suggest that equations incorporating the use of cystatin C, renal mass, and body surface area may provide a more accurate assessment of GFR.[3] Whereas a new equation, the so-called U25 equation, utilizes age and sex-specific constants to improve the estimation of measured GFR in children with CKD, this equation has not been studied in neonates.[4]

Epidemiology

There is little information on the incidence and prevalence of CKD in neonates and infants due to the lack of a uniform definition. In one small study, the estimated incidence of CKD was 1: 10,000 live births with a male-to-female ratio of 2.8:1, skewed primarily due to lower urinary tract obstruction as a leading cause of CKD.[5–7]

Most epidemiologic reports focus on the development of end-stage kidney disease (ESKD) in this age group. These studies demonstrate a varying regional incidence of ESKD, and the worldwide incidence is unknown. The European Registry for Children on Renal Replacement Therapy collects data from countries across Europe and recently reported the incidence rate of ESKD in children aged 0 to 4 years to be around 5.2 per million children.[8] Within the International Pediatric Peritoneal Dialysis Network (IPPN) registry, this same age group (0 to 4 years) accounts for approximately 35% of all pediatric patients on peritoneal dialysis.[9] The adjusted incidence rate of ESKD in the less than 1 year age group, as reported by the United States Renal Data System (USRDS), was 23.2 cases per million US population in 2018.[10] Studies show that wealthier countries, those that spend more on health care, and countries where patients pay less out of pocket expenses have higher rates of kidney replacement therapy (KRT) initiation. Thus, much of the variability is likely explained by socioeconomic factors and less by genetic susceptibility to kidney disease.[8]

Pathophysiology

Congenital Causes of Neonatal CKD

Congenital Anomalies of the Kidney and Urinary Tract

Congenital anomalies of the kidney and urinary tract (CAKUT) are the leading cause of birth defects in neonates and infants, with an incidence of 0.4 per 1000 births.[11] They account for approximately 50% to 60% of all cases of ESKD in those children less than 1 year of age.[10,12,13] Posterior urethral valves/obstructive uropathy cause 21% of cases, and renal hypo/dysplasia is responsible for an additional 28% of these cases.[14] Historically, the etiology of CAKUT was considered sporadic, polygenic, or non-heritable. However, several genes are associated with CAKUT development, primarily in an autosomal dominant pattern.[15] Additionally, many pediatric patients with CKD have copy number variations.[16] Many

of these kidney anomalies occur in the setting of co-existing extrarenal manifestations, and those cases often tend to have a higher rate of associated morbidity and mortality.

Polycystic Kidney Disease and Ciliopathies

Cilia are complex, flagella-like organelles found in most mammalian cell types, which have a wide variety of functions. Due to their wide distribution amongst cell types and their diverse functions, mutations in ciliary proteins lead to a variety of pleiotropic clinical manifestations. Mutations in ciliary proteins cause many different kidney diseases, including polycystic kidney disease (PKD), nephronophthisis, and Bardet-Biedl syndrome. The most common renal ciliopathy is autosomal dominant PKD (ADPKD). It affects about 1 in 1000 people, and most cases are caused by mutations in PKD1 and PKD2. While most cases do not present until later childhood or adulthood, severe cases may present in utero and mimic autosomal recessive PKD (ARPKD).[17] ARPKD, caused primarily by PKHD1, is much less common, affecting about 1 in 10,000 to 1 in 40,000 births. It presents in utero or in the neonatal period with large echogenic kidneys. The severity of kidney disease in ARPKD varies from ESKD at birth to slowly progressive CKD, with most children progressing to kidney failure by 20 years of age. Approximately 30% of patients presenting in the neonatal period die primarily from pulmonary hypoplasia.[18] Other ciliopathies such as Bardet-Biedl and Joubert syndrome often present with extrarenal manifestations. Other causes of neonatal CKD include prune belly syndrome, HNF1B disease, congenital nephrotic syndrome, and neurogenic bladder from spinal cord defects.

Acquired Causes of Neonatal Chronic Kidney Disease

Prematurity and Low Birth Weight

Preterm neonates are at high risk for the development of CKD for three reasons: (1) they have a smaller complement of nephrons at birth as a result of disruption of nephrogenesis, which typically continues to 36 weeks; (2) increased risk of acute kidney injury (AKI) due to medications and comorbidities often associated with premature birth; (3) prenatal kidney disease leads to an increased risk of premature delivery either as a result of direct complications such as oligohydramnios, or the presence of extrarenal comorbidities found in syndromic prenatal kidney disease. In a small German study, 53% of children with CKD were premature, a figure significantly higher than the rate experienced by the total infant population of Germany.[19] A publication from the Chronic Kidney Disease in Children (CKiD) cohort also revealed a high prevalence of children with CKD; the lifetime risk for CKD was increased in those neonates who had an abnormal birth history as defined by low birth weight (17%), small for GA (14%), or prematurity defined as GA less than 36 weeks (12%).[20] In a study of more severe CKD, 35% of affected patients were born prematurely, and approximately 50% had a comorbidity, such as cardiopulmonary and/or neurologic involvement.[5]

A systematic review and meta-analysis in 2009 concluded that low birth weight babies (≤5.5 lb) were 70% more likely to develop CKD later in life compared with individuals with normal birth weight.[21] More recent studies show that the incidence of CKD in preterm infants (<37 weeks' gestation) by mid-adulthood is 9.24/100,000 person-years (py), 5.90/100,000 py for those born at 37 to 38 weeks, and 4.64/100,000 py for those born full term. Low birth weight in the setting of prematurity independently increases the risk for CKD.[22]

Decreased nephron number either due to deranged nephron development and/or scarring leads to an increased risk for CKD regardless of etiology and likely underlies much of the increased risk conferred by premature birth. Total GFR is determined by the filtration rate of single nephrons and the number of nephrons present. When the number of nephrons is diminished, single nephron GFR increases as the kidney works to compensate for the low nephron numbers. This compensatory hypertrophy causes glomeruli to function under increased intracapillary hydraulic pressure, which, over time, causes damage to capillary walls. This abnormal process leads to progressive glomerulosclerosis, proteinuria, hypertension, and CKD.[23] The hyperfiltration hypothesis has been applied and confirmed in autopsy data of hypertensive patients [24,25] and has been written about at length with reference to infants with intrauterine growth restriction.[21,26–28] Nephrogenesis continues through 34 weeks' gestation. Premature infants (even those born appropriate for GA) are therefore born with low nephron numbers compared with term infants. Using computer-assisted morphometry, Rodriguez et al. showed that premature infants who survived to at least 36 weeks' postconception had nephron numbers similar to premature infants with short survival, suggesting that the extrauterine environment does not support normal neoglomerulogenesis. In addition, preterm infants with AKI had fewer nephrons than similar infants without AKI.[29]

Acute Kidney Injury

The epidemiology, etiology, and treatment of AKI in neonates are discussed separately in this textbook. Briefly, recent studies show that thirty percent of all neonates in neonatal intensive care units develop AKI.[30] In small studies looking at the risk for CKD development in those who experienced AKI in intensive care units, 10% of all children developed CKD, while 16.6% of neonates had CKD, suggesting that AKI is more likely to lead to CKD in neonates.[30]

In addition to the loss of nephron number and hyperfiltration injury, as discussed above, tubular and vascular endothelial cellular damage occurs with prolonged kidney hypoperfusion in ischemic AKI. Animal models suggest that although tubular recovery occurs, damage to the vascular endothelial cells remains and can lead to interstitial fibrosis and progressive kidney dysfunction.[31] To further delineate the pathophysiology and extent of AKI-related CKD and to provide guidelines for long-term follow-up, further studies such as those being conducted through the Neonatal Kidney Collaborative are imperative.[32] Given the high rate of AKI and subsequent CKD within this population, future studies of interventions (such as ACE inhibition) to decrease the rate of CKD progression should be explored.

Renal Cortical and Medullary Necrosis

Renal cortical and medullary necrosis is uncommon in newborns. When it occurs, it is usually encountered in critically ill newborns as a manifestation of perinatal and postnatal stress leading to end-organ injury. Risk factors associated with renal cortical and medullary necrosis include congenital heart disease, perinatal anoxia, placenta abruption, twin–twin or twin–maternal transfusions, sepsis, infectious myocarditis, vascular malformations, dehydration, prematurity, respiratory distress syndrome, bleeding diathesis, cardiac catheterization, and use of intravenous contrast agents.[33,34] Subsequent medication administration, blood loss, and ischemia can interfere with compensatory mechanisms to maintain kidney

perfusion and can lead to acute tubular necrosis that, depending on the severity of the insult, may then lead to vascular injury and microthrombi formation with subsequent renal cortical and medullary necrosis.

The clinical and laboratory manifestations of renal cortical and medullary necrosis include hematuria, oliguria, rising serum creatinine and BUN, thrombocytopenia, and kidney enlargement, which are nondiagnostic and are associated with many other common neonatal kidney abnormalities. Initially, renal ultrasound results are typically normal but may ultimately show small kidneys that are hyperechoic for age and have a loss of corticomedullary differentiation. A radionucleotide renal scan shows decreased to no perfusion, with delayed or no function.[35] Infants with cortical necrosis may have partial recovery or no recovery at all. Typically, these infants require KRT, short-term or long-term, and even those who recover enough kidney function to be managed without dialysis early on are at risk for the late development of ESKD. Other acquired causes, such as renal vein and renal artery thrombosis, are discussed in the chapter on acute kidney injury.

Clinical Sequelae of Neonatal Chronic Kidney Disease/End-Stage Kidney Disease

Anemia

Anemia is defined as a Hgb concentration less than the 5th percentile of normal for age and sex.[36] The normative values used to define anemia in children older than 1 year are taken from the third National Health and Nutrition Examination Survey[37] database, whereas the norms for infants younger than 1 year are derived from other reference sources (Table 78.1).[38] More recently, Kidney Disease: Improving Global Outcomes (KDIGO) defined anemia as Hgb less than 11.0 g/dL in children 0.5 to 5 years with CKD.[39]

Anemia is a frequent complication of CKD in infants and children, and the prevalence of anemia increases with worsening stages of CKD, with up to 93% of pediatric patients with CKD stage 5 having anemia or receiving treatment for anemia. Anemia may lead to decreased tissue oxygen delivery, increased cardiac output, cardiac enlargement, ventricular hypertrophy, congestive heart failure, and impaired immune responsiveness.[40–42] Anemia is associated with faster CKD progression,[43] increased risk of hospitalization due to cardiovascular disease in hemodialysis patients,[44] and has a negative impact on health-related quality of life.[45]

The pathophysiology of anemia in infants and young children with CKD is primarily the result of a decrease in the kidney production of erythropoietin, iron deficiency, or both.[46] Other potential contributing factors include a shortened red blood cell life span, secondary hyperparathyroidism, hypothyroidism, folate and vitamin B_{12} deficiency, chronic inflammation, and hemoglobinopathies. In the early stages of CKD, iron deficiency tends to be common.[47] The cause of absolute iron deficiency is multifactorial and can be related to poor intake, gastrointestinal blood loss, and repeated phlebotomies for laboratory tests.

Erythropoietin deficiency becomes more prevalent in the later stages of CKD. Children with CKD may be particularly prone to factors that contribute to relative erythropoietin resistance, including hyperparathyroidism, aluminum toxicity, and hemolysis.[48] The decreased erythropoietin production from the kidney leads to anemia through increased apoptosis of red blood cell precursors in the bone marrow, often accompanied by iron deficiency related to the factors listed above, as well as increased production and decreased excretion of hepcidin.[49,50]

The National Kidney Foundation's Kidney Disease Outcomes Quality Initiative (KDOQI) guidelines recommend checking Hgb in all patients with CKD at least annually. In those children found to be anemic, the initial work-up should include red blood cell indices, reticulocyte count, white blood cell count with differential, platelet count, and iron parameters (serum iron, total iron binding capacity, and serum ferritin). These guidelines also recommend targeting a Hgb level between 11.0 and 12.0 g/dL.[39] Iron and erythropoiesis-stimulating agents (ESAs) such as recombinant erythropoietin-alfa (EPO), darbepoetin, an analogue of erythropoietin with a longer half-life, and methoxy polyethylene glycol-epoetin beta (continuous erythropoietin receptor activator, CERA) are the key elements of anemia management in CKD.[51]

Iron therapy typically consists of the provision of oral elemental iron in doses ranging from 2 to 3 mg/kg/day up to 6 mg/kg/day in two to three divided doses.[52] In hemodialysis (HD) patients, intravenous iron administration is often recommended because of inadequate iron absorption after oral administration coupled with increased losses of blood and iron during HD treatments. Levels of serum ferritin greater than 100 ng/mL and transferrin saturation values greater than 20% are believed to reflect adequate iron stores in patients with CKD.[52]

All ESAs have equal efficacy and a similar safety profile[53,54], and both epoetin and darbepoetin have been studied in premature infants.[55] When treated with an ESA, infants and young children require larger doses than older children and adults, despite having a higher capacity for hematopoiesis. This difference is thought to be related to additional nonhematopoietic erythropoietin binding sites, which are thought to be important for the inhibition of apoptosis. Also, EPO needs tend to increase at times of rapid growth, such as in infancy.[49] Children receiving HD typically require more EPO than patients receiving PD as a result of the blood loss that routinely occurs with HD.

TABLE 78.1 Hemoglobin Levels in Children Between Birth and 24 Months of Age

Age	Mean Hb (g/dL)	−2 SD*
Term (cord blood)	16.5	13.5
1–3 days	18.5	14.5
1 week	17.5	13.5
2 weeks	16.5	12.5
1 month	14.0	10.0
2 months	11.5	9.0
3–6 months	11.5	9.5
6–24 months	12.0	10.5

*Values 2 SDs below the mean are equivalent to less than the 2.5th percentile.
Data taken from normal reference values.
From National Kidney Foundation. KDOQI clinical practice guidelines and clinical practice recommendations for anemia in chronic kidney disease. *Am J Kidney Dis.* 2006;47:S88.

Malnutrition and Growth Failure

The origin of malnutrition in children with CKD is multifactorial; nausea and vomiting, delayed gastric emptying, gastroesophageal reflux, and protein energy wasting (PEW) all contribute. Seventy-five percent of patients exhibit GI complaints.[56] The International Society of Renal Nutrition and Management (ISRNM) defines PEW as reduced body mass index, reduced muscle mass, short stature, and specific biochemical evidence. Modified PEW scores that incorporate these parameters are associated with an increased risk for hospitalization, disease progression, and neurocognitive complications in pediatric patients.[57–59] Studies show that each week of stagnant weight leads to compounding weight percentile loss.[60] While being underweight is associated with higher mortality in children on PD, so too does being overweight. Almost 20% of pediatric patients initiating PD have been found to be overweight and being overweight in infancy was associated with higher mortality rates in patients receiving peritoneal dialysis.[61]

Children with CKD often experience some degree of growth failure, which may occur in children with mild-moderate CKD (CKD stage 3).[62] According to data collected by the USRDS, greater than 50% of all infants with ESKD had short stature, defined as height below the 3rd percentile for age.[10] Growth failure associated with CKD is especially concerning during infancy, and delayed growth is regularly present at the time of dialysis initiation.[12,42] Young children on dialysis have often failed to grow normally, despite meeting 100% of the recommended daily allowance of caloric and protein intake.[63,64]

Growth and nutritional outcomes of infants with ESKD have improved over time, likely because of advances in medical, nutritional, and surgical therapies.[65–67] In particular, multiple reports have described improved longitudinal growth and sustained catch-up growth in infants with CKD in whom recombinant growth hormone (rhGH) treatment was initiated in the first year of life. To that end, guidelines support the use of rhGH in infants greater than 6 months old and with CKD who have a height velocity less than the 25th percentile for more than 3 months, despite addressing all of the potentially modifiable risk factors (e.g., nutrition, acidosis, bone abnormalities).[68] Similar results have, on occasion, also been obtained with intensive nutritional regimens.[69–71] Finally, growth outcomes regularly improve after kidney transplantation in young patients.[72,73]

Nutritional Assessment

Frequent monitoring of the patient is mandatory in view of the importance of nutritional status to the outcome of the infant and young child with CKD. Collaboration with a pediatric renal dietician is beneficial to assist in the nutritional evaluation and treatment strategy.[74] An age-related schema for parameters and frequency of nutritional assessment have been published by the Pediatric Renal Nutrition Task Force (Table 78.2).[75]

Neonates with CKD are also at nutritional risk and may need more frequent monitoring if they are preterm or are characterized by any of the following: low birth weight (<2500 g), a birthweight *z* score less than −2 SD for GA, polyuria, or associated renal salt wasting. Nutritional intervention is indicated in children with CKD when there are findings of an impaired ability to ingest or tolerate oral feedings, a BMI less than the 5th percentile of height-for-age, an acute weight loss of 10% or more, or a length/height ratio more than 2 SDs below the mean.

Nutritional Management

Both spontaneous energy and protein intake decrease as CKD progresses. Energy intake is the principal determinant of growth during infancy. Energy requirements should, in turn, be 100% of the estimated energy requirement for chronologic age.[60,76,77] Similarly, targeted protein intake should be 100% of the suggested daily intake (SDI) (Table 78.3). The SDI includes the average recommended amount of a nutrient from all the available published values ± 2 SDs.[78] There is no evidence that strict dietary protein restriction has any nephroprotective effect,[79] and aggressive restriction has been noted to compromise the growth of infants with CKD.[80] Because moderate dietary protein restriction reduces the accumulation of nitrogenous waste products, decreases acid load, and helps to lower dietary phosphorus intake (which helps preclude bone-mineral and cardiovascular complications), it is

TABLE 78.2 Recommended Parameters and Frequency of Nutritional Assessments for Children With Chronic Kidney Disease Stages 3–5 and 5d

	AGE <1 YEAR (FREQUENCY IN WEEKS)		AGE 1–3 YEARS (FREQUENCY IN MONTHS)	
	CKD 3–5	CKD 5D	CKD 3–5	CKD 5D
Height or length-for-age percentile or SDS	6	2–4	2	1
Height or length (percentile or SDS)	8	4	3	2
Height or length velocity–for-age percentile or SDS	NA	NA	3	2
Estimated dry weight and weight-for-age percentile or SDS	6	4	2	1
BMI-for-height-age percentile or SDS	NA	NA	2*	1*
Head circumference–for-age percentile or SDS	6	4	2	2

*Use weight for length in children up to age 3 if unable to obtain standing height.

BMI, Body mass index; *CKD*, chronic kidney disease; *SDS*, standard deviation score.

Modified from the Pediatric Renal Nutrition Task Force: Nelms CL, Shaw V, Greenbaum LA, et al. Assessment of nutritional status in children with kidney diseases-clinical practice recommendations from the Pediatric Renal Nutrition Taskforce. *Pediatr Nephrol*. 2021;36:995–1010.

TABLE 78.3 Energy and Protein Requirements for Infants With Chronic Kidney Disease Stages 2–5D

Month	SDI Energy (kcal/kg/day)	SDI Protein (g/kg/day)	SDI Protein (g/day)
0	93–107	1.52–2.5	8–12
1	93–120	1.52–1.8	8–12
2	93–120	1.4–1.52	8–12
3	82–98	1.4–1.52	8–12
4	82–98	1.3–1.52	9–13
5	72–82	1.3–1.52	9–13
6–9	72–82	1.1–1.3	9–14
10–11	72–82	1.1–1.3	9–15
12	72–120	0.9–1.14	11–14

Modified from Pediatric Renal Nutrition Task Force. Shaw V, Polderman N, Renken-Terhaerdt J, et al. Energy and protein requirements for children with CKD stages 2–5 and on dialysis-clinical practice recommendations from the Pediatric Renal Nutrition Taskforce. *Pediatr Nephrol.* 2020;35:519–531.

TABLE 78.4 Nutritional Complications of Chronic Kidney Disease in Infancy

	Pathophysiology Mechanisms	Complications	Management Strategies	References
Fluid intake	Polyuric (CAKUT): Concentrating defect Oligoanuria (ARPKD/advanced kidney failure): Inability to excrete necessary fluid intake for nutrition leading to salt and water retention	Polyuria: Hypotension Dehydration Oligoanuria Hypertension Edema	Polyuric: Increase free water Oligoanuria: Increase the caloric density of formula/breastmilk to provide adequate calories in lesser volume, diuretics	
Sodium wasting	Extracellular volume contraction PD: Increased sodium removal	Hypotension, constipation, poor linear growth PD: cerebral edema and intradialytic hypotension increase the risk of cortical blindness	Increase salt supplementation in the form of sodium bicarbonate (if acidotic) or sodium chloride, often with PD patients typically requiring from 5 to 8 mEq/kg/day Frequent blood pressure monitoring PD patients: dialysis sodium balance study	77,81
Potassium	• Poor GFR leads to decreased filtration • Aldosterone resistance in tubule • Volume contraction • Medication-induced: ACEI/ARB, potassium-sparing diuretics	Constipation Cardiac dysrhythmia Muscle weakness	Acute management: kayexalate, diuretics, calcium gluconate, albuterol, insulin/glucose, bicarbonate, volume repletion, dialysis Chronic: Low potassium formula,* diuretics, bicarbonate if acidotic, volume repletion, dialysis Future: Patiromer	76,82–85

*Note that breastmilk is lower in potassium than non-renal commercial formulas. Low potassium formulas such as Renastart are now so low in potassium they usually may not be given alone.
ACEI, Angiotensin-converting enzyme inhibitor; *ARB*, angiotensin II receptor blocker; *ARPKD*, autosomal recessive polycystic kidney disease; *CAKUT*, congenital anomalies of the kidney and urinary tract; *GFR*, glomerular filtration rate; *PD*, peritoneal dialysis.

appropriate to gradually lower protein intake toward 100% of the SDI as CKD progresses toward the need for dialysis.[60]

Supplemental nutritional support with tube feedings (e.g., nasogastric, gastrostomy, gastrojejunostomy) is indicated when the voluntary intake by the infant fails to meet energy requirements and the child is failing to grow as expected. Tube-fed infants must continue to have some oral stimulation and oral intake to mitigate the chance of persistent oral aversion.[76] In patients receiving either peritoneal or hemodialysis, the dietary protein requirements are increased to account for dialysis-related protein losses.

Fluid and Electrolyte Derangements

CKD leads to derangements in several solutes, vitamins, and minerals. In CKD, nutritional issues arise related to fluid balance, sodium handling, and potassium intake (Table 78.4). In turn, understanding the daily recommended intake (DRI) for sodium and potassium (Table 78.5) and consideration of the type of kidney disease is key. Nonglomerular disease secondary to posterior urethral valves or renal dysplasia often leads to polyuria and substantial urinary losses of water and salt. In contrast, diseases such as ARPKD lead to oligoanuria and water and salt

TABLE 78.5 Dietary Reference Intake for Sodium, Chloride, and Potassium in Healthy Children

	SODIUM (mg/day)		CHLORIDE (mg/day)		POTASSIUM (mg/day)	
Age	AI	Upper Limit	AI	Upper Limit	AI	Upper Limit
0–6 months	120	ND	180	ND	400	ND
7–12 months	370	ND	570	ND	700	ND
1–3 years	1000	1500	1500	2300	3000	ND

AI, Adequate intake.
Modified from National Kidney Foundation. KDOQI clinical practice guideline for nutrition in children with CKD: 2008 Update. *Am J Kidney Dis.* 2009;53:S49.

retention. All forms of kidney disease in infancy may result in hyperkalemia through decreased filtration and/or aldosterone resistance. Management of these nutritional complications often involves changes to the amount and composition of formula or breastmilk and sometimes the need for additional medications (see Table 78.4).

Metabolic acidosis is a common manifestation of CKD in children and an important negative influence on growth. Metabolic acidosis leads to changes in bone composition and decreases in 1,25-$(OH)_2$D synthesis, in addition to endogenous growth hormone and rhGH resistance.[86] Based on the experience of successfully enhancing the growth of infants and children with isolated renal tubular acidosis with alkali therapy,[87] it is recommended that children with CKD be treated to achieve a serum bicarbonate level of at least 22 mmol/L.[76] A publication from the CKiD study revealed that a serum bicarbonate level of less than 18 mmol/L was a risk factor for poor growth.[88] In other studies, decreased serum bicarbonate was associated with faster progression of kidney disease and development of secondary hyperparathyroidism, while a resolution of acidosis was associated with decreased CKD progression.[89,90]

Chronic Kidney Disease Mineral and Bone Disorder

Infants with CKD often develop alterations in bone and mineral metabolism leading to CKD mineral and bone disorder (CKD-MBD) which contributes to poor growth and short stature. Renal osteodystrophy refers specifically to bony changes that are characterized by abnormalities of bone turnover and mineralization. CKD-MBD is broader and includes systemic dysregulation of bone and mineral metabolism, including the development of extra-skeletal calcifications. Although limited studies have focused specifically on CKD-MBD in those with CKD during infancy, investigations in pediatric patients on dialysis have revealed that greater than 60% had some dysregulation of bone turnover and 48% had decreased bone mineralization.[91] Secondary hyperparathyroidism occurs in 30% to 45% of patients with CKD stage 2 to 4 and in 50% to 60% of pediatric dialysis patients.[67]

While a complete understanding of the pathophysiology of CKD-MBD remains to be elucidated, the complex interplay of phosphate, calcium, parathyroid hormone (PTH), vitamin D 1,25-dihydroxy vitamin D3 (1,25D), and fibroblast growth factor-23 (FGF23) has a significant role. Worsening CKD leads to increases in phosphate retention which stimulates FGF23 and PTH and decreases 1,25 vitamin D. The decreased 1,25 vitamin D leads to further increases in PTH and lower calcium absorption.[92] In turn, management of CKD-MBD requires optimization of calcium and phosphorus balance and attention to age-appropriate serum phosphorus levels.

Recently, the Pediatric Renal Nutrition Taskforce published guidelines for the management of calcium and phosphorus in children with CKD. Infants should receive the SDI for phosphorus and should receive no more than two times the SDI for calcium. Calcium-containing phosphate binders contribute to the SDI for calcium. When hyperphosphatemia or hyperparathyroidism exists, phosphorus intake must be restricted. If phosphorus remains elevated, phosphate binders taken with food should be initiated. To lessen the risk for vascular calcification, one must not exceed twice the SDI for calcium between dietary intake and binder intake if calcium-containing binders are used. Sevelamer, a non-calcium-containing phosphate binder, should be considered in this setting.[78]

Patients with secondary hyperparathyroidism, despite adequate calcium intake, phosphorus restriction, and the use of phosphorus binders, are typically also treated with vitamin D analogs such as calcitriol, if inactive (nutritional) vitamin D levels are normal. These medications may cause hypercalcemia and hyperphosphatemia, and should this occur, medications such as cinacalcet, a calcimimetic, may be used to lower PTH. This medication may induce hypocalcemia, and thus frequent monitoring of phosphorus, calcium, and PTH should be initiated if using activated vitamin D or calcimimetics.[92] Infants with advanced CKD and secondary hyperparathyroidism may experience improvement in the management of CKD-MBD after the initiation of KRT.[63,65]

Management

Kidney Replacement Therapy

ESKD is an uncommon disorder in children less than 4 years of age, with an incidence of 5.2 to 10.3 per million age-related population.[8] Neonatal ESKD is even less common, with an incidence of approximately 0.32 per 100,000 live births.[7] A large international registry study of neonates on KRT showed that neonates made up between 6.8% and 18.3% of all infants less than 2 years of age on dialysis. Although kidney transplantation is the nearly universal goal for children who develop ESKD, data from the most recent North American Pediatric Renal Trials and Collaborative Studies (NAPRTCS) annual report revealed that 80% of children under 2 years old who received a kidney transplant also received chronic dialysis before transplantation.[73]

Peritoneal Dialysis

Peritoneal dialysis (PD) is the preferred chronic dialysis modality for infants with ESKD. A publication from NAPRTCS showed that greater than 90% of patients 0- to 1-year-old who received chronic dialysis received PD at dialysis initiation.[14,93] The mechanics of chronic PD (CPD) are similar to those discussed for acute

PD in the chapter on AKI. While the CPD prescription initially mirrors that of acute PD, the fill volume is subsequently titrated upwards during CPD to a goal of 600 to 800 mL/m^2 body surface area in infants (<2 years) during each exchange; the duration of the dwell is adjusted to reach predefined adequacy metrics in terms of solute and fluid removal.[94]

The complications of PD are similar in acute and chronic PD. The single most serious complication is peritonitis, which occurs more frequently in infants than in older children.[93,95,96] The recent USRDS report showed that infection requiring hospitalization had an incidence of 0.99 per person-year in those less than 1 year of age, and rates of admission for infection have increased over time in those less than 1 year.[10] G-tube placement following PD catheter insertion and nephrectomy at the time of PD catheter placement both increase the risk of peritonitis and mandate the use of prophylactic antibiotic therapy prior to those procedures.[96] Whereas gram-positive organisms account for many infections, gram-negative episodes of peritonitis are common in infants and young children.[97] Empiric therapy for peritonitis should, in turn, always provide coverage for gram-positive and gram-negative organisms.[98] Other CPD-related complications that occur most frequently during infancy include anterior ischemic optic neuropathy and sudden blindness secondary to hypovolemia, excessive loss of sodium and protein across the peritoneal membrane, and hernia formation.[66,99,100]

Hemodialysis

The use of HD during infancy is most often dictated by the presence of a medical condition (e.g., omphalocele, gastroschisis, diaphragmatic hernia, bladder exstrophy) that compromises the ability to use the peritoneal membrane as a dialyzing membrane. Additional reasons for the use of HD are often related to metabolic conditions that accompany kidney failure.[101] The HD procedure during infancy is complicated due to the infant's small size and the associated challenges related to vascular access and safe fluid removal, and limited clinical experience has revealed a high incidence of patient morbidity.[63,102,103] Recent studies of complications in infants on chronic HD reveal improvements in central venous catheter longevity but continued high rates of hypertension, psychomotor retardation, and hospitalization.[104–106]

Transplantation

The topic of kidney transplantation in patients who develop ESKD as neonates or young infants is complicated, and a lengthy discussion is beyond the scope of this chapter. In short, transplantation is a viable alternative for these young patients, and it is their best hope for long-term survival. In a review of NAPRTCS data, 20% of 0- to 1-year-olds and 24% of 2- to 5-year-olds with ESKD received a preemptive (e.g., no prior dialysis) transplant. The overall transplant rates and graft survival rates for some of the largest and most recent studies of outcomes in neonates or young children with ESKD are listed in Table 78.6.

Infants with HD and PD have shown similar mortality and transplantation rates.[101] Graft survival in patients who developed ESKD as neonates or infants (31 days to 1 year) has improved,[14] and the rates of graft survival for the two groups are similar. These improvements in transplant rates and graft survival likely contribute to the improved overall survival of these patients and likely will improve further as outcomes with respect to other comorbidities, such as cardiovascular disease and growth, also improve.

What is often most important for the neonatologist is recognition of the need to develop a collaborative strategy with members of the pediatric nephrology, surgery, and urology teams for the management of congenital structural abnormalities of the urinary tract that are present in the patient with severe CKD/ESKD, the majority of whom will ultimately require dialysis and transplantation.[107]

Outcomes

Neurocognitive Impairment

Impairment of normal kidney function in infancy, a crucial time of neural development, raises concerns regarding the neurodevelopmental outcomes in children with CKD/ESKD. Advanced CKD has been linked to poor neurocognitive function in the areas of attention, memory, and inhibitory control.[108,109] Small studies examining the effect of ESKD during infancy on neurocognitive development have suggested that there may be minor delays in intellectual and metacognitive function but that most children without other comorbidities do not experience significant developmental delay.[110] Other comorbidities of CKD (e.g., anemia, iron deficiency, hypertension, cerebral vascular accidents, adverse effects of therapy) have been implicated in the neurodevelopmental impairments seen in many patients, and these comorbidities may explain the association between the duration of CKD and impaired executive function.[110–113] Genetic syndromes involving the central nervous system may also influence neurocognitive outcomes for these patients.[114] A recent meta-analysis of 34 studies that examined the effects of CKD on neurocognitive ability in children and adolescents showed that severity of disease and duration of disease did lead to deficits in many aspects of neurocognition, but that age of onset did not contribute to these deficits.[115]

Hospitalization

The majority of neonates and infants with CKD or ESKD will require frequent hospitalization throughout childhood. For those infants in the first year of life who must initiate dialysis, the incidence of hospitalization in days per person-year is as follows: 13.1 for nonsurgical reasons; 8.5 for surgical reasons unrelated to catheter revision; and 1.9 for catheter revisions based on data derived from the USRDS. This rate of hospitalization was higher compared to all other age groups.[10] In one study from outside the US of 18 children requiring chronic HD by 2 years of age, the median hospitalization rate per patient was 8.2 admissions per year, with the duration of hospitalization ranging from 63 to 399 days.[63] Another study divided 698 children requiring chronic dialysis within the first 2 years of life into those initiating dialysis by 1 month of age and those initiating dialysis between 1 month and 24 months of age. Approximately 80% of children in both groups required hospitalization at some point in the 13-year follow-up period. Among children ever hospitalized, those initiating dialysis as neonates were hospitalized more frequently than those children who initiated dialysis later (mean number of hospitalizations 54 vs. 39; $P < .001$). In addition, the average duration of the hospital stays was longer in the younger children.[7]

Survival

The long-term survival of neonates with ESKD appears to be approaching that of older infants and young children. In several recent studies examining medium-term survival of neonates, infants,

TABLE 78.6 Outcomes of Large Studies of Neonates and Infants With End-Stage Kidney Disease

Study	Vidal et al. 2012	Van Stralen et al. 2014	Carey et al. 2015	Vidal et al. 2017	Sanderson et al. 2019
Age range	<1 year	≤1 month	<1 year	<1 year	<1 year
Population	PD *N* = 84	ESKD *N* = 264	PD *N* = 241(neonates) *N* = 387 (infants) EC = 1992–1999 LC = 2000–2012	ESKD *N* = 1063; (917 PD, 146 HD)	PD *N* = 1723
Database(s)	Italian Registry of Paediatric Chronic Dialysis	ESPN/ERA-EDTA, IPPN, Japanese, ANZDATA	NAPRTCS	ESPN/ERA-EDTA	USRDS
Comorbidities	28% overall	73% overall • 20% NDD • 12% pulmonary • 18% CV	NR	NR	NR
Growth	Length SDS: −1.65, body weight SDS: −2.31	63% GR	NR	NR	NR
Survival and hospital rate	Survival 90.5% Hospitalization rate 1.2 days per CPD month	Survival 81% (2 years) 76% (5 years)	73% overall Neonates 70.0% (EC) 91% (LC) Infants 75.8% (EC) 84.6% (LC)	83.9% overall	Survival 79.1%
Transplant rates	20%	21.9% (2 years) 54.9% (5 years)	Neonates 39% (EC) 68% (LC) Infants 53% (EC) 65% (LC)	70.2% (5 years)	61.5%
Graft survival	NR	84.2% (5 years)	3-year survival Neonates 86.3% (EC) 84.2% (LC) Infants ~80% (EC) 92.1% (LC)	NR	NR

ANZDATA, Australian and New Zealand Dialysis and Transplantation; *Cr-EDTA GFR*, chromium-51-EDTA glomerular filtration rate; *CV*, cardiovascular; *EC*, early cohort; *ESKD*, end-stage kidney disease; *ESPN/ERA-EDTA*, European Society of Paediatric Nephrology/European Renal Association-European Dialysis and Transplant Association; *F*, female; *GR*, growth retardation; *HD*, hemodialysis; *IPPN*, International Pediatric Peritoneal Dialysis; *LC*, late cohort; *M*, male; *NDD*, neurodevelopmental delay; *NR*, not reported; *PD*, peritoneal dialysis; *HD*, hemodialysis; *SD*, standard deviation; SDS, standard deviation score; *Z*, zone.

or young children who started dialysis, the overall 3- to 5-year survival rates ranged from 70% to 90% (see Table 78.6).[7,12–14,101,116] Neonates who received chronic dialysis had only slightly lower survival rates compared with older infants and children.[7,13,14] The main reasons for death included infection and cardiovascular disease. Concomitant neurologic disease and other comorbidities were associated with increased mortality.[5,12] Studies that examined multiple time periods have shown improved survival for neonates and infants in more recent years.[7,13,14] Although the recent large studies suggest a good medium-term survival and support the recommendation for KRT in these complex patients, more long-term data are clearly needed to be able to provide parents and families with the best possible prognostic information and to enhance patient therapies and outcomes.

Ethics of Initiating or Withdrawing Kidney Replacement Therapy

Using the conceptual framework for medical decision-making that classifies anticipated therapy and outcomes as clearly beneficial, clearly futile, or of uncertain benefit, KRT for neonates and very young infants has long been considered of uncertain benefit due to unclear long-term outcomes. In turn, medical providers have often deferred to family members with regard to decisions about dialysis initiation and withdrawal in young patients. However, as dialysis is more routinely offered to neonates and infants with ESKD and more published data on improved medium and long-term outcomes have become available, consideration has

been given to classifying this therapy as clearly beneficial.[117] This improvement in outcomes has raised the question of whether dialysis therapy should be refused or withheld in infants, especially for those without other comorbidities.[118] Nevertheless, few studies have directly examined the changing attitudes of medical providers on this issue.

Geary and colleagues initially conducted an international survey on the attitudes of pediatric nephrologists regarding the management of ESKD during infancy nearly two decades ago.[111] More than 200 physicians from eight countries replied to a series of questions pertaining to the provision of KRT to neonates younger than 1 month of age versus those 1 to 12 months old. At that time, 93% of respondents stated that they would offer dialysis to some patients less than 1 month of age, 41% would offer it to all patients less than 1 month of age, and 50% believed it was usually ethical for families to withhold KRT. In a follow-up study conducted by the same group using an almost identical survey 10 years later, 98% of pediatric nephrologists responded that they would offer dialysis to some patients less than 1 month of age, although only 30% of pediatric nephrologists would offer it to all neonates. Additionally, there was a 25% increase compared with the earlier survey results in those who thought it was the parent's right to "usually" refuse KRT in neonates.[119] In both studies, physicians responded that they more routinely provided dialysis to infants in the 1-month to 12-month age group and thought it was less acceptable for families to refuse dialysis initiation for children of this age.[111,119] The factors that most often influenced the decision to initiate or withhold KRT were the presence of coexistent serious medical disorders and the anticipation of significant morbidity for the child.[111,119] Evidence for this age-related variation in philosophy and practice has been seen in other surveys as well.[120]

Factors to consider when making the decision regarding initiation or withdrawal of KRT during infancy include underlying comorbidities, quality of life concerns, allocation of resources, legal issues, and, most importantly, the opinions of the hospital team and the parents. Palliative care involvement early in cases of neonatal CKD/ESKD facilitates medical decision-making for families and improves care coordination while attempting to mitigate suffering for the patient and family and helping frame quality-of-life discussions.[121] Future research into the ethical issues surrounding infant dialysis should focus on separating isolated kidney failure from kidney failure in the setting of comorbid conditions, the cost financially, socially, and emotionally, and the informed consent process around the initiation of chronic dialysis.[117] The role of hospital ethics committees in the process remains extremely variable. In the end, clinicians and parents often struggle bravely to reach a compassionate decision with as much agreement as possible. Principles of practice that may provide valuable assistance in this process have been published.[122]

Suggested Readings

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Glomerulonephropathies and Disorders of Tubular Function

ELIZABETH YU AND KARYN YONEKAWA

KEY POINTS

- Nephrotic syndrome (NS) comprises persistent heavy proteinuria, hypoalbuminemia, edema, and hyperlipidemia.
- Genetic abnormalities of structural or regulatory proteins within the glomerular basement membrane and/or podocyte lead to primary congenital NS (CNS).
- Treatment of CNS does not involve immunosuppression and is aimed at minimizing symptoms and preventing serious complications.
- Infections causing secondary NS include human immunodeficiency virus, syphilis, toxoplasmosis, hepatitis B, malaria, rubella, and cytomegalovirus.
- Renal tubular acidosis (RTA) causes a non-anion gap metabolic acidosis. It is important to evaluate for other sources of bicarbonate (HCO_3^-) loss before initiating a work-up for RTA and to note that immature tubular function in premature infants may cause a self-limited moderate metabolic acidosis in the first 2 weeks of life.
- Inherited renal tubulopathies are rare and can be distinguished from each other in part by differences in serum potassium levels, presence of metabolic acidosis or alkalosis, presence of hypertension, and urine findings.
- Fanconi syndrome is a condition of diffuse proximal tubule dysfunction resulting in polyuria and wasting of HCO_3^-, amino acids, uric acid, phosphate, glucose, and low-molecular-weight proteins. Fanconi syndrome may be due to an isolated defect or can be a part of a broader genetic syndrome.
- Primary nephrogenic diabetes insipidus may present during pregnancy as polyhydramnios. In the newborn period, treatment should focus on maintaining adequate fluid balance.

Glomerulonephropathies

Generally, glomerulonephropathies are considered when nephrotic syndrome (NS)—the constellation of persistent heavy proteinuria, hypoalbuminemia, edema, and hyperlipidemia—is present. Greater than 40 mg/m^2 body surface area per hour of protein excretion on a 24-hour urine collection is consistent with nephrotic range proteinuria. Alternately, an untimed "spot" urine protein-to-creatinine ratio can be used when a 24-hour urine collection is impractical. In this setting, nephrotic range proteinuria is defined as a ratio of ≥2 mg protein/mg creatinine.[1]

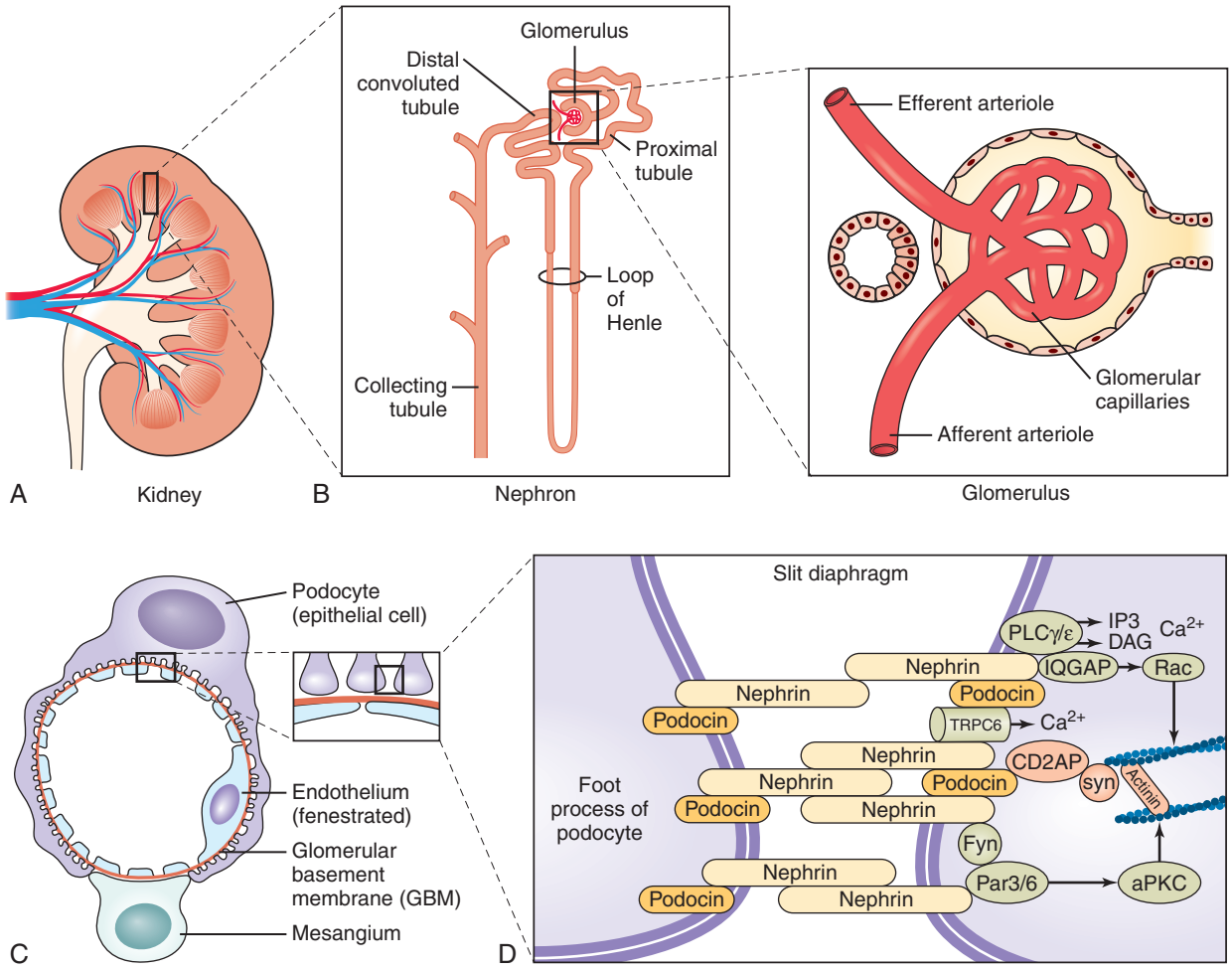
Congenital Nephrotic Syndrome

Congenital nephrotic syndrome (CNS) is defined as NS onset within the first 3 months of life. Infantile NS refers to NS onset within the first year of life. Both congenital and infantile NS can be divided into primary and secondary causes, with primary being the most common. Genetic abnormalities of structural or regulatory proteins within the glomerular basement membrane and/or podocyte lead to primary CNS.[2] Podocytes are cells with extensive foot processes that wrap around the capillaries of the glomerulus to prevent loss of proteins from the capillary into the urinary space. They are anchored in place by many proteins that make up the slit diaphragm, located between podocytes (Fig. 79.1). When there are abnormalities of the podocyte or slit diaphragm proteins, large molecules (such as proteins) may leak into the urinary space leading to proteinuria. Secondary CNS can be a presentation of congenital or perinatal infections or related to underlying metabolic disease.[3] Diagnosis may be made prenatally when a positive family history is present or because of an elevated amniotic fluid alpha fetoprotein indicating fetal proteinuria. In infants, clinical concerns (e.g., edema, failure to thrive, developmental delay) or NS complications, such as thromboembolism or infection, may lead to work-up and diagnosis.[4] Genetic screening identifies the genetic abnormality in greater than 85% of CNS patients.[5] Renal biopsy is not generally recommended due to success and availability of genetic screening, and because biopsy findings are commonly nonspecific. The most common light microscopy findings are mesangial hypercellularity and sclerosis, dilated proximal tubules, microcystic changes, or focal segmental glomerular sclerosis (FSGS), but these are not pathognomonic. Electron microscopy demonstrates diffuse foot process effacement.[3,6–8] Although most children with CNS require kidney transplant at a young age, there are case reports of CNS with spontaneous resolution or delayed progression, likely related to mild genetic mutations.[9]

Primary Congenital Nephrotic Syndromes

Finnish-Type Congenital Nephrotic Syndrome (MIM #256300)

Mutations in the nephrin protein encoding gene *NPHS1* are the most common cause of autosomal recessive (AR) CNS. It



• **Fig. 79.1** (A) The kidney contains approximately one million nephrons. (B) The nephron is composed of a glomerulus, proximal tubule, loop of Henle, distal tubule, and collecting duct. (C) The glomerular filtration barrier is composed of fenestrated endothelial cells, a basement membrane, and epithelial cells (podocytes). (D) Podocytes have structures called foot processes. The area between the foot processes is called the slit diaphragm. Proteins such as nephrin and podocin are essential for proper functioning of the slit diaphragm and filtration barrier. *actinin*, α-Actinin-4; *aPKC*, atypical protein kinase C; *CD2AP*, CD2 adaptor protein; *DAG*, diacylglycerol; *IP3*, inositol 1,4,5 triphosphate; *IQGAP*, IQ motif containing GTPase activating protein 1; *PLC*, phospholipase C; *syn*, synaptopodin; *TRPC6*, transient receptor potential-like channel 6. (Adapted from Chiang CK, Inagi R. Glomerular diseases: genetic causes and future therapeutics. *Nat Rev Nephrol*. 2010;6:539–554.)

is referred to as CNS of the Finnish type (CNF), owing to an incidence of approximately 1 in 8000 live births in the Finnish population.[3] Nephrin is an essential component of the podocyte slit diaphragm.[6] The classic presentation includes placental enlargement, premature delivery, and proteinuria.[3] Patients may have microscopic hematuria in addition to massive proteinuria, and typically present at birth or within the first 3 months of life.[6] Cardiac abnormalities, such as pulmonary stenosis or patent ductus arteriosus, have been reported in patients with *NPHS1* mutations, although severity and frequency are not well defined.[10] Renal ultrasound images demonstrate large echogenic kidneys with poor corticomedullary differentiation. These imaging findings are similar among all forms of congenital and infantile NS.[3]

Congenital Nephrotic Syndrome Type 2 (MIM #600995)

Podocin protein mutations in the *NPHS2* gene lead to the second most common form of AR CNS.[2,11] This is the most common gene implicated in CNS in central European populations.[12] Podocin is essential for targeting nephrin to the slit diaphragm of the podocyte, and protein absence leads to early onset, severe NS.[3] Clinical presentation is often slightly later than for *NPHS1* mutations, with NS commonly presenting between 4 months and 1 year of age.[13] Additionally, progression to end-stage kidney disease (ESKD) is thought to occur more gradually, with a mean time of 6.6 years from diagnosis to development of ESKD.[12] Ultrasound findings are similar to other forms of congenital and infantile NS.

Wilms Tumor Suppressor Gene Mutation Syndromes (MIM #194072, 136680, 194080)

Mutations of the Wilms tumor suppressor gene (*WT1*) may result in isolated diffuse mesangial sclerosis or be part of a syndrome associated with CNS such as Wilms tumor, aniridia, genitourinary abnormalities, and intellectual disabilities (WAGR), Frasier,

or Denys-Drash syndrome. On average, ESKD occurs at 3 years of age but can occur in infancy, and disease severity is partially dependent on mutation type. Patients with *WT1* mutations are at risk for Wilms tumors and gonadoblastomas and should be screened for these malignancies. Patients should also be screened with karyotype testing and, possibly, further genitourinary imaging, as they may have ambiguous genitalia or other genitourinary tract anomalies such as cryptorchidism in males or uterine abnormalities in females.[8]

Pierson Syndrome (MIM #609049)

Pierson syndrome is an AR disorder caused by mutations in *LAMB2*, which codes for the beta 2 chain of laminin, a basement membrane protein integral to the glomerular and ocular basement membranes, retina, and neuromuscular junctions.[14] Children with Pierson syndrome classically present with NS and ophthalmic findings (often microcoria) and neurodevelopmental abnormalities such as hypotonia. Age of presentation varies depending on the mutation type, but many present within the first 3 months of life.

Other Primary Causes of Congenital Nephrotic Syndrome (MIM #251300, 161200)

Galloway-Mowat syndrome is a rare AR condition in patients with NS and central nervous system anomalies, primarily microcephaly, with a genetic defect in the *WDR73*, or *OSGEP*, *TP53RK*, *TPRKB*, and *LAGE3* genes.[15,16] Case studies of other conditions such as nail–patella syndrome (an autosomal dominant [AD] condition associated with *LMX1B* mutations) may present with renal involvement, nail dysplasia, glaucoma, and bony anomalies classically identified by the presence of iliac horns. Though significant proteinuria may be present, this is not typically a presentation of NS.[17]

Management of Primary Congenital Nephrotic Syndromes

Primary CNS does not respond to the immunosuppressive treatments used for idiopathic NS. Given the abnormal or absent functional proteins in the basement membrane or slit diaphragm, this group of diseases is generally treated with symptomatic management targeted at minimizing protein loss. Definitive treatment is kidney transplant, but supportive therapies are used until the child is of appropriate size. The basic principles of management include maintenance of intravascular volume, minimization of proteinuria, adequate nutritional support, and maintaining electrolyte balance.

Albumin infusions are sometimes given to replace protein loss and restore intravascular volume. Diuretics are used in combination with intravenous albumin infusions to avoid severe edema. Treatment frequency varies based on patient characteristics. Medications to decrease the glomerular filtration rate by modulating renal blood flow can be used in an attempt to limit protein loss. Angiotensin-converting enzyme (ACE) inhibitors and nonsteroidal anti-inflammatory drugs (NSAIDs) can be used for this purpose either alone or in combination.[18,19] If medical management is insufficient to maintain stable serum albumin concentrations and prevent severe edema, then unilateral or bilateral nephrectomy would be indicated.

Neonates with CNS are at risk for many serious complications and should be monitored vigilantly. This is in part due to nonspecific urinary protein loss (e.g., immunoglobulins, coagulation proteins), which leads to the higher incidence of sepsis, thrombosis, and failure to thrive seen in this population. Serious bacterial infections are among the leading causes of death in infants with CNS.[5] Management with intravenous immunoglobulins (IVIGs) is not routinely recommended due to rapid loss into the urine.[20] However, IVIG supplementation may be helpful in conjunction with antibiotics during severe bacterial infections.[5]

Neonates with CNS will experience failure to thrive without nutritional supplementation, which often requires tube feedings. In general, fluid intake is limited by use of concentrated formulas. Recommended diet consists of high caloric (i.e., 130 kcal/kg/day) and protein intake (i.e., 4 g/kg/day), with low sodium intake (<0.5 to 2 g/day depending on age), along with the guidance of a renal dietician as part of the multidisciplinary team.[1,5] Commonly, hypothyroidism is found due to thyroid binding protein loss in the urine and is treated with levothyroxine.[5]

Approximately 10% of children with CNS will experience thrombotic complications due to urinary loss of antithrombin III and protein S, as well as increased thrombocytosis and plasma levels of factors V and VIII.[21] No standard guidelines exist regarding when nephrotic children should receive anticoagulation. However, hypercoagulability is of particular concern in children requiring central venous access, and this subgroup should be placed on antithrombotic therapy.[5]

Complications less often reported include anemia and copper deficiency. Smaller studies report refractory anemia related to transferrin and transcobalamin loss.[22] Ceruloplasmin loss leading to copper deficiency and subsequent neutropenia and refractory anemia has been reported.[23]

Most children progress to need kidney replacement therapy. At present, patients with CNS who receive a kidney transplant are expected to do as well as children with ESKD due to other etiologies.[3] A small percentage of patients, particularly with CNF, have a return of proteinuria after transplantation due to antibody formation against the previously absent nephrin protein.[24]

Secondary Causes of Congenital Nephrotic Syndrome

Although primary forms of CNS are most common, clinicians should consider secondary causes in children with negative genetic testing or clinical findings that point to other etiologies. For example, children with signs of metabolic disease in addition to their proteinuria should be evaluated for mitochondrial cytopathies. Those with nipple inversion and other dysmorphisms should be evaluated for congenital disorders of glycosylation.[25] Congenital and perinatal infections may cause CNS, a finding more common in developing countries. Implicated infections include human immunodeficiency virus, syphilis, toxoplasmosis, hepatitis B, malaria, rubella, and cytomegalovirus. Infection-related NS may respond with appropriate antimicrobial treatment, depending on the degree of glomerular damage.

Other Glomerular Diseases

Neonatal glomerulonephritis (GN) is uncommon and primarily described in case reports. GN is defined by proteinuria and hematuria accompanied by varying degrees of acute kidney injury, oliguria, and hypertension. Rarely, maternal antibody transfer can lead to membranous nephropathy in a neonate because of

absence of maternal neutral endopeptidase and subsequent antibody production following exposure to fetal neutral endopeptidase. The infant may experience transient glomerulopathy or may develop chronic kidney disease.[26] Additionally, nephritis may be the result of transfer of maternal antibodies related to systemic lupus erythematosus to the infant.[4]

Renal Tubular Disorders

The renal tubule regulates and modifies the glomerular filtrate to avoid excess salt and water loss in the urine. Defects along the tubule lead to varying pathology. The proximal tubule is in charge of bulk water and electrolyte reabsorption. The distal nephron delicately regulates the final urinary product with close regulation of acid-base balance and water reabsorption. In neonates, genetic causes of tubular dysfunction predominate; however, medications and other secondary causes of tubular dysfunction can occur. Adequate evaluation of renal tubular disorders in neonates can be complicated by the use of intravenous fluids, parenteral nutrition, or electrolyte supplementation, as these modify serum and urine studies. In addition, the renal tubules of premature infants have high urinary sodium excretion that improves over the first month of life.[27] This immature tubular function may cause self-limited moderate metabolic acidosis within the first few weeks of life.[28]

Renal Tubular Acidosis

Renal tubular acidosis (RTA) is suspected in children with a nonanion gap, hyperchloremic metabolic acidosis. There are four types of RTA (Table 79.1). Type 1 RTA is due to a distal nephron defect, type 2 is due to a proximal tubule defect, type 3 is a mix of type 1 and type 2 defects, and type 4 is due to hypoaldosteronism or aldosterone resistance.

Children with RTA present with failure to thrive, emesis, and polyuria. It is important to evaluate for other sources of bicarbonate (HCO_3^-) loss, such as diarrhea, as this should be addressed before pursuing a work-up for RTA. Baseline testing of serum electrolytes, blood gases, and a urine sample should be obtained while the child is acidotic. Evaluation of the serum anion gap (AG) is helpful to determine if a high AG acidosis or a normal AG acidosis is present. The AG is calculated by subtracting the serum anions from the serum cations.

$$AG = Na^+ - (Cl^- + HCO_3^-)$$

Normal values vary by institution, based on instrumentation and methodology. A high AG usually indicates increased unmeasured anions, whereas a normal AG acidosis is usually due to increased serum chloride. RTA is associated with a normal AG acidosis.

TABLE 79.1 Characteristics of Renal Tubular Acidosis Types

	Inheritance	Acid/Base	Potassium	Other Findings
Proximal Tubule				
Proximal RTA (type 2 RTA)	AD or AR or acquired	Metabolic acidosis	N or ↓	
Fanconi syndrome (diffuse proximal RTA)	Inheritance depends on underlying disease or acquired	Metabolic acidosis	N or ↓	Aminoaciduria Phosphaturia Glycosuria Uric aciduria Low-molecular-weight proteinuria
Loop of Henle				
Bartter syndrome	AR	Metabolic alkalosis	↓	Hypercalciuria Hypochloremia
Distal Tubule				
Distal RTA (type 1 RTA)	AD or AR or acquired	Metabolic acidosis	N or ↓	Hypercalciuria Nephrocalcinosis Urinary pH >5.5
Pseudohypoaldosteronism type 1	AD or AR	Metabolic acidosis	↑	Hyponatremia Elevated serum renin and aldosterone
Pseudohypoaldosteronism type 2	AD	Metabolic acidosis	↑	Hypertension Elevated serum aldosterone-to-renin ratio
Gitelman syndrome	AR	Metabolic alkalosis	↓	Older age Hypocalciuria Hypomagnesemia
Liddle syndrome	AD	Metabolic alkalosis	↓	Hypertension Low serum renin and aldosterone

AD, Autosomal dominant; *AR*, autosomal recessive; *N*, normal; *RTA*, renal tubular acidosis.

Urinary studies may help differentiate between the types of RTA. Normal renal response to metabolic acidosis will involve lowering urine pH and eliminating urinary ammonium (NH_4^+). The urinary anion gap ($U_{Na+} + U_{K+} - U_{Cl}^-$) is an approximate indirect measure of NH_4^+ concentration as NH_4^+ cannot be measured directly.[29] The urinary anion gap becomes negative with high urinary NH_4^+ and Cl^- excretion.[30] With low NH_4^+ concentration in the urine ($U_{Na+} + U_{K+} > U_{Cl-}$), the urinary anion gap becomes inappropriately positive. (Of note, in the setting of volume depletion and low urine sodium [≤25 mmol/L], the urine anion gap is also positive as the kidney reabsorbs chloride and has limited ammonia excretion.)[29,31,32] This volume depletion setting may mimic the serum and urine anion gap findings of RTA. Urinary osmolar gap ($U_{Osm} - 2U_{Na+} + 2U_{K+} + (U_{urea\ (mg/dL)}/2.8) + U_{glucose\ (mg/dL)}/18$) also helps determine if adequate NH_4^+ excretion is present. An elevated urinary osmolar gap (>100 mOsm/kg) suggests high NH_4^+ excretion.[30,33]

Distal Renal Tubular Acidosis (Type 1 RTA)

Distal RTA (type 1) is caused by an inability of the collecting duct to excrete adequate hydrogen (H^+) ions. Inherited distal RTA typically presents early in life with hyperchloremic metabolic acidosis, failure to thrive, hypercalciuria, and nephrocalcinosis that is usually seen on ultrasound within the first month of life.[29] Patients may be hypokalemic due to decreased distal hydrogen ion secretion and subsequent increased distal potassium excretion to maintain electroneutrality within the urinary filtrate. Specific AR mutations in the H^+ adenosine triphosphatase have been associated with sensorineural hearing loss as well as distal RTA.[34] Individuals with distal RTA typically have an inappropriately high urinary pH, generally greater than 5.5. The urine anion gap is usually positive, and the urine osmolar gap is low, representing inadequate NH_4^+ excretion. Treatment is with alkali supplementation.

Isolated Proximal Renal Tubular Acidosis (Type 2 RTA)

Proximal RTA (type 2) is not often seen in isolation and usually accompanies more diffuse proximal tubular dysfunction like Fanconi syndrome. The proximal tubule generally reabsorbs 80% of filtered HCO_3^-; therefore, defects lead to massive bicarbonaturia and subsequent acidosis. Serum potassium may be normal or decreased, due to impaired proximal potassium reabsorption in the setting of metabolic acidosis.

While inherited isolated proximal RTA is rare, it can be associated with ocular abnormalities and neurologic findings (i.e., intellectual disability, familial migraine).[29] Age of presentation is variable. Proximal RTA can be distinguished from distal RTA by the absence of hypercalciuria and absence of nephrocalcinosis. In proximal RTA, the urinary pH is often inappropriately high (>5.5) due to bicarbonaturia; however, once the serum HCO_3^- drops, less HCO_3^- will be filtered at the glomerulus, and urinary pH will also drop (<5.5). Treatment is with alkali replacement to correct the metabolic acidosis. Large doses of HCO_3^- supplementation are often needed to maintain balance. As this therapy can often worsen hypokalemia, potassium supplementation is also needed.

Hyperkalemic Tubulopathies (Type 4 RTA)

Hypoaldosteronism

Clinical hypoaldosteronism may be due to either a deficiency in aldosterone production or an inability of the renal tubules to respond to aldosterone (pseudohypoaldosteronism). Aldosterone deficiency can be primary in cases of congenital adrenal insufficiency or secondary due to drugs or infection, and is outside the scope of this chapter.

Pseudohypoaldosteronism Type 1

Pseudohypoaldosteronism type 1 (PHA1) can be AR (MIM #264350) or AD (MIM #177735). AR PHA1 involves a loss-of-function mutation in any one of the three genes (SCNN1A, SCNN1B, and SCNN1G) encoding the epithelial sodium channel (ENaC). This affects all mineralocorticoid targets and can present in infancy with volume depletion, hyponatremia, hyperkalemia, and acidosis. Because of mineralocorticoid resistance, patients have symptoms and laboratory results consistent with hypoaldosteronism, but with elevated renin and aldosterone levels. AD PHA1 is caused by a mutation in the mineralocorticoid receptor gene (NR3C2), and is milder in presentation, renal limited, and clinically may improve later in life.[35] Management requires treatment with Na^+ supplementation and K^+ restriction.

Pseudohypoaldosteronism Type 2 (MIM #145260, 614491, 614492, 614495, 614496)

Pseudohypoaldosteronism type 2 (PHA2), also known as Gordon hyperkalemia-hypertension syndrome, is an AD condition caused by a mutation in the WNK1, WNK4, KLHL3, or CUL3 gene. Patients traditionally present at an older age with normal kidney function, hyperkalemia, hypertension, and mild metabolic acidosis. Hypertension is due to volume overload through mutations in the distal convoluted tubule that increase Na^+ and Cl^- reabsorption. Although renin levels are depressed, aldosterone is not suppressed and, therefore, the aldosterone-to-renin ratio is elevated, which can help in the diagnosis. Low-dose diuretics are the mainstay of therapy, and patients are usually highly responsive to thiazides.[36]

Fanconi Syndrome

Fanconi syndrome is a condition of diffuse proximal tubule dysfunction. Children with Fanconi syndrome have polyuria and wasting of HCO_3^-, amino acids, uric acid, phosphate, glucose, and low-molecular-weight proteins. Fanconi syndrome may be an isolated defect or part of a broader genetic syndrome. In addition to the genetic syndromes described below, many medications can lead to proximal tubular dysfunction. Common offending medications include tenofovir, ifosfamide, carbonic anhydrase inhibitors, and aminoglycosides.

Tyrosinemia (MIM #276700) and classic galactosemia (MIM #230400) can both cause Fanconi syndrome. Both diseases are part of the uniform newborn screening panel performed in the United States, therefore often diagnosed early in life. Tyrosinemia is an AR disorder where toxic metabolites of tyrosine accumulate in the liver and kidney. Early therapy with nitisinone to reduce the production of toxic metabolites can improve renal proximal tubular dysfunction.[37] Classic galactosemia is also AR and presents early in life with emesis, lethargy, hepatomegaly, sepsis, and failure to thrive. Cataracts may also be present in infancy. Proximal tubular dysfunction can accompany galactosemia and exacerbate the clinical picture, although early dietary modification can prevent worsening of disease.[38]

Hereditary fructose intolerance (MIM #229600) is another genetic cause of Fanconi syndrome. Fructose intolerance does not usually present until fructose or sucrose is introduced to the infant's diet. Infants present with emesis and hypoglycemia. On subsequent evaluation the infant may be found to have proximal

tubular dysfunction due to deficient aldolase B within the kidney. Early dietary modification can prevent renal complications.[39]

Lowe syndrome (MIM #309000), also known as the oculocerebrorenal syndrome, is a rare X-linked syndrome that can cause Fanconi syndrome. Congenital cataracts and hypotonia are often detected at birth, with proximal tubulopathy and failure to thrive developing over subsequent weeks to months. Many patients develop chronic kidney disease and subsequent end-stage kidney disease in the second decade of life.[40]

Dent disease (MIM #300009) is another rare X-linked recessive proximal tubulopathy. It is characterized by hypercalciuria and recurrent nephrolithiasis. Urinary phosphate losses can lead to rickets and significant bone abnormalities in infancy.[41]

Fanconi syndrome is also a defining feature of Fanconi-Bickel syndrome (MIM #227810), which is characterized by hepatic and renal dysfunction caused by GLUT2 mutations.[42] This generally presents after a few months of life but can be seen in early infancy.

Nephropathic Cystinosis (MIM #219800)

Cystinosis is the most common cause of primary proximal tubular dysfunction in children. It is an AR lysosomal storage disease caused by a mutation in the *CTNS* gene leading to defects in the transporter cystinosin. This protein is responsible for cystine transport through the lysosome, and malfunction causes systemic cystine accumulation. The renal manifestations are due to cystine accumulation within the proximal tubular cells and present early in life, often leading to diagnosis.[43] Additional clinical features include corneal deposits, hepatomegaly, insulin-dependent diabetes mellitus, weakness, infertility, and hypogonadism. Corneal deposits initially cause light sensitivity and later lead to significant visual impairment.

Cystinosis diagnosis is made by measuring leukocyte cystine levels, detecting corneal deposits, or genetic testing. Genetic testing can also be done prenatally by chorionic villi sampling.[44] Discovery of cysteamine in 1976, now the mainstay of treatment, was a breakthrough in the treatment of patients with cystinosis.[45] Cysteamine decreases cystine concentration within cells, and, although cysteamine therapy cannot reverse renal tubular dysfunction, it can help preserve glomerular filtration rate, delaying the onset of ESKD.[46] Measurement of leukocyte cystine concentration is used to monitor efficacy of therapy. Compliance with treatment is difficult, as the medication has many side effects such as gastrointestinal upset, bad breath, body odor, and skin lesions at high doses. Compliance may be difficult due to need for dosing every 6 hours, although newer delayed release formulations may be easier, given as they are dosed twice daily. Cysteamine eye drops are used to limit corneal deposits, and these require hourly dosing.

Hypokalemic Tubulopathies

Bartter Syndrome (MIM #601678, 241200, 607364, 613090, 602522, 601198)

Bartter syndrome is caused by AR mutations in the thick ascending limb (TAL) of the loop of Henle. There are many known types of Bartter syndrome. Mutations in the genes *SLC12A1, KCNJ1, CLCNKB, CLCNKA, BSND*, and *CASR* have been found. Bartter syndrome is extremely rare, with a prevalence of 1 in 1,000,000 based on adult studies.[47] Bartter syndrome often comes to clinical attention based on poor growth and development, typically at a young age. In Bartter syndrome, impairment of sodium chloride reabsorption in the TAL involving the Na-K-2Cl cotransporter leads to decreased urinary concentrating ability, polyuria, volume depletion, and renin and aldosterone system activation. The secondary aldosterone activation increases Na^+ reabsorption and K^+ and H^+ ion excretion. This physiology can be thought of as analogous to the effect seen with loop diuretics.

Laboratory evaluation will demonstrate hypokalemia, metabolic alkalosis, hypochloremia, and hypercalciuria. Serum magnesium is often normal. Nephrocalcinosis may develop in infancy because of high urinary calcium. Sometimes, differentiation of Bartter syndrome from electrolyte abnormalities related to volume depletion can be difficult. Urinary Cl^- measurement can be very useful, as this should be low (<35 mEq/L) in patients with volume depletion.[48] Bartter syndrome patients uniquely have elevated urinary Cl^- in the presence of hypochloremia.

Bartter syndrome type IV is uniquely associated with sensorineural hearing loss, as the barttin protein is present in the kidney and the inner ear.[49] Genetic testing can confirm the diagnosis and specific mutation type; however, the diagnosis is primarily made clinically. Types I, II, and IV may be detected prenatally as part of the work-up for polyhydramnios. Amniotic fluid alpha fetoprotein should be low in cases of suspected Bartter syndrome.[50]

Early fluid management with adequate volume and electrolyte repletion is necessary to allow appropriate growth.[51] The TAL defect leads to an upregulation of prostaglandin-E_2; therefore, NSAIDs can help counteract this problem and limit fluid and electrolyte losses. Spironolactone and amiloride may also be used to limit distal potassium loss and minimize the need for large-dose potassium supplements.

A more recently described mutation is melanoma-associated antigen D2 (*MAGE-D2*) on the X chromosome and has been associated with a severe but transient form of neonatal Bartter syndrome. This mutation has also been associated with perinatal death.[52] These patients have an earlier, more severe presentation with polyhydramnios around the 20th week of gestation. Because of massive polyhydramnios, infants are often born premature. In the small group of infants studied, polyuria resolved at a median of 4.5 weeks after birth.[52]

Gitelman Syndrome (MIM #263800)

Gitelman syndrome is AR with defects found in the *SLC12A3* gene, which codes for the sodium chloride cotransporter in the distal convoluted tubule. Gitelman syndrome presents with hypokalemia and metabolic alkalosis similar to Bartter syndrome. However, in contrast to Bartter syndrome, it typically presents later in life and with hypomagnesemia and hypocalciuria. This condition can be thought of as analogous to the laboratory findings seen in patients being treated with a thiazide diuretic. Patients commonly present with muscle weakness, paresthesia, paralysis, or tetany. Diagnosis is primarily made based on serum and urine electrolyte measurements and can be confirmed with genetic testing. Treatment involves NSAIDs and drugs that block distal potassium–sodium exchange, combined with potassium and magnesium supplementation.[53]

Liddle Syndrome (MIM #177200, 618114, 618126)

Liddle syndrome is an AD condition where children are hypertensive due to a mutation in the *SCNN1B, SCNN1G*, or *SCNN1A* gene, each encoding a subunit of the ENaC. A mutation in this channel, which is responsible for distal sodium reabsorption, is a gain of function mutation and leads to excess sodium and subsequent water reabsorption. Consequently, Liddle syndrome

is associated with low renin and aldosterone levels, and, classically, hypokalemia and metabolic alkalosis accompany this disease. Therapy of choice is triamterene or amiloride as they directly inhibit ENaC channels.[36] Low sodium diet is also beneficial in minimizing the effects of ENaC activation.

Other Tubulopathies

Nephrogenic Diabetes Insipidus (MIM #304800, 125800)

In children, nephrogenic diabetes insipidus (NDI) is more often primary than secondary. Arginine vasopressin receptor type 2 (*AVPR2*) mutations are inherited in an X-linked recessive pattern and are the most common genetic defect, accounting for 90% of primary NDI.[54] Typically, arginine vasopressin is released from the pituitary in response to volume depletion, and signals to increase water reabsorption. *AVPR2* encodes for arginine vasopressin receptor-2, the receptor for arginine vasopressin on the basolateral surface of collecting duct cells. Receptor activation leads to translocation of aquaporin channels to the apical surface of the nephron for water reabsorption. Mutations in *AVPR2* result in arginine vasopressin resistance because of an inability to appropriately interact with circulating vasopressin.[55] Defects in the aquaporin channel are inherited in an AR pattern and are much less common.[54]

Children with genetic forms of NDI present at a young age with polyuria, failure to thrive, and hypernatremic volume depletion. Infants and small children that do not have the ability to increase their own water intake based on thirst responses are at greatest risk of complications, particularly at times of illness. Long-term complications include developmental delay, growth failure, and chronic kidney disease, all thought to be secondary to repeated volume and Na^+ depletion and rapid fluid shifts. Prenatal polyhydramnios may be appreciated during pregnancy.

Imaging often demonstrates a dilated urinary system because of the high volumes of urine. Elevated arginine vasopressin hormone levels in the setting of hypernatremic volume depletion and polyuria can help differentiate central diabetes insipidus from NDI, as can a desmopressin challenge. Definitive diagnosis is made through genetic testing.

Treatment of NDI in the newborn period focuses on providing adequate fluids. Decreasing the osmolality of the infant's formula helps maintain balance as these children have a limited ability to excrete osmotic loads. Breastfed infants often present later than formula-fed infants due to the lower osmotic and salt loads of breastmilk versus cow's milk based formulas (higher osmolality also necessitating increased urine volume).[54] Thiazide diuretics and nonsteroidal prostaglandin-synthetase inhibitors aid in management. Thiazide diuretics block sodium chloride reabsorption in the distal tubule leading to mild volume depletion, stimulating upregulation of proximal tubular sodium, and water reabsorption to overcome some of the massive water loss in the distal nephron. Nonsteroidal prostaglandin-synthetase inhibitors increase urinary concentrating ability, and are particularly effective when combined with a thiazide diuretic.[56] Gastrointestinal upset may limit usage, however.[57] Recently, sildenafil has been presented as a possible treatment to reduce proteinuria in therapy-resistant patients.[58] These current therapies are primarily aimed at symptomatic relief, but new research is investigating protein folding and increased protein expression to better target the cause of disease.[54]

Suggested Readings

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Urinary Tract Infections and Vesicoureteral Reflux

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KEY POINTS

- The presentation of urinary tract infections (UTIs) in neonates differs from that seen in older children.
- The type and route of infection also differ in neonates, when compared with older children.
- An appropriate urine sample for diagnosis is needed, but treatment should not be delayed.
- A febrile neonate in whom a UTI is suspected should be evaluated for sepsis, including blood and possible spinal fluid cultures.
- The risk factors for UTIs in neonates are uncircumcised males, white race, high-grade vesicoureteral reflux (VUR), a maternal history of UTI, bladder bowel dysfunction, instrumentation of the urinary tract, and the existence of congenital anomalies of the kidney and urinary tract.
- After a documented neonatal UTI, a radiologic work-up is warranted to detect anatomic anomalies.
- VUR is diagnosed in 20% of neonates with a proven UTI.
- Voiding cystourethrogram is the gold standard for the diagnosis of VUR and should be performed on high-risk neonates.
- Treatment of VUR should be tailored to each patient's individual risk of UTI recurrence, with the goals of preventing future UTIs and renal scar formation.

Urinary Tract Infection

Epidemiology

Neonates, by virtue of their immature immune system, are at a higher risk for urinary tract infections (UTIs).[1] In the United States, the prevalence of UTI within the first 30 days of life is estimated at 0.1% to 1%,[2–4] while in developing countries, it is as high as 1.8%.[5,6] UTI is the second most common bacterial infection in children after otitis media.[7]

In febrile children less than 2 months of age without an obvious source of infection, the prevalence of UTI is 5% to 20%.[8–10] There is a male predominance in the neonatal period, with males comprising 70% to 90% of all cases of neonatal UTI before the age of 6 months,[11–13] irrespective of their degree of prematurity.[14] Hispanics and whites are more likely to be diagnosed with a UTI than children of African descent.[15,16]

Pathophysiology

The natural pathophysiology of UTI is dependent on the migration of uropathogenic bacteria through the fecal–perineal–urethral route with subsequent entry into the bladder.[17,18] Iatrogenic instrumentation and hematogenous seeding are alternative modes of entry into the urinary system. Once in the bladder, these uropathogens may ascend into the ureter and kidney in a retrograde manner. The effectiveness of this process is determined by both pathogen and host factors.

Host factors in the neonate often relate to anatomy and involve urogenital malformations such as posterior urethral valves, vesicoureteral reflux (VUR), phimosis, ureteropelvic junction obstruction, and neurogenic bladder dysfunction secondary to spinal dysraphism. These conditions ultimately disturb the washout effect of antegrade urinary flow that would normally clear the uropathogens.[19] Additional host factors include the neonatal immune function and the antimicrobial properties of urine, such as low pH.[20]

Uropathogens have a number of virulence factors that are used to overcome the defense of the host. *Escherichia coli* serotypes with P-fimbriae are commonly isolated in infected urine.[21] These are adhesion proteins at the tip of the bacterium's attachment structures that enhance binding to receptors on the host urothelium.[22–24] These increase the bacteria's ability to ascend the urinary tract, even in the absence of VUR.[25]

Epidemiologic studies have shown that 76% to 94% of pyelonephritic strains of *E. coli* are P-fimbriated, compared with 19% to 23% of strains causing cystitis and 14% to 18% of strains isolated from patients with asymptomatic bacteriuria.[26,27] In one study, P-fimbriated *E. coli* was isolated in only 36% of girls with VUR and recurrent pyelonephritis, as compared with 71% of girls with pyelonephritis without VUR.[28]

However, in the presence of VUR, P-fimbriated *E. coli* is not necessary for infection of the upper urinary tract. Other virulence factors from uropathogenic *E. coli* include the release of alpha hemolysin and cytotoxic necrotizing factor-1,[29–31] as well as the ability to form a protective glycosylated polysaccharide capsule.[32]

Clinical Presentation

The signs and symptoms of UTI in newborns differ from those of older children. The familiar symptoms of dysuria, frequency, urgency, malodorous urine, incontinence, suprapubic pain, and hematuria are often absent or not recognized. The clinician must retain a high index of suspicion for the diagnosis of a UTI in a neonate. In full-term infants the most common presentation is a fever of greater than 38.5°C followed by poor feeding, tachypnea, and lethargy.[13,33] In contrast, premature infants commonly present

with apnea, hypoxia, tachypnea, and fevers with a temperature greater than 39°C.[34] Other nonspecific signs and symptoms, such as abdominal distention, diarrhea, vomiting, and failure to thrive, have also been described in infants with UTIs.[35]

Neonatal jaundice, especially with an onset after 8 days of life, has been associated with neonatal UTIs.[34,36] Mutlu et al. found that the incidence of UTI in children with hyperbilirubinemia was 18%.[37] This is an important association, as Xinias et al. showed that approximately 50% of neonates with UTI-induced jaundice had renal cortical changes on dimercaptosuccinic acid (DMSA) scan.[38]

Bacteriology

Neonatal UTIs are commonly caused by gram-negative rods, with *E. coli* (40% to 72%) and *Klebsiella* species (7% to 40%) responsible for over 80% of cases.[39,40] Enterococcus, a gram-positive coccus, is the third most common organism, with an incidence of 10% to 16%.[3,35]

It is hypothesized that the incompletely developed neonatal immune system not only increases the newborn's risk of infection but also makes the newborn susceptible to additional organisms compared to older children.[41] In neonates with UTIs, group B streptococci have been found to be more common than in older children.[42] Additionally, other gram-positive bacteria, such as coagulase-negative staphylococci, have been associated with UTIs in premature infants, although this remains controversial.[43] Candida, as a causative agent for UTIs, has a reported incidence of 25% to 42% in premature children admitted to the neonatal intensive care unit.[44,45]

Risk Factors

The important risk factors for UTIs in neonates are prematurity, uncircumcised males, white race, high-grade VUR, a maternal history of UTI, instrumentation of the urinary tract, and the existence of congenital anomalies of the kidney and urinary tract.[46,47]

The decreased risk for UTI in circumcised males is supported by two metaanalyses.[10,48] In febrile neonates, the incidence of UTI in uncircumcised males is 20% to 21% compared with 2% in circumcised males and 5% to 8% for females.[10,49] This phenomenon is supported by studies showing a higher concentration of uropathogenic bacteria in the periurethral region of uncircumcised males.[50] Laway et al. prospectively studied the effects of circumcision on the colonization of the periurethral area of 124 children.[51] The authors found that the periurethral region was devoid of *E. coli*, *Llebsiella, Proteus, Pseudomonas*, and *Enterococci* after the children underwent circumcision, whereas these uropathogenic bacteria were present in 68% preoperatively.

In its 2012 Policy Statement on circumcision (current version at the time of this writing), the American Academy of Pediatrics (AAP) states that the health benefits of male newborn circumcision outweigh the risks of the procedure.[52] Benefits include significant reductions in the risk of UTIs in the first year, as outlined above, and reductions in the risk of heterosexual acquisition of human immunodeficiency virus, and the transmission of other sexually transmitted infections. Despite the risk reduction, however, the AAP suggests that the health benefits are not great enough to recommend routine circumcision for all male newborns. Instead, the AAP recommends that clinicians routinely inform parents of the health benefits and risks of circumcision in an unbiased and accurate manner and that the parents should decide whether circumcision is in the best interests of their child.[52]

A maternal history of UTI is a risk factor for neonatal UTI. Milas et al. studied 1200 newborns for a 6-month period and showed that neonates with UTIs were more likely to have mothers that were diagnosed with a UTI during the pregnancy (22.2% vs. 5.2%, $P < .001$).[34] This was further supported by a cross-sectional study by Khalesi et al. who demonstrated a 5.9-fold increased risk for neonatal UTI in newborns where there was a history of maternal UTIs.[53]

The overall reported incidence of genitourinary abnormalities in neonates is 5%.[3] VUR is a common renal abnormality predisposing neonates to UTIs and pyelonephritis. Cleper et al. retrospectively studied 64 neonates with UTIs and found the incidence of VUR to be 20.3%, of which 31% represented high-grade VUR.[54] However, other studies have reported rates between 20% and 50%.[55,56] In male neonates with UTI, the most common anomaly was VUR.[57] In neonates with UTI the incidence of VUR increases to 30% to 50%.[3,58] Although other anatomic abnormalities, such as posterior urethral valves, congenital urethral strictures, ureteroceles, ureteropelvic junction obstruction, and neurologic bladder dysfunction, increase the risk of UTIs, these are less common than VUR. An obstructive pathology represents less than 1% to 2% of the abnormalities found in neonates with UTIs.[59] A detailed discussion on VUR can be found in this chapter under the subheading "Vesicoureteral Reflux."

A calculator was recently developed to help clinicians estimate the probability of UTI in febrile infants at the bedside (https://uticalc.pitt.edu).[60] Although not specific to neonates, it is based on five risk factors that include age under 12 months, white race, female sex (or uncircumcised boy), maximum temperature of 39°C, and the absence of another source for fever. The calculator was validated in a cohort of 2000 children and was found to reduce unnecessary testing, to decrease missed UTIs, and to reduce treatment delays.

Evaluation

The diagnosis of UTI in neonates differs from older children. An initial assessment should involve a detailed history including birth and family history and findings on prenatal imaging, if performed. The focused physical examination should include vital signs, general appearance, as well as abdominal and genital examination. In girls, special attention should be paid to the presence of labial adhesions or intravaginal foreign bodies.[61] In boys, special attention should be paid to the presence of phimosis or meatal stenosis, and testicular examination for signs of epididymoorchitis.[62]

In febrile newborns with a UTI, 20% to 30% have associated bacteremia.[33,63,64] For this reason, a comprehensive work-up for sepsis should be performed in all febrile neonates.

Urine Sample

In children age 2 to 24 months, a UTI is defined as pyuria on urinalysis and a urine culture with >50,000 colony forming units/mL (CFU/mL) of a single organism.[65] Neonates void frequently, which can lead to lower colony counts on urine culture. Therefore, 10,000 CFU/mL is sometimes used as a cutoff for the diagnosis of neonatal UTI.[4,66]

The easiest method of obtaining a specimen from neonates is with a bag applied to the perineum. However, this carries a high contamination rate with a false-positive urine culture rate of 75%, and its only validity is to rule out a UTI in the event of a negative culture.[67] As always, the culture result must be correlated to a urinalysis, looking for signs of inflammation.

A superior method in neonates is urethral catheterization. This yields a sensitivity of 95% and a specificity of 99% for urine culture.[68,69] An even more accurate, although more invasive, method is suprapubic aspiration, with a contamination rate of 1%.[70] This may be a more appropriate option for boys with tight phimosis and girls with labial adhesions.[71] Topical anesthetics such as Eutectic Mixture of Local Anesthetic may be used to reduce pain associated with suprapubic aspiration in children.[72]

Dipstick urinalysis is an inexpensive and rapid screening tool and tests for urine nitrites and leukocyte esterase. Nitrites in the urine imply the presence of nitrate reductase, which is common among gram-negative uropathogens. Positive detection of leukocyte esterase, which is released by white blood cells (WBCs), is a surrogate for pyuria. In a retrospective study of 13,030 febrile infants, the sensitivity and specificity of a dipstick urinalysis were 91.7% and 90.4%, respectively. This was comparable with microscopic urinalyses obtained from the same sample population.[73]

Microscopic analysis completes the simple dipstick analysis by quantifying the WBCs, red blood cells, and presence of bacteria. Pyuria is the presence of five or more WBCs per high power field (HPF) in a centrifuged sample or greater than 10 WBC/HPF in a noncentrifuged sample if a counting chamber is used,[74] although these definitions may be less reliable in neonates.[75]

Renal Imaging

Renal imaging after UTI is driven primarily by a need to rule out underlying renal or urinary tract abnormality, or for the assessment of renal injury. The 2011 American Academy of Pediatrics clinical practice guideline, reaffirmed in 2016, recommends when to obtain renal bladder ultrasound (RBUS) and VCUG following febrile UTI for children aged 2 to 24 months. However, there is no consensus about when to obtain imaging in neonates.[65,76] Traditionally, routine imaging with ultrasonography, VCUGs, and 99Tc-DMSA renal scans were all recommended for neonates with a febrile UTI.[77] Yet, in the past two decades there has been a shift towards more selective usage of DMSA scans and VCUGs, due to concern for ionizing radiation exposure to neonates and their invasive nature relative to RBUS.

Renal Bladder Ultrasound

In the acute UTI phase, RBUS is the first-line imaging tool for the investigation of a neonate with a UTI. It is safe, noninvasive, inexpensive, and readily available, and should be routinely performed to evaluate for urinary tract anomalies. These include obstruction, renal structural anomalies, nephrolithiasis or calcification, and perinephric abscesses or abdominal masses.[46] In the authors' practice, a deferred RBUS after the UTI resolves should also be obtained for more accurate interpretation of anatomy, without the potential for false positive findings associated with tissue edema or endotoxin-induced dilation.

RBUS is a less sensitive imaging modality for the diagnosis of VUR.[78] In a retrospective review of 197 neonates, Wallace et al. found that the sensitivity of RBUS for VUR in infants younger than 2 months of age who were diagnosed with UTI was only 33% for grade I-V VUR, but improved to 86% when limited to grade IV–V.[79] Furthermore, a Cochrane meta-analysis of 42 studies looked at the accuracy of RBUS and/or DMSA in detecting VUR.[80] The authors found that RBUS lacked accuracy to detect either VUR or high-grade VUR, with summary sensitivity estimates ranging from 0.44 to 0.59 and summary specificity estimates ranging from 0.78 to 0.79. Thus, RBUS cannot replace VCUG in detecting VUR, nor can it serve to rule in or rule out VUR or high-grade VUR.

Voiding Cystourethrography

Voiding cystourethrography (VCUG) has the unique ability to provide detailed information about both the anatomical and functional status of the urinary tract. During the procedure, a nonballoon urinary catheter is placed using sterile technique for retrograde filling of the bladder with contrast dye. During cyclic filling, multiple spot images are obtained in the anteroposterior, oblique, and lateral positions, as well as urethral images during voiding. The AAP Sections on Radiology and Urology initiated a consensus standardized VCUG protocol in 2016 to improve the quality of VCUGs performed and to standardize the reporting of findings.[81]

The arguments for routine VCUG in neonates with a febrile UTI revolve around the higher incidence of VUR in this population, which requires aggressive and timely management.[54,57] The discussion against the routine use of VCUG is based on evidence that renal scarring is more common with grades IV and V VUR,[64] while the majority of VUR detected on routine VCUG is grade I–III.[82] AAP guidelines from 2011, reaffirmed in 2016, recommend that infants with initial febrile UTI undergo a RBUS, but state that a VCUG should not be routinely carried out unless indicated by the presence of abnormal sonographic findings (i.e., hydronephrosis, scarring) or atypical/complex clinical circumstances. The guidelines also suggest that additional work-up be considered if a child experiences recurrent febrile UTIs.[65,76] Given the lack of sensitivity and specificity of RBUS to detect VUR,[80] RBUS and VCUG are frequently considered complementary studies. They each impart important, but different, information. In line with this debate, the AAP Section on Urology expressed strong opposition to the AAP guidelines with respect to the omission of VCUG in the evaluation of infants with febrile UTI.[83] Failure to effectively screen children with a febrile UTI for VUR might place young children with VUR at untoward risk of both recurrent UTIs and potential renal injury.[84] It is the authors' opinion that the decision to perform a VCUG in a neonate should be individualized to the patient, with a thorough discussion with the parents regarding the risks, benefits, and alternatives to the imaging modality. A VCUG should be performed in high-risk neonates such as those with UTIs caused by bacteria other than *E. coli*, those with renal bladder ultrasound abnormalities, and uncircumcised males. If a decision is made to perform a VCUG, one should consider performing it 2 to 4 weeks after the infection to ensure that the infection is appropriately treated before instrumentation, although early VCUG may also be appropriate.[54]

Contrast-Enhanced Voiding Urosonography

Contrast-enhanced voiding urosonography (ceVUS) has emerged in the past decades as a replacement for the diagnosis and follow-up of VUR. It is a dynamic, real-time imaging technique that involves injection of an ultrasonic detectable microsphere-based

contrast intravesically via a urinary catheter.[85] CeVUS was first introduced in the 1990s as a means of diagnosing VUR without exposure to ionizing radiation inherent in VCUGs.[86] The current second-generation commercial ultrasound contrast agent, which has improved stability compared to the first generation, was developed in 2001.[87] However, owing to regulatory restrictions in the United States, the intravesical use of commercial contrast agents continues to be much more common in Europe than in the United States.[88] In a 2009 survey of pediatric urologists who belonged to the American Academy Pediatric Section of Urology, 98% indicated a preference for the use of VCUG for evaluating VUR despite awareness of the risks of radiation exposure in the pediatric population.[89]

Similar to a VCUG, ceVUS allows both morphologic and functional evaluation of the urinary tract. A 2018 literature review reported on aggregate findings of 45 comparative studies that assessed the diagnostic accuracy of ceVUS in the detection and evaluation of VUR, using VCUG as the standard reference.[90] The diagnostic accuracy of ceVUS in diagnosing VUR among children is comparable to VCUG, specifically for high-grade VUR and in younger children. Among studies using the second-generation contrast, the median sensitivity was 86.26 (interquartile range [IQR] 81.13 to 97), and the median specificity was 90.99 (IQR 84 to 98). No serious adverse events were reported in any of the studies.[90] However, one must account for the technical expertise and operator dependence required for the procedure. In a prospective study of 39 patients, Velasquez et al. noted a technical learning curve related to poor contrast mixing and the need to titrate the contrast agent, but noted that over a short period of time, a center previously naïve to ceVUS can quickly demonstrate concordance between ceVUS and VCUG.[91]

99Tc-Dimercaptosuccinic Acid Renal Scan

The DMSA renal scan is performed by injecting radiolabeled DMSA through an intravenous catheter. DMSA uptake by the kidneys is visualized with a gamma camera approximately 2 hours later, enabling the radiologist to evaluate uptake of DMSA by different regions of the kidney.[80] The debate surrounding the routine use of a DMSA scan is twofold; first, whether it predicts VUR and second, if it should be performed at all in neonates.

A Cochrane meta-analysis of 42 studies looked at the accuracy of RBUS and/or DMSA in detecting VUR.[80] The summary sensitivity and specificity estimates for DMSA to detect all grades of VUR were 0.75 (95% CI 0.67 to 0.81) and 0.48 (95% CI 0.48 to 0.57), respectively. Thus, DMSA cannot replace VCUG in the detection of VUR or high-grade VUR.

DMSA scan is the current gold standard for assessment of renal parenchymal injury in children with a history of febrile UTI.[46] It is more sensitive for renal scarring than RBUS, which misses a substantial proportion of such cases. If a DMSA scan is to be performed, a delayed one at 4 to 6 months after UTI resolution allows the acute inflammatory reaction to subside, at which point any persistent cortical defects can be assumed to represent permanent renal scarring.[92]

The decision for a DMSA scan should be shared with the parents, taking into account the risk for renal scarring based on the ultrasound findings and grade of VUR (i.e., abnormal renal parenchyma or VUR grade ≥III). Demonstration of renal scarring increases the risk for further renal deterioration,[93] and may influence surgical decision-making in patients with surgically correctable conditions such as VUR.

Management

For a febrile neonate, a septic work-up should be performed, including complete blood count, blood culture, urinalysis, urine culture and lumbar puncture. UTIs in the newborn are considered complicated and are associated with higher incidences of bacteremia (16% to 31%).[58,94] As such, empiric therapy with broad-spectrum antibiotics should be initiated without delay, after blood, urine, and cerebrospinal fluid (CSF) cultures are obtained. The goal of early treatment is to prevent sequelae such as urosepsis and renal scarring.

Local antibiotic resistance patterns and maternal antibiotics history should be used to determine choice of antimicrobial medication for empiric therapy.[95,96] The antibiotic should then be changed to one with less broad activity, according to the bacterium's antibiotic sensitivities.[97]

Parenteral ampicillin and gentamicin are the recommended empiric antibiotic regimen for neonates. Doses are dependent on the weight and age of the infant.[98] An alternative regimen described by the European Association of Urology includes ampicillin with ceftazidime.[99] In a retrospective review of 103 infants aged 0 to 60 days hospitalized with UTIs in a single institution in the United States, empiric antibiotics demonstrated organism susceptibility in 87.6% of cases.[100] Ampicillin and gentamicin were identified as the most common empiric antibiotic combination, with the combination of ampicillin and cefotaxime being the second most common.[100]

In young neonates, parenteral therapy should be continued for 7 to 14 days, followed by oral therapy for a total of 14 to 21 days.[99] For older and more mature neonates with negative blood and CSF cultures, parenteral antibiotics should be continued for 3 to 4 days, followed by oral therapy for a total of 7 to 14 days.[98] Routine follow-up urinalysis and culture after the resolution of UTI symptoms are not necessary unless clinically indicated.[101]

Neonatal candidiasis, which includes candiduria and/or candidemia, occurs predominantly in the neonatal intensive care unit. It is associated with significant mortality and neurodevelopmental impairment in extremely-low-birth-weight (ELBW) infants who weigh under 1000 g.[102] Recent studies have highlighted the significance of candiduria in the absence of candidemia in this specific population.[45,103] In a multicenter prospective observational cohort of ELBW infants in neonatal intensive care units, mortality was similar in those from whom Candida was isolated from only the blood (28%) or only from the urine (26%).[45] The authors suggest that Candida isolated from any normally sterile body fluid, including urine by suprapubic aspiration or in/out catheterization, should be treated as evidence of systemic disease. The clinical data are consistent with animal model studies, in which Candida injected into the blood of rodents was first isolated from urine.[104] When small amounts of Candida were injected, blood cultures were often negative while urine cultures were more frequently positive. For EBLW neonates, and neonates in the neonatal intensive care unit, candiduria should prompt a consultation with a pediatric infectious disease specialist for further work-up (blood cultures, lumbar puncture, abdominal ultrasound) for disseminated Candida infection and warrants treatment.[102] Treatment of neonatal candidiasis typically involves either amphotericin B deoxycholate or fluconazole.[102] For healthy term neonates, there are no specific guidelines from the Infectious Diseases Society of America, possibly in light of the rarity of candiduria in the healthy neonate.

Antibiotic Resistance

In the United States, the incidence of ampicillin- and gentamicin-resistant *E. coli* in the neonatal population reaches upwards of 75% and 17%, respectively.[105,106]

Vesicoureteral Reflux

Epidemiology

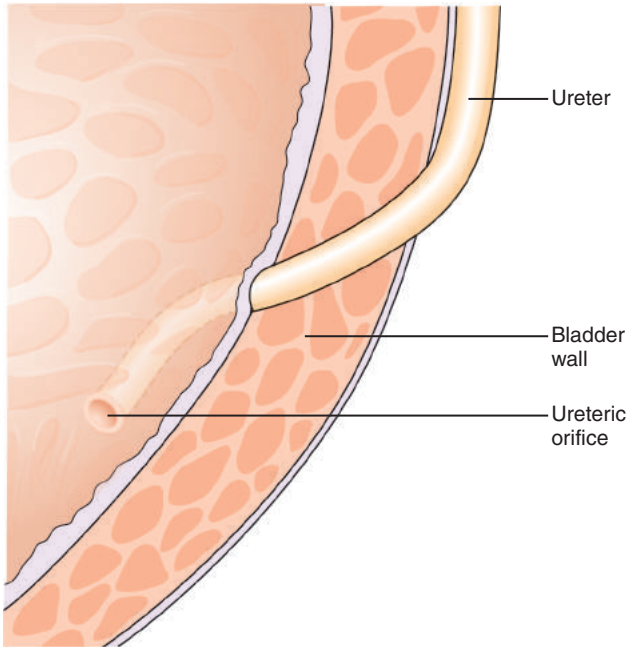
VUR is defined as the retrograde flow of urine from the bladder to the upper urinary tract. VUR is associated with approximately 20% of neonatal UTIs.[3,54] *Klebsiella pneumoniae* has a higher incidence in neonates with VUR presenting with a UTI compared with those without VUR.[54]

Pathophysiology

Primary VUR is thought to result from the abnormal structure and function of the ureterovesical junction, whereas secondary VUR is acquired as a result of increased intravesical pressure. Normally, the ureters enter the bladder obliquely, where a certain length of the ureter courses intramurally and submucosally. This creates a flap-valve mechanism where the distal ureter is compressed as the bladder fills with urine, thus normally preventing urine backflow. (Fig. 80.1 demonstrates this relationship.)

The lateral displacement of the ureteric orifice in the bladder in patients with VUR is thought to occur from an abnormal ureteric bud origin along the mesonephric duct, where the extent of lateral displacement correlates with the degree of associated renal dysplasia.[107]

The International Reflux Study grading system is universally accepted and used when grading reflux.[108,109] The grade of VUR is strongly associated with outcomes such as spontaneous resolution, recurrence of UTI, and renal scarring.[110] (Fig. 80.2 describes the appearance of each grade along with an example of each on VCUG.)



• **Fig. 80.1** Normal Course of the Ureter in the Bladder. Notice how the ureter courses through the bladder wall (intramural portion) and under the mucosa (submucosal portion) to expel urine into the bladder through the ureteric orifice.

Management

Surgical intervention is rarely required in the neonatal period, excluding obstructive abnormalities. If surgery is required, it is reserved for infants with high-grade VUR associated with recurrent breakthrough UTIs and renal scarring. A comprehensive discussion regarding the surgical management of VUR after the neonatal period is beyond the scope of this chapter.

Overall, the management of VUR diagnosed in the neonate after a UTI after the initial evaluation includes close follow-up with renal bladder ultrasounds and selective use of continuous antibiotic prophylaxis (CAP).

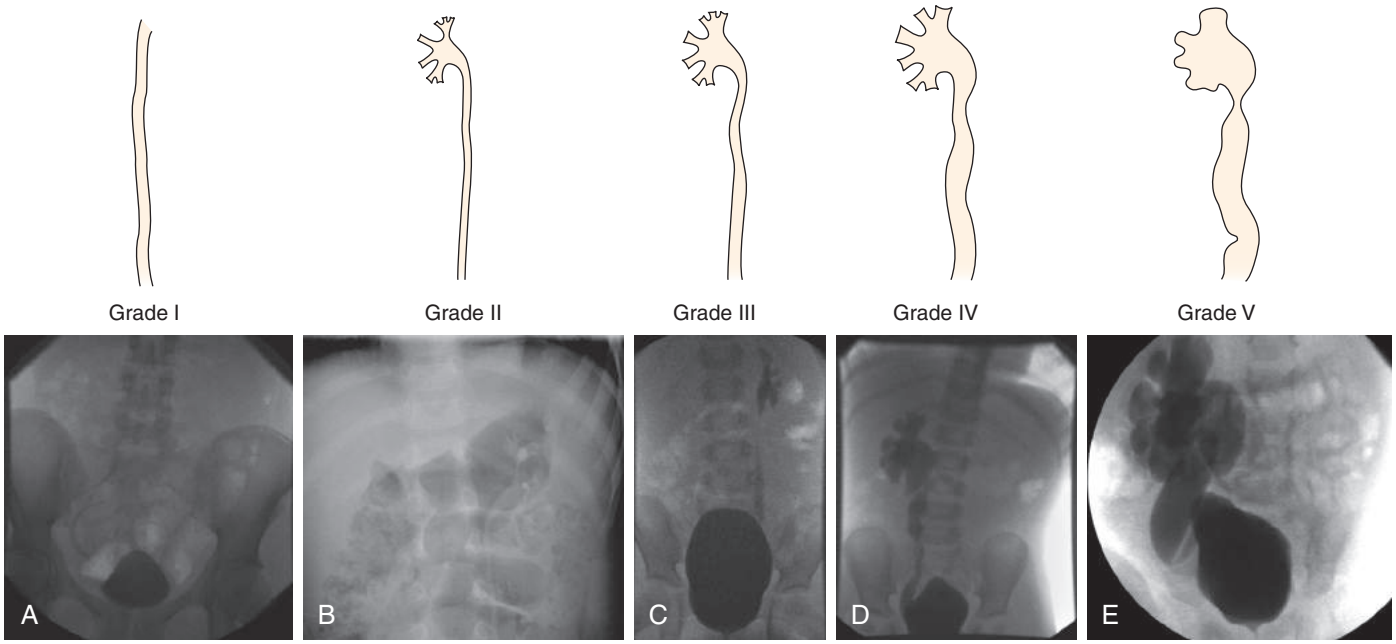
Continuous Antibiotic Prophylaxis

The basis for CAP stems from studies in the 1970s showing that the use of long-term antibiotics reduces the risk of repeat positive urine cultures, in comparison with no prophylaxis.[111,112] In the past decade, a number of randomized controlled trials (RCTs) have been conducted to elucidate the role of CAP in preventing UTI recurrences and renal scars. However, evidence for the efficacy of CAP in the neonatal population is lacking. The following discussion is based on study populations older than 1 month of age. Table 80.1 summarizes these RCTs and their findings.

There have been variations in results, attributed to significant differences in study designs, including patient inclusion and exclusion criteria.[113] In 2014, the findings of the Randomized Intervention for Children with Vesicoureteral Reflux (RIVUR) trial were published, showing CAP reducing risk of UTI recurrence by 50%.[114] The findings of a similarly double-blinded, placebo-controlled trial from the Prevention of Recurrent Urinary Tract Infection in Children with VUR and Normal Renal Tracts (PRIVENT) Investigators showed a more modest effect but with CAP similarly providing benefit, with the number needed to treat to prevent an infection being 14.[115] Other studies fail to show a significant difference in UTI recurrence rate and renal scar formation between those treated with CAP, surveillance, or ureteral reimplantation.[116–121] In systematic reviews and metaanalyses, researchers have also reported mixed results, with some suggesting that CAP is effective,[122] while an updated Cochrane review in 2019 concluded that although CAP may reduce the risk of repeat symptomatic UTI in children with recurrent infections, the benefit may be small.[123]

The 2014 RIVUR trial is the largest randomized, placebo-controlled, double-blind, multicenter study to date investigating the efficacy of trimethoprim-sulfamethoxazole as the CAP agent compared with placebo in young children with VUR.[114,124] This trial, sponsored by the National Institute of Diabetes and Digestive and Kidney Diseases, showed a significantly lower rate of UTI recurrence in children receiving CAP compared with placebo (12.8% vs. 25.4%, $P < .001$). As aforementioned, CAP reduced the risk of UTI recurrence by 50% (hazard ratio 0.50, 95% confidence interval 0.34 to 0.74).[114]

Nevertheless, the results and implications of the RIVUR trial have been widely debated. Critique of the trial includes the homogeneity of the study population. Females and those with VUR grades I–III comprised 91.9% and 91.7% of the study population, respectively.[113] The authors acknowledge that a benefit from



• **Fig. 80.2** International Reflux Study Grading With Voiding Cystourethrogram Examples. (A) In grade I, urine refluxes only into the ureter without reaching the renal pelvis or calyces (notice that it only appears upon voiding in this voiding cystourethrogram example). (B) In grade II, urine refluxes into the ureter, renal pelvis, and calyces, without any dilatation of the collecting system (notice the sharp contours of the fornices). (C) In grade III, urine refluxes into a mildly dilated and tortuous ureter, the renal pelvis, and calyces, with mild blunting of the fornices (notice how the forniceal contours are less sharp). (D) In grade IV, urine refluxes into a moderately dilated and tortuous ureter, the renal pelvis, and calyces, with complete obliteration of forniceal angles but maintenance of papillary impressions. (E) In grade V, urine refluxes into a grossly dilated and tortuous ureter, the renal pelvis, and calyces, with blown fornices where papillary impressions are no longer visible.

CAP was demonstrated; however, the number needed to treat (NNT) of 8 translates to a total of 5840 doses of antibiotic to prevent one UTI recurrence.[113] The AAP practice guidelines were recently reaffirmed by the AAP, which suggests that the results of the RIVUR trial may not be clinically significant.[76] Wang et al. conducted a reanalysis of the RIVUR trial by stratifying children with VUR into low- and high-risk categories.[125] Low-risk patients included circumcised males and females with grade I–III VUR and had no bladder bowel dysfunction (BBD). High risk patients included the following: (1) uncircumcised males with grade I–III VUR with or without BBD, (2) females with grade I–III VUR with BBD, or (3) females and males with grade IV–V VUR with or without BBD. The reanalysis demonstrated that high risk children benefit more, with a NNT of 5 compared to 18 in low-risk children.[125] These findings support a selective, risk-based approach for CAP management.

Antibiotic Resistance on Continuous Antibiotic Prophylaxis

Children being treated with prolonged administration of antibiotics have been found to have an increased risk of resistance.[46] A recent metaanalysis of six RCTs investigating antibiotic prophylaxis for children with VUR included data on microbial resistance.[126] A total of 1299 patients with VUR were included in the final analysis, contributing 224 first recurrent UTIs. Of first recurrent UTIs, 86% were *E. coli*, which was not statistically significant between control (86%) and prophylaxis (85%) groups ($P = .74$). Patients being treated with prophylaxis were more likely to have a multidrug-resistant (33% vs. 6%, $P < .001$) result on their first recurrent UTI, and they were subsequently more likely to receive a broad-spectrum antibiotic (68% vs. 49%, $P = .004$).

Neonatal Continuous Antibiotic Prophylaxis

In cases of febrile UTI and VUR, the American Urological Association (AUA) recommends CAP in children under 1 year of age, in contrast to its recommendations for older children, where a more selective approach is taken.[127] In addition to the AUA recommendations, the recommendations of the European Association of Urology and European Society of Paediatric Urology both include younger age as a particular consideration for prophylaxis, due to the nonspecific clinical presentation for UTI, the difficulty in getting urine specimens, the higher possibility of a need for hospitalization for intravenous antibiotics administration, and an increased risk of septicemia.[99]

The decision to start CAP should be made on an individual and selective basis, based on risk for UTI recurrence, in consultation with parents. The use of a clinical calculator (iReflux) developed by Hidas et al. for determining a child's individualized risk for UTI recurrence may help in this decision.[128] However, it is the authors' opinion that in children with high-grade VUR (grades III–V), especially in uncircumcised males, CAP should be initiated (Table 80.2). Unlike in older children, ampicillin, amoxicillin, and cephalexin are the commonly used antibiotics for CAP in neonates with VUR.[129] Trimethoprim-sulfamethoxazole should be avoided because of the immaturity of the newborn liver and kidneys, which cause slower metabolism and excretion. The prophylactic dose of antimicrobials is one-fourth to one-half of the therapeutic dose for acute infection.[129]

TABLE 80.1 Randomized Controlled Trials Evaluating the Effect of Continuous Antibiotic Prophylaxis on Urinary Tract Infection Recurrence Rates and Renal Scar Formation

Authors	Publication Date	PATIENTS INCLUDED: Age	PATIENTS INCLUDED: VUR Status (Grade)	Randomization	Rate of UTI Recurrence	Renal Scar Formation
Jodal et al.	2006	<11 years	III–IV	CAP vs. ureteral reimplantation	CAP = reimplantation Febrile CAP > girls > boys	CAP = reimplantation
Garin et al.	2006	3 months –18 years	0–III	CAP vs. surveillance	CAP = surveillance	CAP = surveillance
Roussey-Kesler et al.	2007	1 month –3 years	I–III	CAP vs. surveillance	CAP = surveillance (except boys with grade III reflux: CAP < surveillance)	
Montini et al.	2008	2 months –7 years	0–3	CAP vs. surveillance	CAP = surveillance Grade III = independent predictor febrile recurrence	CAP = surveillance
Pennesi et al.	2009	<30 months	II–IV	CAP vs. surveillance	CAP = surveillance	CAP = surveillance
Craig et al. (PRIVENT Investigators)	2009	<18 years	0–IV (60% 0–II)	CAP vs. placebo	CAP < placebo ARR 6% NNT 14	CAP = placebo
Brandstrom et al.	2011	1–2 years	III–IV	CAP vs. endoscopy vs. surveillance	CAP < surveillance girls Endoscopic < surveillance girls, not boys	CAP < surveillance girls
RIVUR Trial Investigators	2014	<6 years, 8% boys	I–IV (92% I–III)	CAP vs. placebo	CAP < placebo ARR 12% NNT 8 CAP > placebo resistance	CAP = placebo
Hari et al.	2015	<12 years	I-IV	CAP vs. placebo	CAP > placebo	CAP = placebo

= signifies that these treatments were shown to be equivalent; > signifies that the first listed treatment yields more of the outcome than the second one; < signifies that the first listed treatment yields less of the outcome than the second one.
ARR, Absolute risk reduction; *CAP*, continuous antibiotic prophylaxis; *NNT*, number needed to treat; *RIVUR*, randomized intervention for children with vesicoureteral reflux; *UTI*, urinary tract infection; *VUR*, vesicoureteral reflux.

TABLE 80.2 Risk of Breakthrough Urinary Tract Infection Stratification Groups

Risk Category	Defining Risk Factors	% Found in Population	% 2-Year Risk of Breakthrough UTI	Treatment Suggested
Low	VUR grade I–III in female without BBD Circumcised male	67.0	8.6	Surveillance
Intermediate	VUR grade I–III female with BBD Uncircumcised male VUR grade IV–V female presented as prenatal hydronephrosis	27.0	27.0	CAP
High	VUR grade IV–V female presented as UTI	6.0	62.0	CAP and endoscopic or surgical correction

BBD, Bladder bowel dysfunction; *CAP*, continuous antibiotic prophylaxis; *UTI*, urinary tract infection; *VUR*, vesicoureteral reflux.
Data from Hidas G, Billimek J, Nam A, et al. Predicting the risk of breakthrough urinary tract infections: primary vesicoureteral reflux. *J Urol.* 2015;194:1396–1401.

Suggested Readings

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81

Systemic Hypertension

JOSEPH T. FLYNN JR.

KEY POINTS

- There are numerous influences on normal blood pressure (BP) in neonates, including gestational age, birthweight, and maternal factors such as preeclampsia.
- As in older children, identification of hypertension (HTN) in the neonate is dependent on proper BP measurement technique.
- While the differential diagnosis of systemic HTN in the neonate is broad, common causes include catheter-related thromboembolic phenomena, chronic lung disease, kidney disease, and iatrogenic causes. A focused diagnostic evaluation should lead to correct identification of the underlying cause in most neonates.
- Therapy for HTN in the neonate is largely empiric because of a lack of data on outcomes of elevated BP in neonates and exclusion of neonates from clinical trials of antihypertensive medications.

Advances in the ability to care for premature infants have led to an increased awareness of systemic hypertension in the neonatal intensive care unit (NICU). However, there is also uncertainty regarding normative blood pressure (BP) in neonates and the best therapeutic approach to elevated BPs, mostly because of a lack of rigorous evidence. This chapter will review the many factors that influence BP in the neonate, present what we know about normal neonatal BP, and then discuss differential diagnosis of hypertension (HTN), the optimal diagnostic evaluation, and antihypertensive therapy.

Factors that Influence Neonatal Blood Pressure

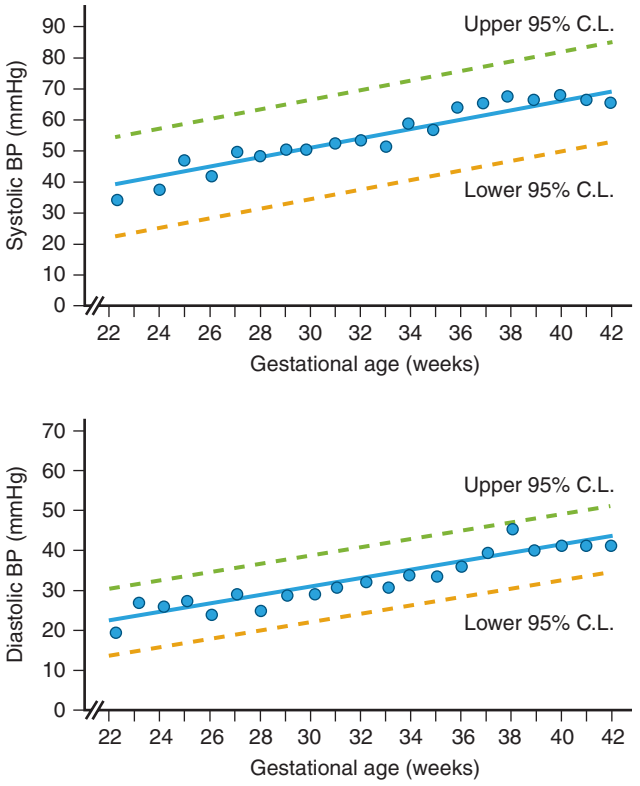
Many studies have examined the factors that influence BP patterns in normal and premature infants. In a study of BP in over 600 infants of various birthweights (BWs) and gestational ages (GAs) admitted to 14 Philadelphia-area NICUs, Zubrow et al.[1] made a series of observations. First, they found that BP at birth is closely correlated with GA (Fig. 81.1) and BW (Fig. 81.2). Then after delivery, there is a predictable increase in BP over the first 5 days of life that is independent of these factors. Thereafter, BP continues to rise gradually, with the most important determining factor being postmenstrual (postconceptual) age (Fig. 81.3). A more recent study of stable NICU infants showed a similar pattern, with BPs in each GA category of premature infants increasing at a faster rate during the first week of life, with subsequent slowing[2]; the rate of rise was more rapid in preterm infants than in term infants (Fig. 81.4). The rate of change in BP has also been shown to be slower in infants born small for GA compared to those born appropriate for GA.[3]

Several maternal factors have emerged that also may have important influences on neonatal BP, including antenatally administered medications and maternal health conditions. Regarding antenatal steroids, while an initial case series suggested that antenatal glucocorticoid exposure led to increased BP in the first week of life,[4] a later randomized controlled trial of corticosteroids versus placebo showed no difference in infant BPs between groups.[5] Inadvertent exposure to agents affecting the renin-angiotensin-aldosterone system (RAAS) during pregnancy is well-known to result in neonatal hypotension, particularly when the exposure occurs in the third trimester.[6] There is some suggestion in the literature that chorioamnionitis and HELLP (hemolysis, elevated liver enzyme levels, low platelet count) syndrome may also be associated with lower infant BPs. On the other hand, maternal preeclampsia appears to be associated with higher offspring BP during the first month of life.[7] Higher infant BPs have been correlated with maternal body mass index greater than 30 kg/m^2 and low socioeconomic status in a study of Nigerian infants[8] and in an Australian study of premature infants born to mothers with diabetes or neonates with abnormal uteroplacental perfusion as evidenced by placental pathology.[9] While these factors may not have caused hypotension or HTN per se, it is clear that many prenatal and postnatal processes combine to influence BP in the newborn period.

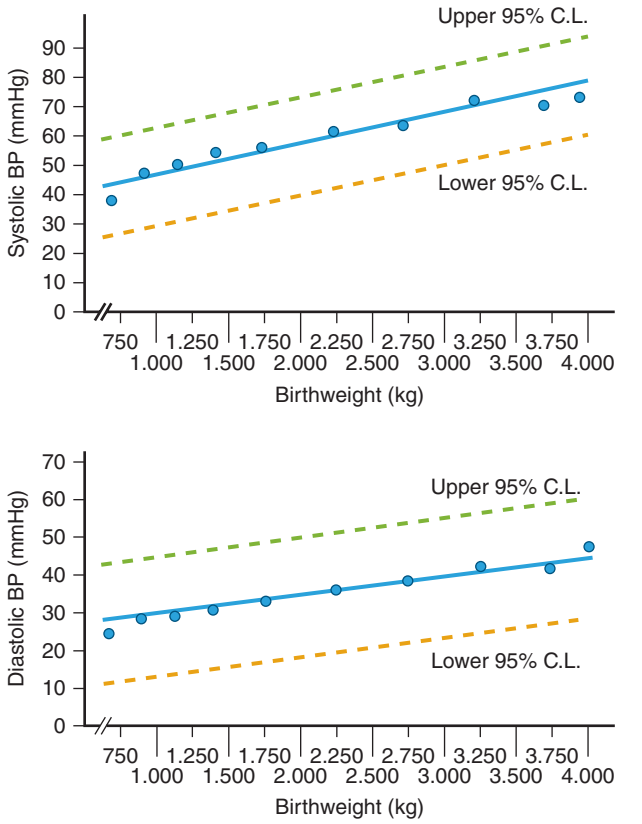
Definition of Hypertension

While the definition of HTN in an older infant or child is clear, it is more difficult to define HTN in newborns and preterm infants given the changes in BP that normally occur in the first few weeks of life discussed earlier.[10] Dionne et al. have summarized available BP data on preterm neonates and have published a table of BPs that is helpful in categorizing an infant's BP as normal or elevated (Table 81.1). Their data were recently endorsed in the 2017 American Academy of Pediatrics (AAP) Clinical Practice Guideline (CPG) on childhood hypertension as the most appropriate reference values for neonates up to 44 weeks post-menstrual age.[11] Sadly, there is a definite gap in knowledge regarding normal BP over the subsequent 12 months of life; the only available data are from the 1987 Second Task Force Report,[12] but they do not correlate well with the revised normative childhood BP data in the 2017 AAP CPG.

For relatively stable infants still in the NICU, a pattern of elevated readings using the data of Dionne et al.,[10] particularly if >99th percentile, should be sufficient to make the diagnosis of



• **Fig. 81.1** Correlation between gestational age and neonatal blood pressure (BP). C.L., Confidence limit. (From Zubrow AB, Hulman S, Kushner H, et al. Determinants of blood pressure in infants admitted to neonatal intensive care units: a prospective multicenter study. *J Perinatol*. 1995;15:470–479.)



• **Fig. 81.2** Correlation between birthweight and neonatal blood pressure (BP). C.L., Confidence limit. (From Zubrow AB, Hulman S, Kushner H, et al. Determinants of blood pressure in infants admitted to neonatal intensive care units: a prospective multicenter study. *J Perinatol*. 1995;15:470–479.)

HTN. If the infant is critically ill and continuous BP monitoring reveals sustained BP elevation over several hours, then HTN should be diagnosed, and appropriate investigation and intervention should be initiated. For older infants and NICU graduates who are being followed up as outpatients, at least three elevated readings should be documented over 1 to 2 weeks before a diagnosis of HTN is made.[12]

Epidemiology

Although one recent series found that 28% of very low birth weight (VLBW) infants had at least one elevated BP reading documented during their NICU stay, the actual incidence of HTN in neonates is very low, ranging from 0.2% in healthy newborns to between 0.7% and 2.5% in high-risk newborns.[11] However, in a recent publication from the Assessment of Worldwide Acute Kidney Injury Epidemiology in Neonates (AWAKEN) study, while the proportion of diagnosed HTN was 1.8% in infants admitted to the NICU, they found that another 3.7% had undiagnosed HTN.[13] This was estimated based upon BP values from a literature review conducted by the AWAKEN investigators themselves, as opposed to the values recommended by the AAP CPG, again highlighting the need for more robust normative data for neonatal BP.

Certain categories of infants are at significantly higher risk, however. For example, HTN is relatively common in patients with a history of umbilical artery (UA) catheterization (3%) and those with bronchopulmonary dysplasia (BPD) (as high as 43%). In one series it was also associated with patent ductus arteriosus and intraventricular hemorrhage. On the other hand, HTN is so uncommon in otherwise healthy term infants that routine BP determination is not even recommended.

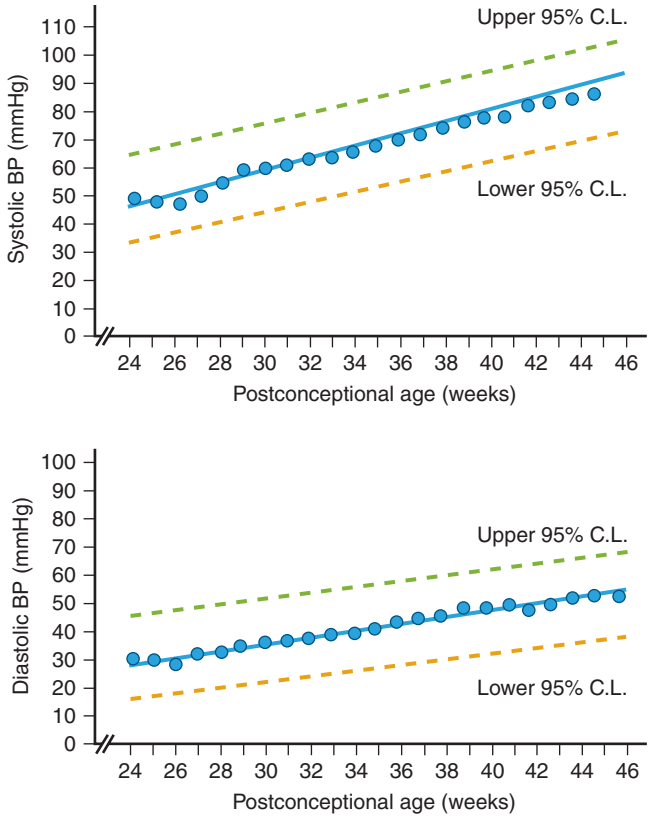
Fewer data are available on the incidence of sustained HTN in NICU graduates. In their classic study, Sheftel et al.[14] performed BP measurement in infants followed up in a neonatal follow-up clinic and found that 8.9% were hypertensive according to criteria used at that time. A later report by some of the same authors demonstrated an incidence of 2.6%.[15] Secondary causes such as those discussed later were found in most cases. More recently, an incidence of elevated BP of 2.9% was seen in a cohort of infants from the neonatology follow-up clinic at the University of Iowa Children's Hospital;[16] elevated BP was associated with the cytochrome P450 genotype (*CYP2D6*) in that cohort. Given these data, it is recommended that BP screening be incorporated into the long-term follow-up of NICU graduates.

Pathophysiology

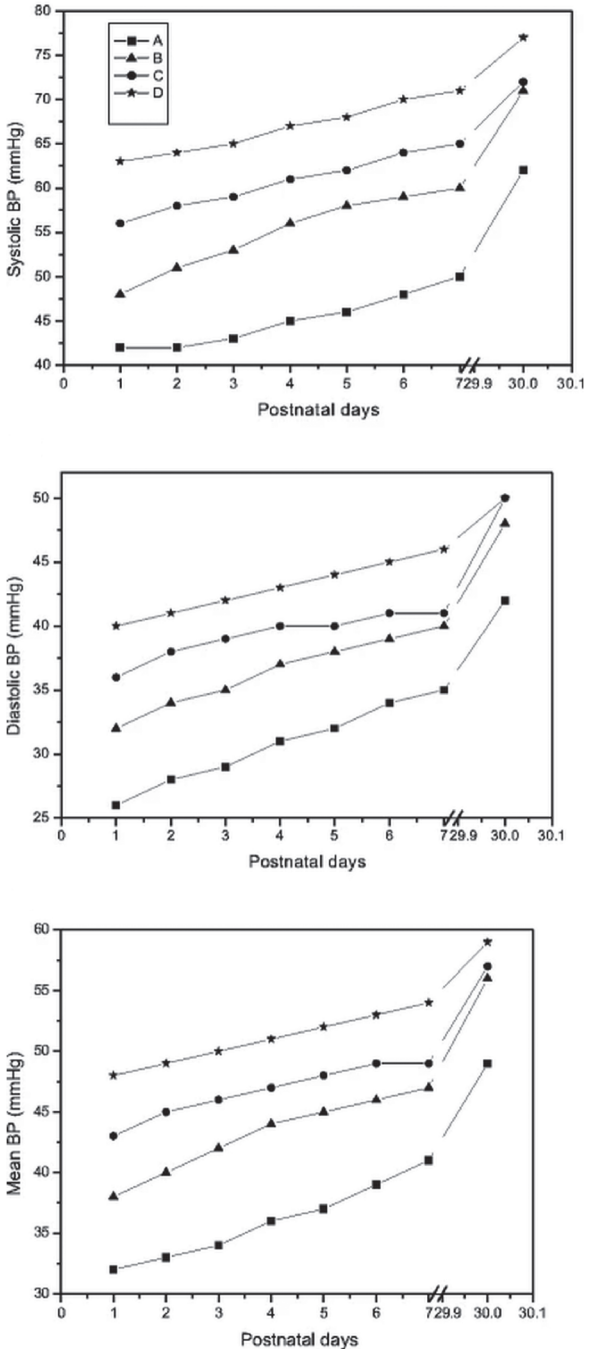
While the differential diagnosis of HTN in the neonate or older infant is extensive (Box 81.1), the most important categories of causes of neonatal HTN include renovascular HTN, BPD, and congenital and acquired kidney disease.

Renovascular Causes

The most common cause of neonatal renovascular HTN is aortic or renal thromboembolism related to UA catheterization.



• **Fig. 81.3** Correlation between postmenstrual (postconceptual) age and neonatal blood pressure (BP). C.L., Confidence limit. (From Zubrow AB, Hulman S, Kushner H, et al. Determinants of blood pressure in infants admitted to neonatal intensive care units: a prospective multicenter study. *J Perinatol*. 1995;15:470–479.)



• **Fig. 81.4** Increase in systolic (A), diastolic (B), and mean blood pressure (C) during the first month of life in groups of infants classified by estimated gestational age: A (≤28 weeks), B (29 to 32 weeks), C (33 to 36 weeks), and D (≥37 weeks). (From Pejovic B, Peco-Antic A, Marinkovic-Eric J. Blood pressure in non-critically ill preterm and full-term neonates. *Pediatr Nephrol*. 2007;22:249–257.)

HTN may develop either while the catheter is in place or long after its removal and may be associated with a history of elevated serum creatinine or gross hematuria. Associated signs may include acute kidney injury (AKI) in patients with bilateral involvement, hematuria, and loss of femoral pulses and blood flow to the lower extremities in patients with extensive aortic thrombosis.

Studies using ultrasound report an incidence of UA-related thromboembolism ranging from 14% to 35%, whereas studies using angiography document incidences up to 64%. Autopsy studies have shown an incidence of UA-related thromboembolism between 9% and 28%, although major clinical symptoms of UA-related thromboembolism occur in just 1% to 3% of infants.[17] Trauma at the time of insertion of an UA catheter caused by endothelial injury is postulated to be the cause of aortic thrombus formation, with clot embolization to the kidneys, causing localized areas of infarction and increased renin release.

Although several studies that have examined the duration of line placement and line position ("low" vs. "high") as factors involved in thrombus formation, the data have not been conclusive. The Cochrane Neonatal Group has attempted to resolve this controversy.[18] It analyzed 11 randomized clinical trials and one study using alternate assignments to compare the incidence of morbidity and mortality for high versus low UAC catheter tip placement. The placement of a catheter tip was defined as 'high' when it was in the descending aorta above the diaphragm and 'low' when it was placed in the descending aorta above the bifurcation but below the renal arteries. The reviewers concluded that high catheter position causes fewer clinically obvious ischemic complications. With regard to HTN, however, it was concluded that it seems to appear with equal frequency among infants with high or low catheter placement.[18]

Congenital vascular anomalies responsible for neonatal renovascular HTN include stenosis or hypoplasia of the renal artery and segmental intimal hyperplasia. These conditions may involve the aorta and the renal arteries. Unilateral renal artery stenosis

TABLE 81.1 Blood Pressure (mmHg) Percentiles for Neonates up to 44 Weeks' Postmenstrual Age

Postmenstrual Age	50th Percentile	95th Percentile	99th Percentile
44 Weeks			
SBP	88	105	110
DBP	50	68	73
MAP	63	80	85
42 Weeks			
SBP	85	98	102
DBP	50	65	70
MAP	62	76	81
40 Weeks			
SBP	80	95	100
DBP	50	65	70
MAP	60	75	80
38 Weeks			
SBP	77	92	97
DBP	50	65	70
MAP	59	74	79
36 Weeks			
SBP	72	87	92
DBP	50	65	70
MAP	57	72	77
34 Weeks			
SBP	70	85	90
DBP	40	55	60
MAP	50	65	70
32 Weeks			
SBP	68	83	88
DBP	40	55	60
MAP	49	64	69
30 Weeks			
SBP	65	80	85
DBP	40	55	60
MAP	48	63	68
28 Weeks			
SBP	60	75	80
DBP	38	50	54
MAP	45	58	63
26 Weeks			
SBP	55	72	77
DBP	30	50	56
MAP	38	57	63

This table provides estimated values for blood pressure percentiles after 2 weeks of age in infants from 26 to 44 weeks' postmenstrual age. The 95th and 99th percentile values are intended to serve as a reference to identify infants with persistently elevated blood pressure.
DBP, Diastolic blood pressure; *MAP*, mean arterial pressure; *SBP*, systolic blood pressure.
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• BOX 81.1 Differential Diagnosis of Hypertension in the Neonate

Renovascular
- Thromboembolism
- Renal artery stenosis
- Midaortic coarctation
- Renal venous thrombosis
- Compression of renal artery
- Idiopathic arterial calcification
- Congenital rubella syndrome

Kidney Parenchymal Disease
- Congenital/chronic
  - Polycystic kidney disease
  - Multicystic dysplastic kidney
  - Tuberous sclerosis
  - Ureteropelvic junction obstruction
  - Kidney hypodysplasia
  - Congenital nephrotic syndrome
  - Renal tubular dysgenesis
- Acquired/acute
  - Acute tubular necrosis
  - Cortical necrosis
  - Interstitial nephritis
  - Hemolytic–uremic syndrome
  - Obstruction (stones, tumors)

Pulmonary
- Bronchopulmonary dysplasia
- Pneumothorax

Cardiac
- Thoracic aortic coarctation

Endocrine
- Congenital adrenal hyperplasia
- Hyperaldosteronism
- Hyperthyroidism
- Pseudohypoaldosteronism type II

Medication Related
- Infant
  - Dexamethasone
  - Adrenergic agents
  - Vitamin D intoxication
  - Theophylline
  - Caffeine
  - Pancuronium
  - Phenylephrine
- Maternal
  - Cocaine
  - Heroin

Neoplasia
- Wilms tumor
- Mesoblastic nephroma
- Neuroblastoma
- Pheochromocytoma

Neurologic
- Pain
- Intracranial hypertension
- Seizures
- Familial dysautonomia
- Subdural hematoma

Miscellaneous
- Total parenteral nutrition
- Closure of abdominal wall defect
- Adrenal hemorrhage
- Hypercalcemia
- Traction
- Extracorporeal membrane oxygenation
- Birth asphyxia
- Fluid overload

may cause a reversible syndrome characterized by hypokalemic alkalosis, hyponatremia, and increased echogenicity of the contralateral kidney.[19]

HTN may rarely result from calcium infiltration of the arterial wall. Generalized arterial calcification of infancy types 1 and 2 (GACI1, Online Mendelian Inheritance in Man [OMIN] catalog #208000; GACI2, OMIM # 614473) are rare disorders characterized by calcium deposits in all layers of the arteries, including the aorta and the coronary arteries, and in the heart valves. Even though these are caused by different genetic mutations (ENPP1 in GACI1 and the ABCC6 gene in GACI2,[20] the disorders are phenotypically similar. HTN is severe and typically fails to respond to standard antihypertensive medication or even to nephrectomy; biphosphonates, calcium antagonists, or prostaglandin infusion may be successful, although reported mortality rates are high.

Other causes of renovascular HTN include neonatal renal arterial embolism in the absence of UA catheterization, intramural hematoma of the renal artery, renal venous thrombosis, and external compression of the renal artery by a hydronephrotic kidney, adrenal hemorrhage, and urinoma. Finally, a neonate with HTN as a result of an aneurysm of the abdominal aorta has

been reported[21]; this fortunately rare condition may present with intractable congestive heart failure.

Bronchopulmonary Dysplasia

Many infants with BPD develop HTN. This phenomenon was first described in the mid-1980s by Abman.[22] In a study of 65 infants discharged from a NICU, the incidence of HTN in infants with BPD was 43%, versus 4.5% in infants without BPD. The investigators were unable to identify a clear cause of HTN but postulated that hypoxemia might be involved. More than half of the infants with BPD who developed HTN did not display it until after discharge from the NICU, further highlighting the need for measurement of BP in all NICU "graduates," and particularly in those with chronic lung disease.

The findings of Abman et al., have been reproduced by other investigators, including Alagappan and Malloy,[23] who found that HTN was twice as common in VLBW infants with BPD compared with all VLBW infants. Because all of the hypertensive infants required supplemental oxygen and aminophylline, development of HTN appeared to be correlated with the severity of pulmonary disease. Anderson et al.[24] have demonstrated that the more severe the BPD (defined as a greater need for diuretics and bronchodilators), the higher the likelihood of the development of increased BP. BPD was also noted as a common diagnosis in a case series from Houston, Texas,[25] and infants from the Iowa cohort mentioned previously[16] were more likely to have elevated BP if they required ongoing oxygen therapy after NICU discharge. These observations reinforce the impression that infants with severe BPD are clearly at increased risk and need close monitoring for the development of HTN.

Congenital and Acquired Kidney Disease

HTN is a common complication of congenital kidney anomalies, including hypoplastic and dysplastic kidneys, and obstructive lesions causing hydronephrosis. It is well known that both autosomal dominant polycystic kidney disease and autosomal recessive polycystic kidney disease (ARPKD) may present in the newborn period with severe nephromegaly and HTN.[26,27] With ARPKD, the median age of onset of HTN has been reported to be 16 days; most affected infants will be discovered to be hypertensive during the first year of life. The most severely affected infants with ARPKD are at risk of development of congestive heart failure from severe, malignant HTN.

Although much less common than in polycystic kidney disease, HTN has also been reported in infants with multicystic dysplastic kidneys.[28,29] This is somewhat paradoxical, as such kidneys are usually thought to be nonfunctioning. The case has been made that HTN in such patients is the result of another coexisting abnormality such as parenchymal scarring.[30] Another possible explanation is increased renin production by macrophages within the dysplastic kidney.[31]

Kidney obstruction may be accompanied by HTN, even when there is no compression of the renal vasculature. This has been seen, for example, in infants with congenital ureteropelvic junction obstruction and sometimes may persist following surgical correction of the obstruction. HTN has also been described in infants with congenital primary megaureter. Ureteral obstruction by other intraabdominal masses may also be accompanied by HTN. The mechanism of HTN in such instances is unclear, although activation of the RAAS may be involved.

HTN as a result of acquired kidney disease occurs less commonly in the NICU than that as a result of congenital abnormalities. However, AKI from nephrotoxic or hypoxic acute tubular necrosis, interstitial nephritis, or cortical necrosis may be accompanied by significant HTN, usually as a result of fluid and sodium overload or activation of the RAAS. Atypical hemolytic–uremic syndrome, which has been described in both term and preterm infants,[32] is usually also accompanied by HTN. Such HTN may be extremely difficult to control, requiring treatment with multiple agents.

Genetic Causes

Genetic forms of HTN that may present in the neonatal period fall into two broad categories: HTN resulting from a single-gene disorder and HTN occurring as one feature of a malformation syndrome. Single-gene disorders causing HTN with reported cases in the neonatal period include Liddle syndrome, glucocorticoid-remediable aldosteronism, and pseudohypoaldosteronism type II (Gordon syndrome). A summary of the major features of these disorders is presented in Table 81.2; for a detailed discussion of monogenic HTN, the reader should consult other references.[33–35]

Malformation syndromes that may cause HTN include Williams syndrome (renal artery stenosis), Turner syndrome (aortic coarctation), neurofibromatosis (renal artery stenosis, coarctation), and Cockayne syndrome. Usually, the HTN in these syndromes presents beyond the neonatal period, but infantile presentations with HTN have been described.

Miscellaneous Causes

Coarctation of the Aorta

Coarctation of the thoracic aorta has been reported in numerous case series of neonatal HTN. Although usually detected in the newborn period based on decreased pulses and lower BPs in the lower extremities compared with the upper extremities, the similarity of upper and lower extremity BP readings in early infancy means that echocardiography is needed for definitive diagnosis.[36] Coarctation repair early in infancy seems to lead to an improved long-term outcome compared with delayed repair; however, recurrence of hypertension later in life is common.[37]

Endocrine Disorders

Endocrine disorders, particularly congenital adrenal hyperplasia, hyperaldosteronism, and hyperthyroidism, constitute easily recognizable clinical entities that have been reported to cause HTN in neonates. Several adrenal disturbances can induce HTN directly; they should be differentiated from Liddle syndrome. Hyperthyroidism is associated with systolic HTN and sustained tachycardia and, sometimes, with episodes of supraventricular tachycardia. An interesting link between phthalate exposure and neonatal HTN reportedly mediated by activation of the mineralocorticoid receptor has recently been reported in a large single-center case series.[38] This association has yet to be confirmed by other investigators.

Tumors

Tumors, including neuroblastoma, Wilms tumor, and mesoblastic nephroma, may present in the neonatal period and may produce HTN, either because of compression of the renal vessels or ureters

TABLE 81.2 Major Features of Monogenic Forms of Hypertension

	OMIM Catalog #	Inheritance Pattern	Age	Potassium Level	Renin Level	Aldosterone Level	Genetic Defect	Therapy
Apparent mineralocorticoid excess	218030	AR	I, C, A	Decreased or normal	Decreased	Decreased	Loss of function in 11-β-HSD2 gene	Spironolactone, eplerenone
Glucocorticoid remediable aldosteronism	103900	AD	I, C	Decreased or normal	Decreased	Decreased or normal	Chimeric gene; fusion of aldosterone synthase and 11-β-hydroxylase genes	Amiloride, triamterene, glucocorticoids
Congenital adrenal hyperplasia	202010	AR	I	Decreased or normal	Decreased	Decreased	Loss of function in 11-β-hydroxylase or 17α-hydroxylase gene	Spironolactone, eplerenone
Liddle syndrome	177200, 618114, 618126	AD	C, A	Decreased or normal	Decreased	Decreased	Gain of function in ENaC gene	Amiloride, triamterene
Pseudohypoaldosteronism type II (Gordon syndrome)	145260, 614492, 614495, 614496	AD, AR	A, C	Increased or normal	Decreased	Increased or normal	Gain of function in WNK1 gene; loss of function in WNK4 gene	Thiazide

A, Adulthood; *AD*, autosomal dominant; *AR*, autosomal recessive; *C*, childhood; *ENaC*, epithelial sodium channel; *11-β-HSD2*, 11-β-hydroxysteroid dehydrogenase2; *I*, infancy; *OMIM*, Online Mendelian Inheritance in Man; *WNK*, WNK lysine-deficient protein kinase.

or because of production of vasoactive substances such as renin or catecholamines. Neurologic causes of HTN include intracranial HTN, drug withdrawal, seizures, pain, and familial dysautonomia. Seizures are common complications of severe HTN; in turn, BP may increase transiently during seizure episodes. Appropriate pain relief should be given before and after surgical procedures.

Iatrogenic Causes

Iatrogenic causes of neonatal HTN are common and usually obvious but are important to consider. If the infant is hypervolemic secondary to excessive administration of sodium or fluids, intake should be restricted, and a diuretic should be administered. It is imperative to eliminate hidden sources of sodium, such as isotonic saline used to flush an arterial line and sodium-containing medications (e.g., antibiotics). If HTN is induced by a medication, one may consider withholding it, decreasing the dose, or using an infusion instead of repeated injections. As noted earlier, dexamethasone is a relatively common cause of BP elevation[39,40]; if this occurs, a decision must be made regarding the possible benefits of continued steroid treatment versus the risks of HTN. HTN induced by pancuronium is probably related to catecholamine release; BP may normalize after substitution of vecuronium for pancuronium.

Extracorporeal Membrane Oxygenation

HTN develops in 11% to 92% of neonates receiving extracorporeal membrane oxygenation[41,42] and may result in serious complications, including intracranial hemorrhage and increased mortality. Despite extensive investigation, the exact pathogenesis of this form of HTN remains poorly understood. Fluid overload, altered kidney sodium and water handling, and altered baroreceptor function have all been proposed as causative factors. Nicardipine infusions are commonly used to treat this form of HTN.[43]

Postsurgical

Finally, HTN may develop after surgery, specifically following repair of various abdominal wall defects. Including omphalocele and gastroschisis.[44] HTN appearing after primary closure for bladder exstrophy may be related to traction for skeletal immobilization.[45]

Evaluation

The first step in the evaluation is to make sure the BP is being measured correctly; this is discussed in detail in the next section. After that, it is important to determine whether the infant is persistently hypertensive or if the BP rises only during periods of agitation, pain, crying, feeding, or performance of procedures. Only in infants with correctly measured BP and persistent BP elevation should the "diagnosis" of HTN be considered and diagnostic work-up be initiated.

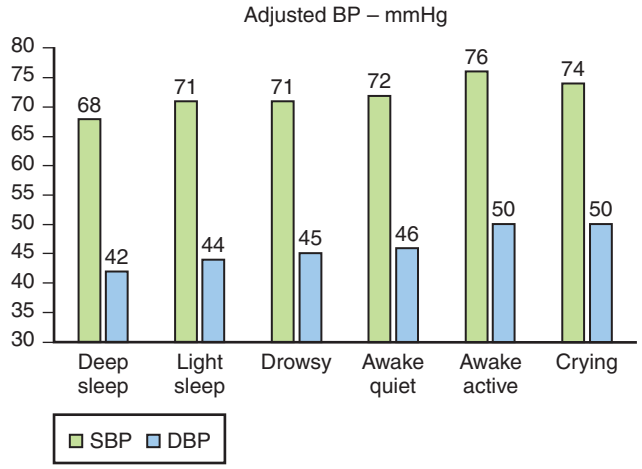
Blood Pressure Measurement

Many aspects of BP measurement in neonates are addressed in a recent systematic review of the literature[46]; therefore, only a few key points will be covered here. While direct measurement of BP via an indwelling arterial catheter is the most accurate method of BP measurement in neonates, the most commonly

used method in most NICUs, especially in stable infants, is the oscillometric technique, which directly measures mean arterial pressure (MAP) based on the oscillations of the arterial wall as the cuff is deflated. Although the readings obtained by oscillometric devices may differ between 1 and 5 mmHg compared with directly measured BP, these devices are usually sufficiently accurate for routine clinical use.[47] Oscillometric devices are clearly useful for measuring BP in infants without indwelling arterial catheters (especially those who require frequently repeated BP measurement) and in infants who have been discharged from the nursery.

Selection of a proper sized cuff is crucial for correct indirect BP measurement. The cuff bladder length should encircle 80% to 100% of the arm circumference; a cuff bladder with a width-to-length ratio of 0.45 to 0.55 is recommended.[46] A full range of "neonatal" cuff sizes will need to be available for outpatient follow-up of NICU graduates. If BP is measured in the calf, it is important to use a wide enough cuff; cuffs designed for use in the upper arm may be too narrow to use on the calf, resulting in a falsely high BP reading.

BP increases when an infant is awake, in the knee–chest position, with crying, pain, and during physical examination and procedures, or during feeding. A recent study from Japan of BP in 3148 newborns demonstrated the effects on BP of various states of activity (Fig. 81.5).[48] Even pacifier use during sleep has been shown to increase BP.[49] Because of these factors, it is important to follow a standard approach for BP measurement in neonates; the protocol described by Nwankwo et al.[46,50] is appropriate. The protocol involved BP measurements at least one and one-half hours following their last feeding or medical intervention, or just before feeding. An appropriately sized cuff (sizes 2 to 4) was applied to the right upper arm. The infant was then left undisturbed for at least 15 minutes or until the infant was sleeping or in a quiet awake state prior to BP measurement.[50] Measurements in NICU graduates should be obtained only when the infant is asleep or calm.[51]



• **Fig. 81.5** Effect of infant state on blood pressure (BP). *DBP*, Diastolic blood pressure; *SBP*, systolic blood pressure. (From Satoh M, Inoue R, Tada H, et al. Reference values and associated factors for Japanese newborns' blood pressure and pulse rate: the babies' and their parents' longitudinal observation in Suzuki Memorial Hospital on intrauterine period (BOSHI) study. *J Hypertens*. 2016;34:1578–1585.)

History and Physical Examination

In most neonates suspected of having HTN, a relatively focused history can be obtained, paying attention to determining whether there were any relevant prenatal exposures and to the particulars of the infant's clinical course and any concurrent medical conditions/complications of prematurity. The procedures that the infant has undergone (e.g., umbilical catheter placement) should be reviewed, and the current medication list should be scrutinized. Easily identifiable causes of HTN such as fluid overload or medication-induced HTN should be able to be identified at this stage, and appropriate countermeasures should be taken to correct the problem.

The physical examination should similarly focus on identifying obvious problems that may be causing the high BP. While four-extremity BP readings are commonly recommended as a screen for aortic coarctation, this may not be sufficient, as arm and calf BP readings may be similar in neonates.[36] The general appearance of the infant should be assessed, with particular attention paid to the presence of dysmorphic features. Careful cardiac and abdominal examinations should be performed, as specific findings (heart murmur, unilateral flank mass, ambiguous genitalia, etc.) can help direct subsequent diagnostic testing.

Laboratory Testing and Imaging

If no iatrogenic or other obvious correctable cause of HTN has been found, laboratory testing and diagnostic imaging will be needed, beginning with a urinalysis and screening chemistry tests (Table 81.3). If there is any suspicion of urinary tract infection, urine obtained by suprapubic aspiration or bladder catheterization should be sent for bacterial and fungal culture. Ultrasonography of the kidneys, adrenal glands, aorta, and bladder, with a flow study (i.e., Doppler ultrasonography) of the aorta and the renal arteries and renal veins, is usually appropriate. A chest radiograph may be useful if one has not been obtained recently. An echocardiogram will be needed to confirm the diagnosis of aortic coarctation. Nuclear renal scans, angiography, magnetic resonance imaging, or computed tomography are generally less useful in neonates because of immature kidney function and/or size considerations but may be indicated in specific patients.

Plasma renin activity (PRA) measurement is often included as part of the laboratory evaluation of neonatal hypertension. The PRA is most helpful if it is extremely low—in such cases, a single-gene disorder affecting kidney sodium transport should be suspected (see the preceding discussion on monogenic HTN). Elevated PRA is less helpful, as it may be secondary to the administration of diuretics or adrenergic medications or may be secondary to severe respiratory disease; mild elevations of PRA may be seen in normal infants given the role of renin in kidney development.

Other blood studies listed in Table 81.3 should be reserved for selected infants when there is a suggestion from the history, physical examination, and/or screening studies that a specific secondary cause of HTN may be present. Usually, it is best to obtain the assistance of the appropriate subspecialist (nephrologist, endocrinologist, oncologist, etc.) before such studies are ordered.

Management

As already mentioned, treatment of neonatal HTN should begin with correction of any iatrogenic causes such as fluid overload or medications that increase BP. After that, a decision needs to be

TABLE 81.3 Diagnostic Studies

Generally Useful	Useful in Selected Infants
Urinalysis (with or without culture)	Thyroid studies
Complete blood count and platelet count	Aldosterone
Electrolytes	Cortisol
Blood urea nitrogen, creatinine	Urine vanillylmandelic acid/homovanillic acid
Calcium	Plasma catecholamines/metanephrines
Plasma renin	Echocardiogram
Chest x-ray	Abdominal/pelvic ultrasonography
Renal ultrasonography with Doppler measurement	Voiding cystourethrogram
	Renal angiography
	Nuclear scan (DTPA/MAG-3)

DTPA, Diethylenetriaminepentaacetic acid; *MAG-3*, mercaptoacetyltriglycine.

made as to whether antihypertensive medications are indicated. Clearly, infants with severe, symptomatic HTN should receive immediate treatment. However, in those with less severe HTN, particularly those in whom the high BP does not appear to be causing any immediate problem, the necessity of initiating treatment with antihypertensive medications is often unclear. We do not know whether HTN in such infants has any impact on long-term outcomes, and we certainly do not know at what BP target-organ effects begin to appear. Furthermore, no clinical trials of antihypertensive medications have ever been conducted in neonates, so there are no data on efficacy or safety in this population. Thus, a great deal of individual judgment must be brought to bear in deciding to treat HTN in the NICU.

Critically ill infants with acute onset of severe HTN should generally be treated with an intravenous agent administered by continuous infusion, as this will allow the greatest control of the rate and magnitude of the BP reduction. These infants should have their BP lowered by no more than 25% of the planned reduction in the first 8 hours to prevent cerebral ischemia.[52] On the other hand, relatively well infants with mild HTN may be treated with oral antihypertensive agents. Recommended doses for intravenous and oral antihypertensive drugs in neonates can be found in Table 81.4.

The choice of oral antihypertensive medication to use in hypertensive neonates is somewhat controversial. Available data indicate that hypertensive infants are treated with a wide variety of classes of antihypertensive agents.[41] Whereas angiotensin-converting enzyme (ACE) inhibitors are considered the drugs of choice for adults and children with HTN secondary to kidney disease, and although there is a long history of their use in neonatal HTN, many neonatologists and pediatric nephrologists have serious concerns about the potential major side effects, such as excessive hypotension, AKI,[53] and CNS ischemia. There may also be adverse effects on the completion of kidney development in premature neonates.[54] Other medications, such as a β-blocker or, in the case of a hypertensive crisis, a potent vasodilator, should be tried first. The advantage of a β-blocker such as propranolol is that it reduces the secretion of renin and the release of norepinephrine; however, it may also cause bronchoconstriction, making its use problematic in some infants.

Of the available vasodilators, the calcium channel blockers isradipine and amlodipine have found widespread use in neonates.[10] Older vasodilating agents such as hydralazine and minoxidil may also be useful in selected infants or when the newer agents are not available. Clonidine is yet another potential choice. All oral antihypertensive medications excepting propranolol and amlodipine need to be compounded into suspensions by the hospital pharmacy.

Of the many intravenous antihypertensive agents available, nicardipine has emerged as the most useful for management of severe neonatal HTN, including HTN in neonates who are on ECMO.[43,55,56] It can be precisely titrated to the desired antihypertensive effect, and its use may be continued for prolonged periods without loss of antihypertensive efficacy. A possible issue is the need to infuse nicardipine through a central line. Alternative agents that may be given by continuous infusion include esmolol, hydralazine, labetalol, and sodium nitroprusside. Esmolol and labetalol may be contraindicated in infants with lung disease, and nitroprusside can be used only for limited periods (usually <72 hours) because of the accumulation of thiocyanate.

Intravenous antihypertensives that can be administered by intermittent bolus injection include hydralazine and labetalol. The intravenous ACE inhibitor enalaprilat has been reported to be effective in cases of severe neonatal HTN in one small case series[57]; however, our anecdotal experience suggests that this agent may cause sudden, oliguric AKI similar to that reported for orally administered enalapril.[58] Given this, we do not recommend use of enalaprilat in neonates.

Surgery is indicated for treatment of neonatal HTN in a limited set of circumstances.[59] In particular, HTN caused by urologic causes such as ureteropelvic junction obstruction or aortic coarctation is best approached surgically. On the other hand, most infants with renal artery stenosis will need to be treated medically until they have grown sufficiently to undergo definitive repair of the vascular abnormalities, although case reports of successful intervention in infancy have been published.[60] Infants with HTN secondary to Wilms tumor or neuroblastoma will require surgical tumor removal, possibly following chemotherapy. A case has also been made by some authors for prophylactic removal of multicystic dysplastic kidneys because of the risk of development of HTN,[61] although this is controversial.

Outcomes

The prognosis of neonatal HTN depends on the cause, timing of the diagnosis, presence of complications, and response to therapy. Patients with severe HTN and neurologic, cardiovascular, or AKI requiring kidney replacement therapy have a high mortality rate. Although data are limited, the long-term prognosis for newborns with HTN related to thromboembolism or BPD appears to be good, often with progressive resolution of the HTN within 6 to 12 months of age.[62,63] On the other hand, patients with parenchymal kidney disease, especially those with persistently impaired kidney function following NICU discharge, are likely to have persistent HTN throughout childhood. Infants who undergo repair of aortic coarctation are at long-term risk of persistent or recurrent HTN later in childhood and require continued follow-up.[37]

TABLE 81.4 Recommended Dosing of Selected Antihypertensive Agents in Neonates

Class	Drug	Route	Dose	Interval	Comments
ACE inhibitors	Captopril	Oral	<3 months: 0.01–0.5 mg/kg/dose Maximum 2 mg/kg/day >3 months: 0.15–0.3 mg/kg/dose Maximum 6 mg/kg/day	TID	1. First dose may cause rapid drop in BP, especially if receiving diuretics 2. Monitor serum creatinine and K^+ levels
	Enalapril	Oral	0.08–0.6 mg/kg/day	BID	
	Lisinopril	Oral	0.07–0.6 mg/kg/day	QD	
α and β antagonists	Labetalol	Oral	0.5–1.0 mg/kg/dose up to 10 mg/kg/day	BID–TID	Heart failure, BPD relative contraindications
		IV IV	0.20–1.0 mg/kg/dose (bolus) 0.25–3.0 mg/kg/h	Q4h–Q6 hInfusion	
	Carvedilol	Oral	0.1 mg/kg/dose up to 0.5 mg/kg/dose	BID	May be useful in heart failure
β antagonists	Esmolol	IV	100–500 μg/kg/min	Infusion	Very short acting—constant infusion necessary
	Propranolol	Oral	0.5–1.0 mg/kg/dose Maximum 8–10 mg/kg/day	TID	Monitor heart rate; avoid in BPD
Calcium channel blockers	Amlodipine	Oral	0.05–0.3 mg/kg/dose up to 0.6 mg/kg/day	QD	All may cause mild reflex tachycardia
	Isradipine	Oral	0.05–0.15 mg/kg/dose up to 0.8 mg/kg/day	QID	
	Nicardipine	IV	0.5–4 μg/kg/min	Infusion	Useful for management of severe neonatal HTN, including HTN in neonates who are on ECMO
Central α agonist	Clonidine	Oral	5–10 μg/kg/day up to 25 μg/kg/day	TID	May cause mild sedation
Diuretics	Chlorothiazide	Oral	5–15 mg/kg/dose	BID	Monitor electrolyte levels
	Hydrochlorothiazide	Oral	1–3 mg/kg/dose	QD–BID	
	Spironolactone	Oral	0.5–1.5 mg/kg/dose	BID	
Vasodilators	Hydralazine	Oral	0.25–1.0 mg/kg/dose up to 7.5 mg/kg/day	TID–QID	Tachycardia and fluid retention are common side effects
		IV	0.15–0.6 mg/kg/dose	Q4h	
	Minoxidil	Oral	0.1–0.2 mg/kg/dose	BID–TID	Tachycardia and fluid retention are common side effects; prolonged use causes hypertrichosis
	Sodium nitroprusside	IV	0.5–10 μg/kg/min	Infusion	Thiocyanate toxicity can occur with prolonged (>72 h) use or in renal failure

Use of any of these medications in neonates should be considered off-label.
ACE, Angiotensin-converting enzyme; *BID*, twice daily; *BP*, blood pressure; *BPD*, bronchopulmonary dysplasia; *IV*, intravenous; *K^+*, potassium; *QD*, once daily; *Q4h*, every 4 hours; *Q6h* every 6 hours; *QID*, four times daily; *TID*, three times daily.

Suggested Readings

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82 Developmental Endocrinology

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KEY POINTS

- Developmental disorders of endocrine organs often manifest themselves in the neonatal period.
- The fetal adrenal gland produces large amounts of androgens to be used by the placenta for estrogen biosynthesis.
- Neonatal hypopituitarism may be due to a number of specific gene mutations with or without defects in other cranial structures.
- The thyroid emerges from the pharyngeal floor and then migrates caudally to its final destination in the anterior neck. Abnormalities in thyroid descent result in ectopic thyroid, thyroglossal duct, or cysts.
- Pancreatic β-cell differentiation and proliferation are dependent on insulin-like growth factor 2.
- The placenta actively transports maternal calcium, phosphorus, and magnesium to maintain the high fetal serum levels necessary for the developing skeleton.

Like the development of other fetal organ systems, the development of the endocrine system involves the integration of complex genetic, cellular, and hormonal cues as well as the coordinated action of transcription factors, signaling molecules, and epigenetic regulation. Additionally, endocrine communication is necessary for vital maternal-placental-fetal interaction because endocrine signals are the tools that cells and organs use to communicate. In this chapter, we will first review basic concepts of hormonal systems and then discuss the unique hormonal milieu of the placenta that allows maternal-fetal communication and fetal growth. The development of each of the classic endocrine organs will then be discussed. Finally, the developmental origins of the adult disease hypothesis will be examined.

Endocrine Systems

A few key concepts of cell communication will be presented here to help build a foundation of knowledge to understand hormonal abnormalities in neonates.

In the process of communication, cells emit signals that act locally or distally. If a cell emits signals that are received by the cell itself, this is termed *autocrine regulation*. If a cell emits signals that are received by neighboring cells, this is termed *paracrine regulation*. Finally, if the cell signal travels through the bloodstream to distant cells and organs, this is termed *endocrine regulation*.

A feature of endocrine systems is that the hormonal signals are present in the bloodstream at very low concentrations, and even small changes in hormone concentration can elicit a robust response from receiving cells. The released endocrine hormones can be either water-soluble peptide hormones that must bind to receptors on the extracellular surface of the cell or fat-soluble steroid-like molecules that can pass through the cell membrane directly into the cell's nucleus to bind its receptor to regulate gene transcription. On binding to their specific cell membrane receptor, peptide hormones activate downstream signaling transduction pathways in the cell to induce biological responses. These responses can be immediate; for instance, thyroid-stimulating hormone (TSH), when bound to the TSH receptor on thyroid follicular cells, cause iodine transport into the cell to increase. It can also induce relatively delayed cell responses, such as an increase in gene transcription of enzymes involved in thyroglobulin production. In contrast, steroid-like molecules, such as cortisol, when bound to their respective receptors, induce a biological response by causing an increase in the transcription of various genes. These induce longer-term, broader changes in the target cell function by modulating which genes the cells express and, thus, the proteins they produce.

Many hormonal systems exhibit a hierarchy of hormone signals that allow strict regulation of system function. The hypothalamic-pituitary-end gland system is an example of this multitier hormonal system. In this system, a hormone released by the upstream hypothalamus travels through the portal circulation of the pituitary and binds to the corresponding receptor on the dedicated pituitary cell. Once bound, this receptor induces the cell to synthesize and release the corresponding pituitary hormone. This hormone then travels through the systemic circulation to the endocrine gland to bind to the hormone's dedicated receptor on the target gland. The binding of the hormone to its receptor induces the target organ to release its dedicated end hormone. This hormone then travels through the bloodstream to multiple organs to affect cell function. End hormones use negative feedback to regulate their own production. That is, the hypothalamus and pituitary sense the end hormone concentration, adjusting the release of the upstream regulatory hormone cascade to fine-tune end hormone production. Negative feedback is a unique and consistent property of hormonal systems and is useful in interpreting the adequate response of a hormonal cascade to perturbation in the system.

Endocrine Organ Development and Perinatal Transition

Early in fetal development, fetal hormonal gland development is driven by fetal genotype. However, later in fetal development, the hormonal responsiveness of target tissues is dependent not only on fetal genotype but also on the complex fetal hormonal milieu, which comprises fetal, maternal, and placental hormones. Placental hormones may be influenced by maternal and fetal genotype, as well as maternal prepregnancy and pregnancy health and nutrition. Thus the fetal hormonal environment is dependent on a complex interplay between fetal and maternal factors.

In evaluating hormonal disorders in newborns, one needs to consider both maternal and fetal factors and keep the following principles in mind. First, human fetal endocrine organ development begins predominantly independently of maternal hormones. This independence is possible because the placenta is a barrier to many (but not all) maternal hormones, including steroids, peptides, and glycoproteins. Second, although endocrine organ development may be normal, perturbations in the maternal hormonal milieu may still affect fetal development for the rare hormone that crosses the placenta. An example is disorders of thyroid hormone production. Maternal-fetal transfer of thyroxine (T_4) may result in 25% to 50% of neonatal plasma levels of T_4.[1] This transfer allows children with athyrosis to have good neurodevelopmental outcomes if treatment is initiated within 2 weeks of birth, as the maternal thyroid hormone crossed the placenta and allowed normal fetal neurodevelopment and growth. In contrast, children born to mothers with untreated or undertreated hypothyroidism during pregnancy have poor neurodevelopmental outcomes because of the hypothyroxinemia early in gestation before T_4 production by the fetal thyroid.[2] Third, alterations in transplacental substrate transfer can modify the late development of the fetal and, thus, neonatal hormonal pathways and feedback mechanisms. This can be seen in neonates born from pregnancies with uncontrolled diabetes, in which the transplacental passage of glucose induces robust insulin release and subsequent β-cell hypertrophy in the still-developing pancreas. This leads to transient neonatal hyperinsulinemic hypoglycemia due to the abrupt fall in glucose supply at birth. This is also seen in the placental transfer of hormonal agents or maternal antibodies that affect neonatal endocrine gland function. Examples include the transplacental crossing of maternal TSH antibodies causing neonatal hyperthyroidism. Fourth, when interpreting research on endocrine developmental biology from animal fetal physiology models and gene manipulation studies, one must consider similarities and differences between animal and human fetuses and newborns. The maturational state at birth differs widely among species. For many species, including rodents and some large animals, the maturational state of the newborn is relatively immature (altricial). Organ systems in humans, in contrast, are relatively more developed at birth (precocial). This is especially true in neuroendocrine and most endocrine systems. Thus a study of hormonal physiology in nonhuman species may yield insights, but they may not be immediately applicable to human newborns.

The Maternal-Placental-Fetal Unit

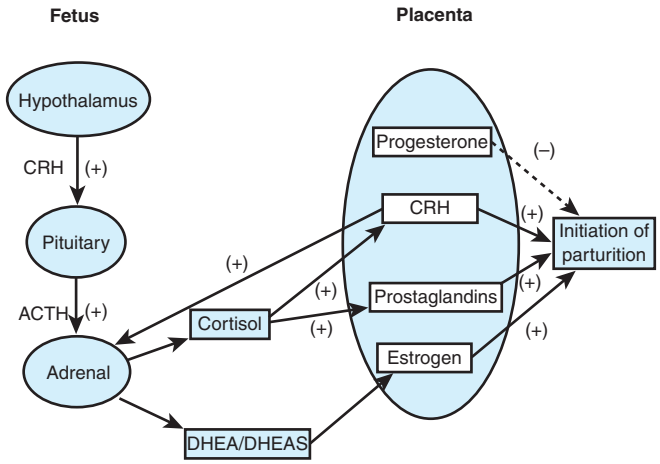
During pregnancy, the mother, fetus, and placenta function in concert as a steroidogenic unit for estrogen and progesterone production. Most steroidogenic activity is exerted in the fetal zone (FZ) of the human fetal adrenal (HFA) gland, where large amounts of adrenal androgens are produced to be used by the placenta for estrogen biosynthesis. Estrogen promotes placental trophoblast differentiation into syncytiotrophoblast and upregulates key enzymes in progesterone biosynthesis.[3]

Cortisol may act as a "two-edged sword" for the fetus; it promotes the maturation of fetal organs necessary for extrauterine life, but it can also adversely influence fetal growth and postnatal development. Therefore, cortisol production in the fetoplacental unit is strictly regulated to protect the fetus from hypercortisolism effects. Fetal access to maternal glucocorticoid is restricted, and a maternal-fetal gradient is maintained by the enzyme 11β-hydroxysteroid dehydrogenase type 2, which converts cortisol to inactive cortisone. Maternal cortisol levels are usually 5 to 10 times higher than fetal cortisol levels. Fetal protection is also achieved through other mechanisms in the HFA by regulation of 3β-hydroxysteroid dehydrogenase/$\Delta^{4,5}$ isomerase type 2, the key steroidogenic enzyme for cortisol biosynthesis, and in fetal membranes by control of 11β-hydroxysteroid dehydrogenase type 1 activity, which converts inactive cortisone to cortisol.[3] A single course of glucocorticoids is accepted as standard therapy for pregnant women at risk of preterm delivery to accelerate fetal lung maturation and reduce morbidity and mortality in preterm infants.[4] However, multiple courses of prenatal glucocorticoids (more than 2) may be associated with decreased weight, length, and head circumference at birth and are currently not recommended because of concerns for maternal and fetal harm.[5]

Placental corticotropin-releasing hormone (CRH) is one of the key determinants of the timing of parturition. Near term, CRH levels increase and directly stimulate the HFA by increasing its responsiveness to adrenocorticotropic hormone (ACTH) and secretion of cortisol and dehydroepiandrosterone (DHEA) and its sulfonated form, precursors of placental estrogen. Cortisol, in turn, stimulates placental CRH production, forming a positive feedback loop and generating more cortisol and estrogen near term. Estrogen upregulates contraction-associated proteins and transforms the myometrium into a contractile state, preparing for successful uterine contractions and parturition. Cortisol also promotes the maturation of fetal organs (e.g., the lung) and stimulates the production of prostaglandins necessary for parturition. Increased levels of estrogen, cortisol, and CRH, together with functional progesterone withdrawal, are thought to contribute to the initiation of parturition (Fig. 82.1).[6,7]

From this perspective, one would expect that the lower cortisol levels seen in preterm infants may have adverse consequences. This is not confirmed. Some studies showed no adverse effects related to low cortisol levels in preterm infants,[8] while others correlated low cortisol levels to increased severity of illness, hypotension, mortality, and development of bronchopulmonary dysplasia.[9] Selective hydrocortisone supplementation could be beneficial for survival in very preterm infants but with possible increased risks of spontaneous gastrointestinal perforation and late-onset sepsis.[10]

Umbilical plasma estradiol and progesterone levels are quite high and fall approximately 100-fold during the first day after birth. The consequences of estradiol and progesterone withdrawal earlier in premature infants remain largely unknown. Pilot studies of estradiol and progesterone supplementation in extremely low-birth-weight infants have shown trends toward increased bone mineralization and a decrease in the incidence of chronic lung disease.[11,12] Adequately powered clinical trials are needed to determine the benefits and risks of hormonal replacement in preterm infants.

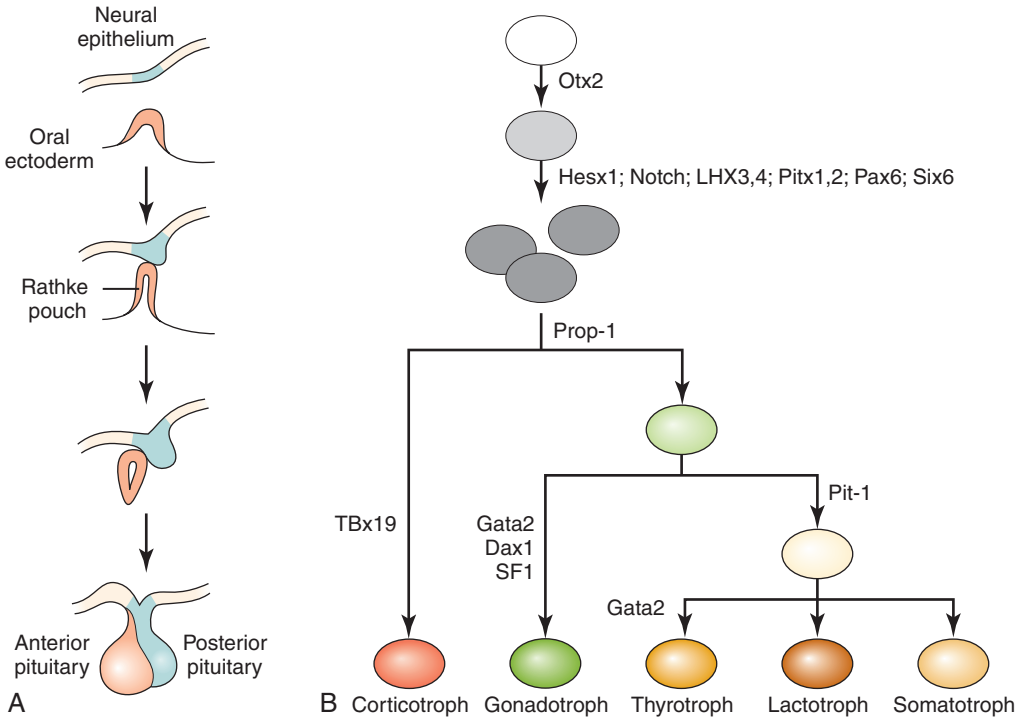


• **Fig. 82.1** Endocrine Cascades in the Fetoplacental Unit That Lead to Initiation of Parturition. Near term, the production of placental corticotropin-releasing hormone *(CRH)* increases and stimulates fetal adrenal glands to produce dehydroepiandrosterone *(DHEA)*/dehydroepiandrosterone sulfate *(DHEAS)* and cortisol. Increased cortisol production stimulates production of placental CRH through a positive feedback loop. DHEA/DHEAS is converted by the placenta to estrogen, promoting the initiation of parturition through the upregulation of contraction-associated proteins. Cortisol and CRH also stimulate the production of prostaglandins necessary for contractions. When "functional withdrawal" of progesterone occurs, coupled with these changes, parturition is initiated. *ACTH*, Adrenocorticotropic hormone.

Hypothalamic and Pituitary Development

The anterior and posterior pituitary glands have different embryonic origins. The anterior lobe arises from the oral ectoderm, and the posterior pituitary arises from the infundibulum of the developing central nervous system. A section of the oral ectoderm thickens very early in development, forming an invagination termed a *Rathke pouch*. As the Rathke pouch invaginates further, the adjacent neural ectoderm evaginates to form the infundibulum (Fig. 82.2A). With further evagination, the Rathke pouch and the infundibulum directly contact each other. This close contact is essential for subsequent anterior and posterior pituitary development. The Rathke pouch later "pinches off" from the remaining oral ectoderm to form the anterior pituitary gland. In humans, this occurs by 5 to 6 weeks of gestation.

The hypothalamus comprises a diverse collection of neurons that regulate pituitary hormone release, thirst, body temperature, blood pressure, and serum osmolality. Although the location of the diverse neurons within the hypothalamus is well delineated, the development of these neuron populations within the hypothalamus has yet to be elucidated. The complexity of the anatomy and neuronal cell types makes it difficult to elucidate the developmental cascade of events. Nevertheless, it is known that the hypothalamic nuclei, which contain the individual neuron types, are fully developed with projections to the median eminence (with subsequent release of hormones into the pituitary portal circulation) by 15 to 18 weeks' gestation.[13]



• **Fig. 82.2** Pituitary Gland Development. (A) Simplified schematic of anterior pituitary development from oral ectoderm via evagination into the Rathke pouch, with subsequent pinching from tissue to separate from oral ectoderm and eventually form the anterior pituitary. (B) The transcription factor cascade is implicated in pituitary development and cell differentiation. *Dax1*, Dosage-sensitive sex reversal, adrenal hypoplasia congenital, X-chromosome factor; *Gata2*, GATA binding protein; *Hesx1*, homeobox1; *LHX3,4*, LIM homebox3,4; *Pax6*, paired box 6; *Pitx1,2*, paired-like homeodomain 1, 2; *Prop-1*, prophet of Pit-1; *Otx2*, orthodenticle homeobox 2; *SF1*, steroidogenic factor 1; *Six6*, sine oculis-related homeobox 6; *TBx19*, T-box 19.

In contrast, knowledge regarding differentiation of the anterior pituitary is well delineated. Differentiation of Rathke pouch cell progenitors into the pituitary cell types (corticotrophs, gonadotrophs, lactotrophs, thyrotrophs, and somatotrophs) is tightly regulated by a cascading series of transcription factors (see Fig. 82.2B). Knowledge of the series of events comes from knockout studies in mice as well as genetic studies in humans with congenital hypopituitarism. The pituitary cell types arise in a temporally and spatially specific pattern as directed by the transcription factors, occurring between week 7 and week 16 of human gestation. If one transcription factor of the developmental series malfunctions or is expressed out of series, as is seen in clinical syndromes of hypopituitarism (Table 82.1), then a very typical pattern of pituitary hormone deficiency is manifest.[14]

Each mature pituitary cell type synthesizes and secretes a corresponding hormone that is regulated by a hypothalamic peptide: corticotrophs secrete ACTH in response to CRH; gonadotrophs secrete luteinizing hormone (LH) and follicle-stimulating hormone (FSH) in response to gonadotropin-releasing hormone (GnRH); lactotrophs secrete prolactin in response to dopamine; thyrotrophs secrete thyrotropin (TSH) in response to thyrotropin-releasing hormone (TRH; and somatotrophs secrete growth hormone in response to growth hormone-releasing hormone and somatostatin.

The mature pituitary cell types contain secretory granules by 10 to 12 weeks' gestation, and the hormones can be measured by 12 to 17 weeks' gestation.[13] The pituitary portal circulation is mature by 12 to 17 weeks, and thus regulation of pituitary hormone release by the corresponding hypothalamic peptide may be operational by that time. However, the negative feedback mechanisms that finely tune hypothalamic-pituitary-end hormone release may not be fully mature until later in gestation. At birth, the negative feedback mechanisms are fully mature, which is crucial in the diagnostic evaluation of infants with suspected pituitary hormone disorders.

The neurohypophysis (posterior pituitary), formed by the evagination of the neuroectoderm, is fully developed by 10 to 12 weeks of gestation and contains granules of arginine vasopressin (AVP) and oxytocin.[15] The neurohypophysis comprises the axonal terminals of hypothalamic neurons whose cell bodies are in the mature paraventricular nuclei and supraoptic nuclei. These neurons generate AVP or oxytocin separately and are thus regulated by separate factors. AVP-releasing neurons integrate signals from plasma osmolality sensors and baroreceptors in the carotid sinus and aortic arch to release AVP and thus regulate serum osmolality and systemic blood pressure. AVP acts on the renal collecting duct via V_2 receptors to induce water reabsorption. AVP also acts on V_{1A} receptors on endothelial cells to increase

TABLE 82.1 Genes Important in Pituitary Development and Implicated in Human Disease

Gene	Involved Pituitary Cell Types	Extrapituitary Phenotype	Radiology Findings	Mode of Inheritance
HESX1	Somatotrophs, thyrotrophs, gonadotrophs, posterior pituitary cells	Septo-optic dysplasia/optic nerve hypoplasia Midline forebrain abnormalities	Pituitary hypoplasia EPP	AD or AR
LHX3	Somatotrophs, lactotrophs, thyrotrophs, gonadotrophs, corticotrophs (variable)	Rigid cervical spine with limited neck rotation	Pituitary hypoplasia	AR
LHX4	Somatotrophs, lactotrophs, thyrotrophs, gonadotrophs, corticotrophs	Abnormality of skull base Cerebellar abnormalities	Pituitary hypoplasia EPP	AD
GPR161	Somatotrophs, thyrotrophs	None	Pituitary hypoplasia EPP	AR
GLI2	Somatotrophs, lactotrophs, thyrotrophs, gonadotrophs, corticotrophs	Polydactyly Cleft lip/palate	Pituitary hypoplasia EPP	AD variable penetrance
PROP1	Somatotrophs, lactotrophs, thyrotrophs, gonadotrophs, corticotrophs (later in life)	May have normal puberty (variable gonadotroph function)	Pituitary hypoplasia	AR
POU1F1 (PIT1)	Somatotrophs, lactotrophs, thyrotrophs,		Pituitary hypoplasia	AD/AR
OTX2	Somatotrophs, thyrotrophs, gonadotrophs	Microphthalmia or anophthalmia	Pituitary hypoplasia EPP	Unknown
SOX2	Somatotrophs, gonadotrophs	Microphthalmia or anophthalmia Sensorineural defects Esophageal atresia	Pituitary hypoplasia	De novo
SOX3	Somatotrophs, lactotrophs, thyrotrophs, gonadotrophs, corticotrophs	Duplication of Xq26–27 in affected males	Pituitary hypoplasia EPP Abnormal corpus callosum	X-linked recessive

AD, Autosomal dominant; *AR*, autosomal recessive; *EPP*, ectopic posterior pituitary.

arterial and venous constriction and thus increase blood pressure. Serum osmolality is tightly controlled between 280 and 295 milliOsmoles (mOsm)/kg water in adults. With even a slight increase in serum osmolality, AVP release increases exponentially to induce renal water reabsorption to a maximum urine osmolality of 800 to 1200 mOsm/kg water (maximum urine osmolality depends on the osmolality of the upstream kidney medulla). In neonates, however, the urine-concentrating ability of the kidney is impaired to a maximum of 300 mOsm/kg water because of the relative immaturity of the renal tubules rather than lack of AVP release; AVP is present in the fetus and neonate, and V_2 receptors are functional.[16]

Diseases of Hypothalamic or Pituitary Maldevelopment

Given the overlapping developmental windows of important cranial midline structures, a diagnosis of congenital hypopituitarism in a neonate should be considered if there are intracranial or extracranial midline defects. For instance, hypopituitarism has been reported in children with cleft lip or palate or central incisors. Hypopituitarism and diabetes insipidus can be encountered in neonates with holoprosencephaly. Children who have intracranial abnormalities along the spectrum of optic nerve hypoplasia, with or without septo-optic dysplasia, commonly have congenital hypopituitarism.

Neonates with congenital hypopituitarism because of a mutation in a gene important in pituitary differentiation may or may not have obvious defects in midline structures (see Table 82.1).[14] In the absence of these clinical signs, hypopituitarism may be suspected in neonates with micropenis and normal testicular descent (caused by growth hormone with or without gonadotropin deficiency), prolonged hypoglycemia (caused by combined growth hormone and cortisol deficiency), or rarely, cholestatic giant cell hepatitis (caused by combined growth hormone, thyroid hormone, and cortisol deficiency). As fetal growth is independent of growth hormones, children with congenital hypopituitarism are of normal size and weight at birth. If a newborn screen uses T_4 levels as the screen for congenital hypothyroidism, then low T_4 levels may be a diagnostic clue; if a newborn screen measures thyrotropin (also known as thyroid-stimulating hormone, TSH) to screen a newborn for congenital hypothyroidism, the central hypothyroidism of hypopituitarism will not be detected. After 2 months of life, a clue to optic nerve hypoplasia may be a disconjugate gaze. If diabetes insipidus is diagnosed, then full evaluation of the remaining pituitary hormones is mandatory.

Adrenal Gland Development

Cells of the adrenal cortex arise from the intermediate mesoderm and can be recognized at the upper pole of the mesonephros by 3 to 4 weeks' gestation. Cells of the adrenal medulla are derived from the ectoderm, and they infiltrate the adrenal cortex cells at 7 to 8 weeks' gestation. Encapsulation of the adrenal gland occurs at 9 weeks' gestation, resulting in the formation of a distinct organ above the developing kidneys. The cortical and medullary cells proliferate rapidly and sort into a central medulla and surrounding cortex.[3]

The HFA cortex acquires two distinct zones: the inner FZ and the outer definitive zone (DZ). DZ cells are in an undifferentiated proliferative state, whereas FZ cells are differentiated, steroidogenic cells. Current data suggest that the HFA cortex is a dynamic organ in which cells proliferate in the periphery (i.e., the DZ) and migrate centripetally, differentiating to form the inner FZ that is unique to primate gestation.[3] There is a third zone between the DZ and FZ, named the *transitional zone* (TZ). Cells in this zone show intermediate characteristics and can produce cortisol, analogous to the zona fasciculata cells of the adult adrenal cortex. By the 30th week of gestation, the HFA cortex manifests a rudimentary form of the adult adrenal cortex; the DZ and TZ begin to resemble the zona glomerulosa and the zona fasciculata, respectively. The third definitive cortical layer, the zona reticularis, is absent at birth and starts developing at 3 years of age to form an extragonadal source of sex steroids. The cells of the inner FZ are the primary site for steroidogenesis during gestation, producing an abundant amount of adrenal androgens, which are the substrates for placental estrogen production.

The weight of the adrenal gland increases 10-fold from the 8th to 10th week of gestation, and the gland continues to grow rapidly thereafter until term. By 30 weeks, it achieves a relative size 10 to 20 times that of the adult adrenal gland. A further doubling in weight occurs after that. Soon after birth, the HFA undergoes rapid involution with rapid disappearance of the FZ and a decrease in androgen secretion. As a consequence, the total weight of the glands decreases by approximately 50%. Remodeling of the postnatal adrenal gland involves a combination of FZ regression and the development of the zona glomerulosa and zona fasciculata. During the first year, the fetal cortex regresses, and adrenal mass diminishes to 2 to 3 g.

The early stages of adrenal development are regulated by several transcription factors, mainly steroidogenic factor 1 (SF1) and DAX1 (aka NR0B1; dosage-sensitive sex reversal, adrenal hypoplasia congenital, X-chromosome factor). SF1-knockout mice manifest adrenal and gonadal agenesis and gonadotropin deficiency.[17] Inactivating DAX1 mutations are associated with adrenal hypoplasia and gonadotropin deficiency in mice and humans.[18]

ACTH secreted by the fetal pituitary is the primary regulator of the development and function of the HFA. Other ACTH-independent regulators include local peptide growth factors and placenta-derived factors, such as CRH and estrogens. The steroidogenic activity in the HFA is characterized by early transient cortisol biosynthesis.[19] The hypothalamic-pituitary-adrenal axis is sensitive to glucocorticoid-mediated feedback at this time. Therefore 46 XX females with steroidogenic defects (such as congenital adrenal hyperplasia) who lack cortisol will have an elevated ACTH level that stimulates overproduction of androgens, resulting in virilization of the female genitalia. Cortisol production is suppressed thereafter until late gestation (because of the lack of 3β-hydroxysteroid dehydrogenase type 2 expression). Near term, the fetal cortisol production rate increases and is similar to that in adults per unit of body weight. During most of gestation, there is extensive production of DHEA and its sulfate, precursors of placental estrogen. This begins at around 8 to 10 weeks' gestation and increases significantly during the second and third trimesters. Aldosterone synthesis in the HFA may be suppressed during midgestation because of the probable lack of *CYP11B2* expression but likely becomes active by the term because 80% of aldosterone in human fetal blood at term appears to originate from the HFA.

The adrenal medulla functions as a classic endocrine (ductless) gland that secretes hormones directly into the bloodstream. It also participates in sympathetic control via preganglionic sympathetic nerve fibers. Pheochromocytoblasts give rise to the medullary pheochromocytes, which are epinephrine- and

norepinephrine-secreting homologues of sympathetic postganglionic cells. By 3 months' gestation, adrenal pheochromocytes secrete epinephrine and norepinephrine into the medullary sinusoids and then into the systemic circulation.[20] The hypothalamic-pituitary-medullary adrenal axis becomes sufficiently functional by midgestation so that fetal stress responses can be independent of the mother's.[21] This fetal catecholamine stress response contrasts with the fetal cortisol output capacity, which is minimally present before midgestation.

Thyroid Gland Development

The thyroid is the first endocrine gland to develop in the embryo. It is derived from contributions of two anlagen: a midline thickening of the pharyngeal floor (median anlage) that gives rise to the T_4-producing follicular cells and paired caudal extensions of the fourth pharyngobranchial pouches (lateral anlagen) that act as the precursor to the parafollicular calcitonin-secreting cells (C cells). The thyroid begins its development by 24 days' gestation when the median anlage forms the thyroid diverticulum that extends from the floor of the buccal cavity to the fourth branchial arch. At 24 to 32 days, the median anlage becomes a bilobed structure. By 50 days, the median and lateral anlagen have fused, and the buccal stalk has ruptured. During this period, the thyroid gland migrates caudally from the pharyngeal floor to the developing hyoid bone and laryngeal cartilage. It reaches its final location in the anterior neck below the thyroid cartilage. During its descent, the thyroid gland retains an attachment to the pharynx by a narrow epithelial stalk known as the *thyroglossal duct* that disappears by 7 weeks of gestation. Abnormalities in thyroid descent result in ectopic thyroid, persistent thyroglossal duct, or cysts. The genes involved in thyroid gland development include *HEX*, *TTF1*, *FOXE1*, *NKX2-5*, *PAX8, GLIS3*, and *TUBB1*.[22–24] Defects in these genes can result in various abnormalities in thyroid gland development and congenital hypothyroidism.

By 10 to 11 weeks, clusters of endodermal epithelial cells form single layers around lumens, the thyroid follicles, in which colloid begins to appear. At 4 weeks, the fetal gland can synthesize thyroglobulin; 6 to 8 weeks later, the thyroid synthesizes thyroid hormones mediated by the incorporation of iodine. This early growth and development appear to be independent of TSH, as secretion of TSH cannot be shown before 10 to 12 weeks. Because of the maturation of the hypothalamic-pituitary-thyroid axis, TSH levels increase rapidly thereafter. Similarly, total T_4 and free T_4 levels increase significantly in the second and third trimesters. The fetal pituitary-thyroid feedback mechanism appears to be fully responsive by 18 to 20 weeks. Hypothyroidism will result in elevated fetal TSH production, whereas hyperthyroidism caused by maternal Graves disease (due to transplacental passage of maternal thyroid-stimulating autoantibodies) will cause suppressed TSH production.

Thyroid hormones T_4 and triiodothyronine are essential for the development and maintenance of normal fetal physiologic processes, especially those of the central nervous system, where thyroid hormones assist in brain maturation throughout gestation.[25] Thyroid hormones regulate genes involved in myelination and neuronal/glial cell differentiation.[26] Delivery of thyroid hormones to the fetal brain is a complex process requiring, at different times, expression of brain thyroid hormone receptors, placental thyroid hormone, and iodide transport, a feedback system that involves the hypothalamic-pituitary-thyroid (HPT) axis, and thyroid hormone metabolism by liver and brain deiodinase enzymes (deiodinase type 2 and deiodinase type 3), which ensure basal levels are sustained.[27]

The fetus is dependent on maternal contribution as the primary source of thyroid hormone during pregnancy, especially during the first trimester.[1] This dependence decreases as the fetal T_4 production increases and the HPT axis matures. At birth, maternal T_4 comprises 30% to 50% of T_4 measured in cord blood. In congenital hypothyroidism due to thyroid agenesis (athyreosis), maternal T_4 lessens the impact on fetal neurologic development. Early neonatal diagnosis and T_4 treatment permit normalized growth and development. In contrast, when the mother is hypothyroid throughout pregnancy, the developmental consequences are more severe.[2] Additionally, substances that negatively affect fetal thyroid development, including thyroid autoantibodies and antithyroid medications, can also cross the placenta if present in the mother. Maternal TSH receptor antibodies (TRAbs) can be stimulating or inhibiting, cross the placenta, bind to thyroid follicular cells, and alter fetal thyroid hormone production, leading to transient neonatal hyperthyroidism or hypothyroidism, respectively.[28]

Prematurity can result in low thyroid hormone levels, and the severity correlates inversely with gestational age because of the immaturity of the HPT system. Fifty percent of preterm infants born before 28 weeks' gestation will have transient hypothyroxinemia of prematurity identified by low T_4 and normal TSH levels that do not require treatment. Very low-birth-weight infants are at higher risk of developing primary hypothyroidism and can have a delayed TSH level rise. Thyroid hormone treatment is indicated in these infants, although hypothyroxinemia of prematurity is commonly a temporary problem.

Reproductive Axis Development

Like other hypothalamic-pituitary-end gland systems, early gonadal development is completely separate from neuroendocrine development and thus will be discussed separately. GnRH neurons, the hypothalamic neurons that induce gonadotrophs to synthesize and secrete LH and FSH, are unique among hypothalamic neurons in that they arise outside the neuroectoderm in the olfactory pit. This occurs at week 6 after conception. The GnRH neuron begins to synthesize and secrete GnRH shortly thereafter. The neurons then migrate via the forebrain, arriving at the hypothalamus by week 9.[29] The location, number of GnRH neurons, and projection to the median eminence are similar to those in adult animals. Migration is supported by the signaling protein products of the genes *ANOS1* (anosmin 1), *PROK1* (prokineticin 1), and *FGF8* (fibroblast growth factor 8), as well as receptor encoding genes such as *FGFR1*, *PROKR2*, and *CHD7*.[30]

Before gestational week 6, the male and female genital duct systems coexist with an indifferent (bipotential) gonad. The specifics of sex-specific gonadal differentiation and associated disorders are discussed elsewhere in this textbook. In brief, the presence or absence of the Y chromosome, and thus the gene *SRY*, is the determining factor directing the development of the bipotential gonad into either testes or ovaries. *SRY* is expressed by week 6 of gestation. *SRY* directs the expression of many genes that direct the differentiation of the bipotential gonad into the Leydig (testosterone-producing) cells of the testes and the Sertoli cells.[31] *SRY* also induces the expression of the gene *SF1*. *SF1*, in turn, acts to induce the expression of the anti-müllerian

hormone, which induces regression of the female (müllerian) duct structures. Müllerian structures include the fallopian tubes, uterus, and upper third of the vagina. In the presence of two normal X chromosomes, ovarian differentiation commences, although less is known about ovarian development. Formation of the bipotential gonad into testes or ovaries is completed by 8 weeks of gestation, while differentiation of the genital duct systems into the male or female internal reproductive organs is complete by week 9 of gestation.[31]

External development of male or female structures is determined by the presence or absence of testosterone (and thus the *SRY* gene). Further conversion of testosterone to dihydrotestosterone (DHT) is necessary for the normal development of the penis and prostate gland. In the absence of DHT, the lower two-thirds of the vagina and the labia minora and majora develop. Placental human chorionic gonadotropin directs testosterone secretion by the ever-expanding Leydig cell population after week 14 of gestation. The testosterone directs the formation of the external genitalia. LH and FSH reach peak levels at midgestation, about 20 to 24 weeks. The testes and ovaries are responsive to LH and FSH by week 20, and some primary follicles are seen in the ovaries. However, placental estrogen predominates, so estrogen secretion by the fetal ovary is thought to be minimal. LH-directed testosterone synthesis is required for normal testicular descent from the abdomen into the scrotal sac in late gestation, as well as for further lengthening the penis in late gestation. By the last half of the third trimester, placental estrogen production provides negative feedback to the axis to cause LH and FSH levels to decrease.[31]

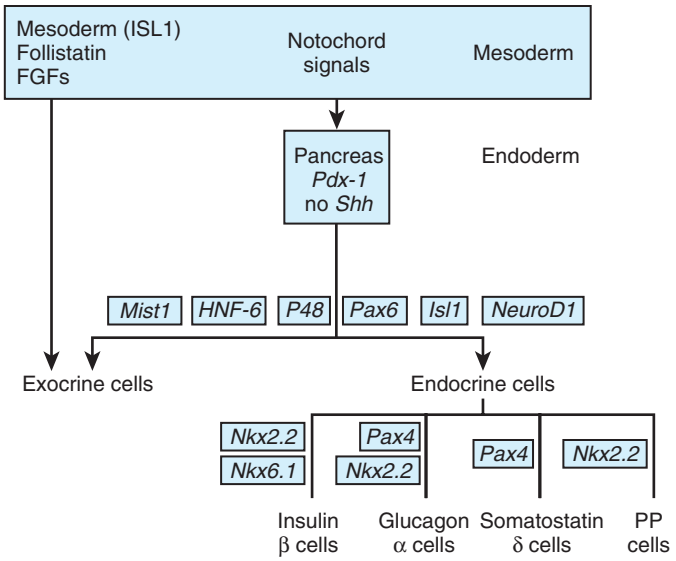
Development of the Endocrine Pancreas

The pancreas is derived from two buds, dorsal and ventral, that arise from the distal foregut endoderm. It contains a distinctive combination of cell lineages. The exocrine tissue comprises acinar cells that secrete digestive fluid and a duct system by which the fluid drains into the intestine. The endocrine part is arranged as discrete islets of Langerhans, which contain distinct cell types secreting different hormones into the circulation (α cells, glucagon; β cells, insulin; δ cells, somatostatin; ε cells, ghrelin; and γ cells, pancreatic polypeptide).[32]

Human pancreas formation is first evident at 26 days' gestation, and it begins with the dorsal bud formation, followed by the appearance of two ventral buds at 30 days. The left ventral bud gradually regresses, whereas the right ventral bud migrates posteriorly and fuses with the dorsal bud on gut rotation at 6 to 7 weeks' gestation. Failure of the left ventral bud to regress could lead to annular pancreas. The dorsal pancreas gives rise to most of the pancreas, including the upper part of the head, the isthmus, the body, and the tail of the pancreas. The right ventral bud gives rise to the inferior part of the head.[33]

Starting from day 45 to day 47 of gestation, the pancreatic epithelium undergoes active growth and branching morphogenesis to give rise to endocrine and acinar cells under the influence of locally acting signals and activation of lineage-specific transcription factors. During this process, the pancreatic epithelium is embedded in the loose mesenchyme and is surrounded by dense peripancreatic mesenchyme. At approximately 7 to 8 weeks, the epithelium begins to ramify and form a lobular pattern.[33]

The first endocrine cells that appear are insulin-expressing cells at 7.5 weeks, and they remain the most prevalent endocrine cell type during the first trimester.[34] This is followed by the appearance of glucagon- and somatostatin-expressing cells at week 8 and pancreatic polypeptide-expressing and ghrelin-expressing cells at week 9. Ongoing β-cell proliferation and differentiation are dependent on insulin-like growth factor type 2 (*IGF2*) expression. Islet formation begins by week 12. Islet volume increases from about 4% to 13% of total pancreatic tissue by term.[33] Targeted gene deletion studies in mice have demonstrated critical roles for several transcription factors in pancreatic endocrine development (Fig. 82.3).



• **Fig. 82.3** Molecular Control of Cell Fate Choices in the Pancreas. *Pax6* and *Isl1* are expressed early, and their disruption leads to the reduction or absence of endocrine differentiation, which suggests that they could be expressed in endocrine progenitors able to give rise to all cell types. In *Isl1*-mutant mice, *Pax6* is not expressed, which suggests *Pax6* is downstream of *Isl1*. Although *Pax4*, *NeuroD1*, *Nkx2.2*, and *NKx6.1* are expressed as early as *Pax6* and *Isl1*, they affect the differentiation of only a subset of lineages. *Pax4* is required for glucagon-producing and somatostatin-producing cell differentiation. *Nxk2.2* is expressed in all islet cells, except somatostatin-producing cells, and its inactivation leads to the absence of the cell types where it is expressed. *Nkx6.1* is itself required for insulin cell differentiation. Although *Mist1* and *Onecut1* (also known as *Hnf6*) are expressed in endocrine cell lineages, their function has not yet been assessed by inactivation experiments. *FGFs*, Fibroblast growth factors; *PP*, pancreatic peptide. (From Grapin-Botton A, Melton DA. Endoderm development: from patterning to organogenesis. Trends Genet. 2000;16:124–130.)

Neonatal insulin kinetics and end-organ sensitivity to insulin appear to be established during the third trimester in preparation for extrauterine fuel metabolism. The maternal environment and fetal genome appear to influence the number and/or function of pancreatic β cells in early life, with lifelong implications for postnatal diabetes.[35] In contrast, insulin gene-knockout mice and human newborns with pancreatic agenesis both experience severe intrauterine growth restriction (IUGR), demonstrating insulin's role in mitogenesis and growth.

Development of Parathyroid Glands and Fetal Mineral Homeostasis

The parathyroid glands arise from the third and fourth pharyngeal pouches. The third pouches develop into the inferior parathyroid glands, and the fourth pouches develop into the superior

glands. Fetal parathyroid glands produce low amounts of parathyroid hormone (PTH) throughout gestation that can be detected by 10 weeks. It is unclear if they also make PTH-related protein (PTHrP), which is mainly produced in the placenta. Other hormones involved in mineral homeostasis include calcitonin, which is detectable in fetal thyroidal C cells as early as 14 weeks. As early as the second half of gestation, the fetal renal tubules can synthesize the active form of vitamin D, 1,25-dihydroxyvitamin D (calcitriol), but the fetal levels remain low.

Mineral and bone metabolism is regulated differently in utero compared with the adult. The placenta meets the fetal mineral need by actively transporting calcium, phosphorus, and magnesium from the maternal circulation to maintain higher fetal concentrations compared with maternal levels. These high levels are necessary for the developing skeleton to accrete a normal amount of mineral by term. A human fetus typically accumulates approximately 30 g of calcium by term, with 80% of that mineral content obtained in the third trimester.

PTH and PTHrP are critical for fetal bone development, regulation of serum minerals, and placental mineral transfer, while calcitriol and calcitonin are not required. PTH and calcitriol circulate at low concentrations in fetal circulation. PTH is suppressed by the high fetal serum calcium level. Low calcitriol concentration is caused by low PTH concentration, high calcium concentration, and rapid clearance.[36]

After birth, there is a loss of active transport of minerals through the placenta, and the neonate has to rely on an enteral intake of minerals. The calcium levels fall after birth, reaching a nadir at 24 to 48 hours, and gradually rise to adult values over several days, likely due to a fall in PTHrP levels and hyporesponsiveness of parathyroid glands. As PTH and calcitriol levels increase postnatally, calcium levels rise with maturation in the function of the kidneys and intestines. However, there may be a significant delay in intestinal maturation in preterm infants, along with an increased demand for mineral accretion, which predisposes them to osteopenia of prematurity.

Hormonal Regulation of Fetal Growth

Hormones that play an important role in postnatal growth, such as thyroid hormones, growth hormone, and gonadal steroids, play a very limited role in fetal growth. As such, neonates with these hormonal disorders are generally born with normal weight and size. Fetal growth is driven by nutritionally dependent growth factors such as insulin or other growth factor systems such as IGF1 and IGF2.

Insulin

Insulin is an important fetal growth factor, as evident by the hyperinsulinemia and macrosomia experienced by infants born to women with uncontrolled diabetes mellitus. In contrast, infants with pituitary agenesis and corresponding lack of insulin are small at birth, with minimal muscle bulk and adipose tissue.[37]

Insulin-Like Growth Factor 1

The importance of IGF-1 in fetal growth is derived from studies of humans and mice with *IGF1* or IGF-1 receptor (*IGF1R*) mutations. Humans with mutations in these genes have IUGR, microcephaly, hypoglycemia, and severe developmental delay.[38] Mice null for the *IGF1* gene or the *IGF1R* gene are 60% of normal weight and die shortly after birth because of impaired lung maturation.

Insulin-Like Growth Factor 2

The importance of IGF-2 to fetal somatic and organ growth is manifest in the phenotypes of Beckwith-Wiedemann syndrome (BWS) and Russell-Silver syndrome (RSS). The *IGF2* gene is normally imprinted such that only the paternal allele is active. In BWS, either there is loss of imprinting, such that the maternal allele is also active, or there are two copies of the active paternal allele (uniparental disomy). Infants with BWS have *IGF2* overexpression and have resultant fetal and postnatal overgrowth. This overgrowth manifests as macrosomia, enlarged tongue, β-cell hypertrophy with hyperinsulinemic hypoglycemia, and an increased risk of certain childhood cancers. In contrast, RSS may be due to duplication of the maternal allele or silencing of the paternal allele via imprinting. Infants with RSS have IUGR with postnatal growth retardation, with adult height four standard deviations below the mean.[38] Other clues to the diagnosis of RSS include hemihypertrophy, clinodactyly, triangular facies, and normal head circumference.

Placental Factors

Human placental lactogen is a major regulator of maternal glucose, amino acid, and lipid metabolism during pregnancy, allowing mobilization of these fuel sources for use by the fetus.[39] Alterations in maternal nutrition induced by starvation or disease also affect fetal growth. Uterine vasoconstriction or vascular insufficiency of the placenta is also associated with poor fetal growth and neonatal IUGR. Vascular compromise of the placenta may be due to maternal hypertension, drug exposure, infection, or placental abruption.

Developmental Origin of Health and Disease

The developmental origin of the health and disease hypothesis is the theory that the perinatal environment (conception to infancy into toddlerhood) directly influences the development of metabolic and cardiovascular disease during adulthood. This hypothesis, also termed the *Barker hypothesis*, was first proposed in the 1990s by David Barker, attempting to account for higher rates of heart disease in more impoverished areas of Britain.[40] Using longitudinal data, he found that London boroughs with historically high neonatal mortality had the highest rates of adult cardiovascular disease, and low birth weight was associated with an increased risk of death from coronary heart disease. Many studies since Barker's original studies have replicated his results in men and women in Europe, North America, South America, and India. In addition to heart disease, low birth weight has been associated with an increased risk of type 2 diabetes, cerebrovascular disease, hypertension, dyslipidemia, altered puberty, and obesity in adulthood.

The association of low birth weight with the metabolic disease led to the proposal that a nutrient supply perceived as limited by the fetus yields a fetal/neonatal "thrifty phenotype" that would allow survival in a nutrient-limited extrauterine environment.[41] The "programming" for an extrauterine life of limited nutrients proves maladaptive in the abundant postnatal nutrition environment. As a corollary to low birth weight, epidemiologic studies find that excess maternal weight during pregnancy or excessive

weight gain in early infancy (whether experienced by low-birth-weight or normal-weight neonates) is also associated with an increased risk of metabolic diseases later in life. At the highest risk of metabolic disease as adults are individuals with low birth weight and excessive weight gain in infancy and childhood.

Other in utero exposures that have been associated with the development of adult disease include increased risk of obesity and mental disorders in offspring of pregnancies complicated by maternal depression, increased risk of metabolic disorders in offspring of mothers with hyperandrogenemia, and increased risk of hypertension in offspring of pregnancies complicated by preeclampsia.[42] A number of mechanisms have been proposed to program later disease by environmental influences. These include epigenetic regulation of gene expression, oxidative stress of tissues resulting in damage to DNA, lipids, and proteins, and direct hormonal effects on development. Epigenetic changes that affect gene expression include DNA methylation, histone modification, chromatin packing, and micro-ribonucleic acid expression. These changes are malleable or permanent and can accumulate over time to influence gene expression in the short term or long term.[42]

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83

Disorders of Calcium and Phosphorus Metabolism

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KEY POINTS

- Neonatal hypocalcemia may be asymptomatic or present with signs of increased neuromuscular excitability, including focal or generalized seizures.
- Neonatal hypocalcemia is classified by the timing of onset, with early and late hypocalcemia having different causes and approaches to evaluation.
- Neonatal hypercalcemia may be asymptomatic if there are only mild elevations in calcium level or may lead to severe symptoms such as failure to thrive, polyuria, lethargy, and seizures.
- The causes of hypercalcemia can differ but are due to the dysregulation of calcium regulatory systems.
- Metabolic bone disease of prematurity is caused by deficiencies in dietary phosphate and calcium, early withdrawal of placental estradiol and progesterone, lack of mobility, and therapy with medications that can increase urinary calcium excretion and contribute to serum mineral imbalance and osteopenia.
- Osteopenia in preterm infants usually appears between 6 and 12 weeks of age, and fractures may be seen. The incidence and severity increase with decreasing gestational age and birthweight and are more common in preterm infants having a complicated medical course and delayed nutrition.

Homeostatic Control of Calcium and Magnesium

Calcium plays two important physiologic roles. Calcium salts in bone provide structural integrity. Calcium ions present in the cytosol and extracellular fluid (ECF) are essential for the maintenance and control of many biological processes, including cell-cell communication, cell aggregation and division, coagulation, neuromuscular excitability, membrane integrity, and permeability, enzyme activity, and secretion. This functional diversity is made possible by the maintenance of a large electrochemical gradient between the ECF ionized calcium (Ca^{2+}) concentration, which is in the 1-mmol/L range, and the resting intracellular (cytosolic) Ca^{2+} concentration, which is about 0.1 µmol/L.

Significant alterations in serum calcium concentration frequently occur in the neonatal period. It is important to evaluate these potential derangements in light of normal dynamic changes that occur during the perinatal transition. After the first 2 to 3 days, normal total serum calcium concentrations vary only slightly with age and a range between 8.8 and 10.6 mg/dL (2.2 to 2.6 mmol/L), with an ionized serum calcium concentration of 4 to 5.6 mg/dL (1 to 1.4 mmol/L). The metric measurement unit conversion factors are 0.2595 and 0.2495, respectively, as used everywhere other than the United States.

Approximately 55% to 60% of the total plasma calcium is diffusible (or ultrafilterable), the remainder being protein bound. Most diffusible calcium is ionized, but about 5% of total circulating calcium is complexed to plasma anions, such as phosphates, citrate, and bicarbonate. Ca^{2+} is the only biologically available fraction of ECF calcium. It is subject to precise metabolic control based on the integrated regulation of calcium fluxes with respect to the intestine, kidneys, and bone. The precise regulation of circulating Ca^{2+} is controlled by calcium itself, through a calcium receptor and several hormones, the most important of which are parathyroid hormone (PTH) and 1,25-dihydroxyvitamin D ($1,25(OH)_2D$).[1–3]

Hypoalbuminemia leads to a decline in total serum calcium, but proportionate increases in the ionized fraction usually maintain serum Ca^{2+} concentration within the normal range. Acute alkalosis (e.g., hyperventilation or bicarbonate infusion) or rapid administration of citrate-buffered blood (e.g., during exchange transfusion, initiation of extracorporeal membrane oxygenation [ECMO], cardioplegia, or organ transplant) may acutely lower serum Ca^{2+} concentration by increasing albumin binding or citrate chelation. These conditions can produce transient clinical manifestations of hypocalcemia but do not lower the total serum calcium concentration.[4] In general, for routine clinical purposes, measurement of total serum calcium concentration often suffices, and the correction formula in the setting of hypoalbuminemia for total serum calcium concentration measured as mg/dL is:

$$\text{Corrected Ca Level} = [0.8 \times (\text{Normal Albumin Level} - \text{Patient's Albumin Level})] + \text{Serum Ca Level}$$

Large amounts of calcium exchange occur in the kidney, bone, and intestine. Although the intestine has considerable calcium absorptive capacity, renal tubular calcium reabsorption usually exceeds intestinal absorption by at least 40-fold. Most of the tubular Ca^{2+} load is reabsorbed in the proximal tubule and thick

ascending limb of the loop of Henle via paracellular, passive flux (coupled with sodium reabsorption) driven by the existing electrochemical gradient. A transcellular pathway in the distal nephron tightly regulates the rest of urinary Ca^{2+} reabsorption. Calcitropic hormones regulate the distal Ca^{2+}-selective, Na^+-independent channels. More than 98% of total body calcium is deposited in the skeleton as hydroxyapatite ($Ca_5[OH][PO_4]_3$); the ECF and soft tissues contain the remainder. A small fraction of skeletal calcium freely exchanges with the ECF and serves as an important buffer of circulating calcium. Consequently, decreased skeletal calcium is a hallmark of most metabolic bone diseases (MBDs).

Magnesium homeostasis is largely mediated through the kidneys. Approximately 80% of total plasma magnesium is filtered through the glomerulus and is reabsorbed mainly in cortical segments of the thick ascending limb of the loop of Henle. Once the maximal tubular reabsorption is exceeded, filtered magnesium is excreted into the urine. Hormones regulate magnesium reabsorption by changing the transepithelial voltage and paracellular permeability of tubular cells. Magnesium is required to maintain normal PTH secretory responses.

Homeostatic Control of Phosphorus

Blood inorganic phosphate concentration varies with age. It is highest during infancy and gradually declines to adulthood. Approximately 10% of plasma inorganic phosphate is noncovalently bound to protein, whereas 90% circulates as ions or as complexes with sodium, calcium, or magnesium. About 80% to 85% of total body phosphorus contributes to mechanical support as part of the hydroxyapatite lattice of bone. The remainder is distributed in the ECF, largely as inorganic ions or complexes, and in soft tissues as phosphate esters. Intracellular phosphate esters and phosphorylated intermediates regulate cell metabolism and gene expression (via phosphorylase, kinase, and phosphatase activities) and generate and transfer cellular energy (i.e., via adenosine triphosphate). Cytosolic and ECF phosphorus levels (approximately 0.1 and 0.2 mmol/L, equivalent to 0.31 and 0.62 mg/dL, respectively) are less stringently regulated than are levels of Ca^{2+} and magnesium (Mg^{2+}).

Dietary phosphate is generally absorbed in proportion to its content in food. Although phosphorus and calcium can be absorbed along the entire length of the small intestine, most phosphate absorption occurs in the jejunum and ileum. In contrast, most calcium absorption occurs in the duodenum. The renal proximal tubule is the principal regulatory site for phosphorus homeostasis.[5] Renal regulation is accomplished primarily by variation of the threshold for phosphate reabsorption (the tubular maximum for inorganic phosphate [TmP]/glomerular filtration rate [GFR]). Hormones (PTH, PTH-related protein [PTHrP], growth hormone) and dietary phosphate reset this theoretical threshold by regulating apical tubular Na^+-TmP cotransporters.[6] Essentially, TmP/GFR is the "setpoint" that defines the fasting serum phosphorus concentration. At lower serum phosphorus levels, most filtered phosphorus is reabsorbed; at higher levels, most filtered phosphorus is excreted. To assess TmP/GFR, a fasting urine specimen is obtained to measure phosphorus and creatinine, along with the simultaneous determination of serum phosphorus and creatinine. A nomogram has been constructed so that TmP/GFR can easily be derived from these values.[7]

The higher serum phosphate levels in infants (e.g., 4.5 to 9.3 mg/dL) compared with those in adults (3.0 to 4.5 mg/dL) reflect infants' greater tubular phosphate resorption. This adaptation permits avid tubular phosphate conservation despite high ambient serum phosphate levels. For this reason, neonatal disorders of chronic hypophosphatemia and/or phosphorus depletion usually result from an inadequate dietary supply (as in preterm infants) or intrinsic (e.g., familial hypophosphatemic rickets) or extrinsic (e.g., hyperparathyroidism) alterations in TmP/GFR. Similarly, chronic hyperphosphatemia usually implies either intrinsic (e.g., renal insufficiency) or extrinsic (e.g., hypoparathyroidism) abnormalities in TmP/GFR.

Parathyroid-Renal Hormonal Axis

In mammals, calcium and phosphate homeostasis is controlled by a parathyroid-renal hormonal axis involving PTH and $1,25(OH)_2D$. The influence of these two hormones on bone deposition, mobilization of minerals, and regulation of intestinal and renal absorption is depicted in Fig. 83.1. Deficiency or excess of either hormone causes hypocalcemia or hypercalcemia, respectively.

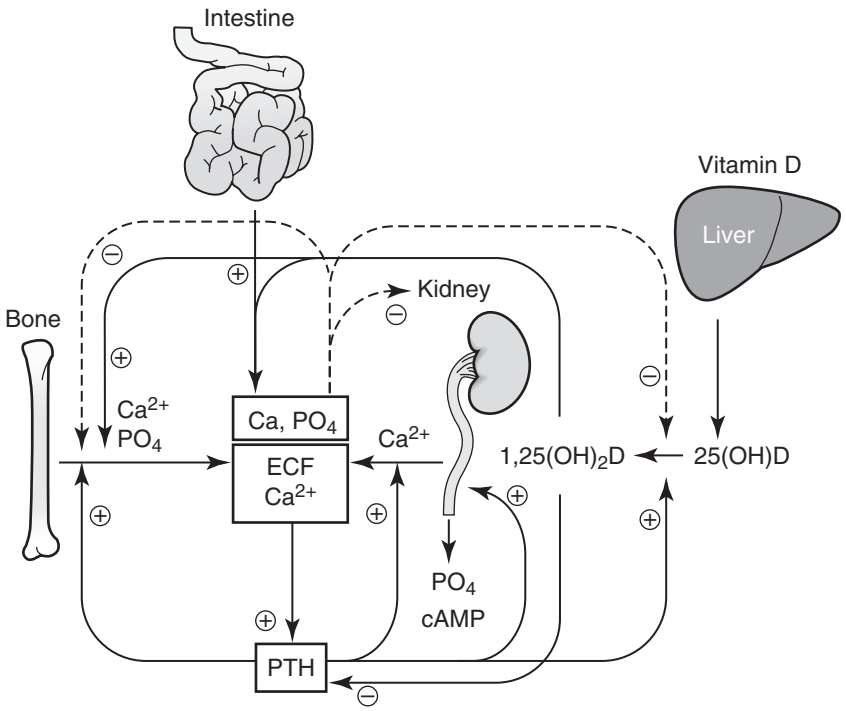
Parathyroid Hormones

PTH mobilizes calcium and phosphorus from bone, stimulates calcium reabsorption in kidneys, inhibits phosphorus reabsorption by reducing TmP/GFR, and stimulates the renal synthesis of $1,25(OH)_2D$, which participates with PTH in calcium reabsorption in kidneys, increases the efficiency of intestinal absorption of calcium and phosphorus, and mobilizes calcium from bones. Therefore PTH secretion causes the serum calcium concentration to rise and the serum phosphorus concentration to be maintained or decline.

PTH is a 9500-Da, single-chain polypeptide. It is synthesized by the four parathyroid glands embedded within the thyroid gland poles, which are derived from the embryonic third and fourth pharyngeal pouches. The messenger ribonucleic acid (mRNA) for PTH (preproPTH) encodes the 84 amino acids of the mature peptide, an amino-terminal (N-terminal) "pre" sequence of 25 amino acids, and a basic "pro" hexapeptide, which is clipped intracellularly. After secretion, the intact PTH molecule, PTH (1 to 84), is further metabolized and rapidly cleared from the circulation, with a half-life of less than 4 minutes. The N-terminal region of the PTH molecule, PTH (1 to 34), binds the PTH receptor and shows full biological activity, whereas the carboxyl terminal (C terminal) has specific, albeit poorly understood, activities in osteoclasts and osteoclastic precursors.

Secretion of PTH fragments by the parathyroid glands and prolonged clearance of the C-terminal PTH metabolites add considerable immunoheterogeneity to circulating PTH. The numerous inconsistencies found in reports on PTH pathophysiology until the late 1980s are due to the use of earlier generation "C-terminal" and "midmolecule" PTH assays. In contrast, current two-site "intact PTH" assays are sufficiently sensitive and specific to detect physiologic levels of biologically active PTH (1 to 84) and to distinguish hypoparathyroid from euparathyroid states. The normal circulating levels of intact PTH range from approximately 10 to 60 picogram (pg)/mL; the maximally stimulated (hypocalcemic) and maximally suppressed (hypercalcemic) levels for normal parathyroid function are about 100 to 150 pg/mL and 2 to 5 pg/mL, respectively.

Parathyroid cells are exquisitely responsive to changes in ambient Ca^{2+} concentration. PTH secretion may be described as an inverse sigmoid hysteretic relationship between serum PTH and



• **Fig. 83.1** Hormonal Regulation of Calcium and Phosphate by Parathyroid Hormone (PTH) and 1,25-Dihydroxyvitamin D (1,25[OH]$_2$D). Decreased Ca^{2+} concentration stimulates PTH and 1,25(OH)$_2$D secretion. Renal, gastrointestinal, and skeletal mechanisms increase Ca^{2+} concentration, inhibiting PTH secretion and closing the negative feedback loop. *cAMP*, Cyclic adenosine monophosphate; *ECF*, extracellular fluid. (From Brown EM, MacLeod RJ. Extracellular calcium sensing and extracellular calcium signaling. *Physiol Rev*. 2001;81:239–297.)

Ca^{2+} with a parathyroid cell setpoint (the Ca^{2+} concentration at which PTH secretion is half maximal) of 1.2 to 1.25 mmol/L. The parathyroid "calcistat" detects perturbations of blood Ca^{2+} concentration as small as 0.025 to 0.05 mmol/L and promptly adjusts PTH secretion. The molecular mechanism that enables specific cells (e.g., parathyroid cells, thyroidal C cells, renal tubular cells, osteoblasts) to sense these minute changes in ECF Ca^{2+} concentration involves a member of the family C of G protein–coupled receptors, Ca^{2+}-sensing receptor (CaSR). ECF Ca^{2+} (and, at lower affinity, Mg^{2+}) binds the CaSR, activates several intracellular effector pathways, and ultimately leads to oppositely directed changes in PTH secretion, and altered renal cation handling CaSR activation inhibits renal cellular Ca^{2+} absorption induced by PTH, as well as passive paracellular Ca^{2+} transport. As described later, loss-of-function (or inactivating) mutations in the *CaSR* gene are responsible for neonatal hyperparathyroidism and familial hypocalciuric hypercalcemia (FHH). In contrast, gain-of-function *CaSR* mutations result in autosomal dominant neonatal hypocalcemia.[8]

PTHrP is a second member of the PTH family, first identified as the cause of humoral hypercalcemia of malignancy. The amino acid sequences of PTHrP and PTH are homologous at the N terminal, and 8 of the first 13 amino acids are identical. Beyond this region, the sequences have little in common. PTHrP is a multifunctional molecule, like neuropeptides such as proopiomelanocortin (the precursor of corticotropin, endorphins, and melanocyte-simulating hormones). The three PTHrP isoforms (139, 141, and 173 amino acids) give rise to several secreted peptide fragments. PTHrP is widely expressed, especially in fetal tissues, and has important local functions in morphogenesis and differentiation. The normal circulating levels of PTHrP are considerably lower than the levels of PTH, and it is doubtful that PTHrP has a major role in calcium homeostasis. Two important exceptions are in the fetus and the lactating woman, for whom PTHrP appears to be an important calcitropic hormone.[9]

The actions of PTH on its two major target organs, kidney, and bone, are mediated through the type 1 PTH/PTHrP receptor (PTHR1),[10] a G protein–coupled receptor belonging to a receptor subfamily that includes receptors for calcitonin, secretin, and corticotropin-releasing hormone. This versatile receptor mediates the actions of its two physiologic ligands in multiple tissues and signals through several second-messenger pathways. The best-characterized effector of PTH action is cyclic adenosine monophosphate (cAMP).

Vitamin D

A main biological function of 1,25(OH)$_2$D is to increase intestinal absorption of calcium and phosphorus. During low calcium intake, increased PTH levels stimulate the renal conversion of 25-hydroxyvitamin D (25[OH]D) to 1,25(OH)$_2$D, which in turn stimulates osteoclast differentiation and bone resorption. Most of the identified biological actions of 1,25(OH)$_2$D are mediated via binding to vitamin D receptor (VDR), a member of the intracellular receptor superfamily. VDR interacts with specific response elements in promoters of vitamin D–responsive genes.

Vitamin D is a secosteroid synthesized in the skin or absorbed from the diet.[11,12] Exposure to sunlight (290 to 320 nm) cleaves the B ring of 7-dehydrocholesterol, or provitamin D, the immediate precursor of cholesterol, to form a sterol, previtamin D.

Previtamin D in the skin undergoes isomerization to the biologically inert vitamin D. Vitamin D enters the circulation bound to vitamin D–binding protein and is transported to the liver, where a mitochondrial cytochrome P450 vitamin D 25-hydroxylase produces 25(OH)D. 25(OH)D (provitamin D) is the major circulating vitamin D metabolite. Because the activity of hepatic 25-hydroxylase is not tightly regulated, the measurement of serum 25(OH)D is a useful assessment of vitamin D stores. In renal proximal tubule cells, mitochondrial 25(OH)D 1α-hydroxylase metabolizes 25(OH)D to the biologically active hormone, $1,25(OH)_2D$. The normal circulating level of 25(OH)D is approximately 10 to 50 ng/mL. The normal circulating concentration of $1,25(OH)_2D$ ranges from 30 to 75 pg/mL, or about 1/1000 that of 25(OH)D.

Serum 25(OH)D levels are increased by sunlight exposure and by vitamin D ingestion and are decreased in vitamin D deficiency and in hepatobiliary disorders. Circulating $1,25(OH)_2D$ levels are increased by hyperparathyroidism and phosphate depletion and are reduced in hypoparathyroidism. $1,25(OH)_2D$ is biologically inactivated through a series of reactions beginning with 24-hydroxylation. $1,25(OH)_2D$ induces the 24-hydroxylase in vitamin D target cells. Hypocalcemia, by increasing PTH levels, suppresses this enzyme. 24-Hydroxylase metabolizes 25(OH)D as well as $1,25(OH)_2D$. In vitamin D–sufficient states, the kidney preferentially 24-hydroxylates the prohormone, 25(OH)D, to 24,25-dihydroxyvitamin D ($24,25[OH]_2D$). In contrast, when vitamin D action is required, 25(OH)D 1α-hydroxylase is preferentially activated for $1,25(OH)_2D$ synthesis.

The parathyroid-renal (PTH–$1,25[OH]_2D$) axis, reminiscent of the hypothalamic-pituitary-adrenal axis, is the principal means for systemic response to a sustained or major hypocalcemic challenge. In this long-loop feedback system, $1,25(OH)_2D$-mediated calcium absorption provides the ultimate feedback on PTH secretion. PTH secreted in response to hypocalcemia is the principal regulator of renal production of $1,25(OH)_2D$, which, in turn, feeds back to suppress PTH gene expression (see Fig. 83.1). Hypocalcemia directly stimulates PTH mRNA transcription. PTH regulates minute-to-minute perturbations of ECF Ca^{2+} concentration. Maximal adjustments of intestinal calcium absorption via the PTH-$1,25(OH)_2D$ axis require 1 to 2 days to become fully operative, so $1,25(OH)_2D$ effects come into play only when hypocalcemic stress persists.

Calcitonin

Calcitonin, a peptide hormone synthesized by thyroid parafollicular C cells (also known as *clear cells*), has an antihypercalcemic effect (i.e., opposite that of PTH). Human calcitonin is a 32-amino-acid chain with a 1,7-disulfide bridge and a C-terminal prolinamide. Alternative splicing of several transcripts from the calcitonin gene produces several polypeptide products, some of which have uncertain calcitropic importance. The primary stimulus for calcitonin secretion is a rise in circulating calcium concentration. Calcitonin lowers serum calcium and phosphorus concentrations mainly by inhibiting bone resorption and increasing calcium excretion in the kidneys (Table 83.1).

Glucocorticoids lower serum calcium concentration by inhibiting osteoclast formation and activity but use for a long time causes osteoporosis by decreasing bone formation and increasing bone resorption. They also decrease intestinal absorption and increase renal excretion of calcium and phosphorus. Because of these mechanisms, glucocorticoids depress hypercalcemia in vitamin D intoxication and subcutaneous fat necrosis (SFN).

Growth hormone increases calcium excretion in the kidney and intestinal absorption. Insulin-like growth factor 1, generated by growth hormone action, stimulates protein synthesis in bone. Insulin increases bone formation, with observed bone loss in patients with uncontrolled diabetes mellitus.

Thyroid hormone excess has been described to be associated with hypercalcemia, hypercalciuria, and osteoporosis. The mechanism of these findings is not entirely clear.

Currently, there is no compelling evidence that the calcitonin-like calcium-lowering hormones are critical regulators of calcium homeostasis in nonpregnant adult humans, perhaps because the low prevailing rate of bone turnover blunts the impact of the antiresorptive actions. However, calcitonin may have important calcitropic functions in pregnant and lactating women and in the fetus and neonate, and in other mammals, particularly rodents, whose bones are constantly growing. In human newborns, the parafollicular C-cell population and serum calcitonin concentrations are much greater than in adults.

Perinatal Mineral Metabolism

During human pregnancy, approximately 30 g of calcium and more than 16 g of phosphorus are transferred transplacentally from the maternal circulation to the growing fetus during the third trimester, when fetal calcium accretion is approximately 140 to 150 mg/kg/day. In humans, a doubling of maternal intestinal calcium absorption and a net increase of calcium accretion into bone compensate for the formidable demand on maternal calcium.[13] A mid-molecule PTHrP hormone[14] expressed principally by the placenta regulates this transplacental calcium pump. TRPV6, a member of the transient receptor potential channel superfamily,

TABLE 83.1 Other Hormones

	Serum Calcium Concentration	Intestinal Calcium Absorption	Renal Calcium Excretion	Bone Effects
Glucocorticoids	Increase	Decrease	Increase	
Growth hormone		Increase		
Insulin-like growth factor 1				Protein synthesis in bone
Insulin				Bone loss
Thyroid hormone excess	Increase		Increase	Osteoporosis

may be the primary calcium channel at the trophoblast apical membrane. Calcium flux across the placenta in *Trpv6*-null mice is reduced by approximately 40%.[15]

Pregnancy

Pregnancy constitutes a unique hormonal milieu that promotes a state of "physiologic absorptive hypercalciuria."[16] Maternal total serum calcium concentration declines slightly during pregnancy, reaches a nadir in the middle of the third trimester, and then increases slightly toward term. The maternal serum phosphorus and magnesium profiles are similar to that of calcium. Maternal serum 25(OH)D concentration varies seasonally and with vitamin D intake, but the vitamin D transport protein concentration increases during pregnancy. Serum $1,25(OH)_2D$ concentrations increase early in pregnancy and continue to rise throughout gestation.[17] The calculated concentration of free $1,25(OH)_2D$ also rises. For many years it was believed that PTH levels also increased steadily throughout pregnancy. However, the use of newer immunometric "sandwich" assays indicates that PTH concentration declines during pregnancy.[17–19] PTHrP levels, in contrast, may be higher in pregnant than in nonpregnant women.[20] The role of circulating calcitonin in pregnancy is uncertain.

$1,25(OH)_2D$ drives enhanced maternal intestinal mineral absorption (reviewed in Kovacs, 2008). After parturition, $1,25(OH)_2D$ concentrations and calcium absorption rates[21] decrease to prepregnancy levels. The interplay of calcitropic and progestational hormones in pregnancy protects the maternal skeleton from demineralization. In contrast, during the relatively low estrogen state of lactation, calcium is mobilized from bone stores, possibly under the influence of PTHrP.[22]

Fetal plasma PTH concentration is low, and calcitonin and PTHrP levels are relatively high. Even these low circulating PTH levels may be functionally important in fetal calcium and magnesium metabolism. There is also a close correlation between maternal and fetal serum 25(OH)D levels, consistent with the transplacental transfer of this metabolite. Hypocalcemia is commonly found in infants born to women with low circulating 25(OH)D levels resulting from poor dietary intake of vitamin D and lack of sunlight exposure. Fetal plasma $1,25(OH)_2D$ concentration is also relatively low, despite robust renal 25(OH)D 1α-hydroxylase activity, whereas the concentrations of $24,25(OH)_2D$ are high. The major function of the fetal kidneys in calcium homeostasis may be the production of $1,25(OH)_2D$ rather than renal tubular regulation of calcium excretion. The high circulating concentrations of calcitonin may support this stimulated fetal 25(OH)D 1α-hydroxylase activity. In contrast, the relatively low circulating fetal $1,25(OH)_2D$ concentrations are a consequence of enhanced placental clearance.[23] Constitutively activated placental 24-hydroxylase activity[24] also preferentially hydroxylates maternally derived 25(OH)D to $24,25(OH)_2D$. This placental capacity to metabolize 25(OH)D and $1,25(OH)_2D$ accounts for the enhanced clearance of fetal $1,25(OH)_2D$, limits access of placenta-synthesized $1,25(OH)_2D$ to the fetal and maternal circulations, and, in effect, partitions the maternal and fetal vitamin D pools.

The Neonate

Placental transfer of calcium ceases abruptly at birth. In healthy term newborns, total calcium concentration and Ca^{2+} concentration decline from nearly 11 mg/dL and 6 mg/dL, respectively, in umbilical cord blood to serum levels of 8 to 9 mg/dL and 5 mg/dL, respectively, by 24 to 48 hours. The nadir of Ca^{2+} concentration may range from 4.4 to 5.4 mg/dL. Concomitant rises in the concentrations of PTH and $1,25(OH)_2D$ stabilize serum calcium concentration as the newborn adapts to extrauterine mineral homeostasis and dietary calcium intake. In preterm infants, calcium absorption from the intestine is nonsaturable and may be vitamin D independent.[25] Serum calcitonin levels increase sharply during the first day and remain elevated compared with those in adults. In the mother, prolactin helps stimulate PTHrP expression in lactating breast tissue. PTHrP is secreted into milk at concentrations 10,000-fold higher than in serum. It is possible that the abundant milk PTHrP content ingested by the neonate is important for mineral regulation. By 2 weeks of life, serum calcium concentration rises to the mean values observed in older children and adults.

During the first week of life, urinary phosphate excretion is significantly higher in preterm newborns than in term newborns but then approximates that of term newborns, possibly owing to accelerated postnatal renal maturation. Calcium excretion is low during the first week when the newborn must compensate for the postpartum fall in serum calcium concentration. After the first several days, calcium excretion increases with a magnitude inversely proportional to gestation. The high urinary calcium-to-creatinine ratio (UCa/Cr) of young infants then steadily declines with age.[26] However, in preterm breastfed infants who are more than 2 weeks old, the UCa/Cr can exceed 2.0.[27] These changes may reflect the relative phosphate deficiency in many preterm infants, which results in an adaptively low urinary phosphate excretion, decreased bone mineralization, and, consequently, relatively high urinary calcium excretion.

Neonatal Hypocalcemia

The definition of hypocalcemia depends on gestational age and birthweight. A precise definition of hypocalcemia, like hypoglycemia, in preterm infants is particularly difficult to formulate. Neonatal hypocalcemia has been defined as a serum calcium level below 8 mg/dL (2 mmol/L) or Ca^{2+} level less than 4.4 mg/dL (1.10 mmol/L) in term infants and preterm infants weighing >1500 g. Hypocalcemia in very low birthweight (VLBW) infants has been defined as a serum calcium level below 7 mg/dL (1.75 mmol/L) or Ca^{2+} level less than 4 mg/dL (1 mmol/L).[28] Under conditions of normal acid-base status and normal serum albumin concentration, total serum calcium and Ca^{2+} levels are linearly correlated, so total serum calcium measurements remain useful as a screening test. However, because Ca^{2+} is the physiologically active fraction in sick infants, it may be preferable to assay Ca^{2+} directly in freshly obtained blood samples. The causes of neonatal hypocalcemia are classified by the timing of onset. Early hypocalcemia occurs in the first 2 to 3 days of life. "Early" and "late" occurring hypocalcemias (Box 83.1) have different causes, usually occur in different clinical settings, and should prompt different approaches to evaluation and management.

Clinical Presentation

Most infants with hypocalcemia are asymptomatic. Hypocalcemic signs in neonates are variable and may not correlate with the magnitude of the decline in calcium level. Calcium ions couple excitation and contraction in skeletal and cardiac muscle, so increased neuromuscular excitability (tetany) is a cardinal feature of hypocalcemia. Such infants are jittery and hyperactive and frequently exhibit muscle jerks and twitches induced by environmental noise

• BOX 83.1 Causes of Neonatal Hypocalcemia

- Early-Onset Hypocalcemia (<48 h of Age)
  - Prematurity
  - Perinatal distress/asphyxia
  - Infants of diabetic mothers
  - Intrauterine growth restriction
- Late-Onset Hypocalcemia (First Week of Life)
  - High phosphate load with or without hypoparathyroidism or vitamin D deficiency
- Neonatal Hypoparathyroid Syndromes
  - Parathyroid agenesis
  - DiGeorge syndrome (22q11.2 deletions)
  - Familial isolated hypoparathyroidism
    - *PTH* mutations
- Autosomal Dominant Hypocalcemic Hypocalciuria
  - Activating mutations of Ca^{2+}-sensing receptor
- Neonatal Hypoparathyroidism Secondary to Maternal Hyperparathyroidism
- Autoimmune Polyglandular Syndrome Type 1 (Autoimmune Polyendocrinopathy–Candidiasis–Ectodermal Dystrophy)
- Hypoparathyroidism Associated With Skeletal Dysplasias
  - Kenny-Caffey syndrome
  - Hypoparathyroidism-retardation-dysmorphism (Sanjad-Sakati) syndrome
  - Osteogenesis imperfecta type II
- Parathyroid Hormone Resistance (Transient Neonatal Pseudohypoparathyroidism)
- Hypomagnesemia With or Without Distal Renal Tubular Acidosis
  - Primary hypomagnesemia
  - Renal tubular acidosis type 1
- Abnormal Vitamin D (1,25-Dihydroxyvitamin D) Production or Action ("Hypocalcemic Rickets")
  - Vitamin D deficiency (secondary to maternal vitamin D deficiency)
  - Acquired or inherited disorders of vitamin D metabolism
  - Resistance to the actions of vitamin D
- Hyperphosphatemia
  - Excessive dietary phosphate
  - Phosphate-containing enemas
  - Rhabdomyolysis-induced acute renal failure
  - Hyperphosphatemic renal insufficiency
- "Hungry Bones Syndrome" (Mineralization Outpacing Osteoclastic Bone Resorption)
- Other Causes
  - Metabolic or respiratory alkalosis
  - Phototherapy
  - Long-chain 3-hydroxyacyl-coenzyme A dehydrogenase deficiency
  - Pancreatitis
  - Sepsis, septic shock
  - Rotavirus gastroenteritis
  - Osteopetrosis and other skeletal dysplasias
  - Pseudohypocalcemia (hypoalbuminemia)
  - Medications
    - Bicarbonate
    - Rapid transfusion or plasmapheresis with citrated blood
    - Furosemide induced
    - Lipid infusions

or other stimuli. Generalized or focal clonic seizures may occur. Other signs of neonatal tetany include poor feeding, hypotonia, apnea, tachycardia, tachypnea, high-pitched cry, and irritability.[29] Occasionally, respiratory or gastrointestinal rather than neurologic signs predominate. Rare presentations include inspiratory stridor caused by laryngospasm, wheezing caused by bronchospasm, or vomiting possibly resulting from pylorospasm, which may cause hematemesis or melena. At times the gastrointestinal signs are severe enough to mimic those of intestinal obstruction.[30] Carpopedal spasm and the Chvostek sign are not as reliably elicited in hypocalcemic newborns as in older children or adults. Hypocalcemia characteristically causes prolongation of the QT interval in the electrocardiogram.[31]

Early Neonatal Hypocalcemia

Hypocalcemia occurring during the first 3 days of life, usually between 24 and 48 hours postpartum, is termed *early neonatal hypocalcemia*. It is an exaggeration of the normal decline in circulating calcium concentration. Early neonatal hypocalcemia is typically encountered in any of four circumstances: prematurity, severe perinatal stress or asphyxia, maternal diabetes, or significant intrauterine growth restriction (IUGR). In addition, phototherapy, maternal hyperparathyroidism, maternal anticonvulsant use, and iatrogenic causes (e.g., transfusion with citrated blood, furosemide use) have also been implicated with early neonatal hypocalcemia.

In preterm infants, there is a steeper and more rapid postnatal decline in serum calcium concentration. The magnitude of the depression is inversely proportional to gestational age. Approximately one-third of premature infants and most VLBW infants have low total serum calcium levels (<7.0 mg/dL) during the first 2 days after birth.[32] However, the fall in Ca^{2+} concentration is not proportional to the fall in total calcium concentration. The ratio of ionized to total calcium in these newborns is higher than at term. This "sparing" of Ca^{2+} may be related to the lower serum protein concentration and pH in prematurity. Multiple factors contribute to the fall in total serum calcium concentration, including low milk intake, impaired response to PTH, and hypoalbuminemia, which does not lower the Ca^{2+} concentration. The sparing effect on Ca^{2+} concentration explains the frequent absence of hypocalcemic signs in preterm infants.

The neonatal parathyroid glands, regardless of the degree of prematurity, can mount an appropriate PTH response to hypocalcemia. Hypocalcemia in extremely preterm newborns[33] or infants undergoing cardiac bypass[34] stimulates increases in serum PTH at least as great as those seen in adults during citrate-induced hypocalcemia.[35] PTH resistance plays an uncertain role in early neonatal hypocalcemia. A several-day delay in the phosphaturic and renal cAMP responses to PTH has inconsistently been reported, suggesting that there might be a maturational delay in renal responses to PTH. Calcitonin usually peaks in 12 to 24 hours of life. Preterm infants' exaggerated rise in calcitonin may also promote hypocalcemia.[36]

Early neonatal hypocalcemia with hyperphosphatemia is frequently observed in severely stressed or asphyxiated infants. Possible mechanisms include increased phosphate load caused by tissue catabolism, renal insufficiency, and acidosis. There is an exaggerated serum calcitonin response and decreased PTH secretion. Low serum Ca^{2+} and elevated serum magnesium levels have been correlated with the severity of hypoxic–ischemic encephalopathy and poor outcome.[37]

Hypocalcemia occurs in at least 20% to 50% of infants of diabetic mothers (IDMs).[38] IDMs show an exaggerated postnatal drop in circulating calcium levels compared with gestational-age controls that typically occur between 24 and 72 hours after birth and are often associated with hyperphosphatemia. The course is usually similar to early neonatal hypocalcemia in preterm infants, although hypocalcemia sometimes persists for several additional days. The greater bone mass and relative undermineralization typical of macrosomic IDMs may increase the neonatal demand for

calcium, producing a deeper and prolonged decline in postnatal serum calcium levels. In addition, magnesium deficiency leads to decreased PTH production and action.[33,39] Maternal glycosuria is associated with urinary magnesium losses, which may lead to significant maternal magnesium deficiency followed by fetal magnesium deficiency.

Neonatal hypocalcemia in IDMs has been associated with the severity and duration of maternal diabetes and inadequate glycemic control. Not surprisingly, preterm IDMs who have sustained IUGR and asphyxia as a result of uteroplacental insufficiency invariably become quite hypocalcemic. Improved metabolic control for pregnant diabetic women can markedly diminish the occurrence and severity of early neonatal hypocalcemia.[40] Healthy IDMs who can start milk feedings on the first day do not require serum calcium monitoring unless suspicious signs (e.g., jitteriness, stridor) are noted.

Hypocalcemia occurs with increased frequency in infants with IUGR. The mechanism is thought to involve a decreased transfer of calcium across the placenta due to uteroplacental insufficiency.

Late Neonatal Hypocalcemia

Late neonatal hypocalcemia develops after 3 to 5 days of life and typically occurs at the end of the first week. It occurs more frequently in term than in preterm infants and is not usually associated with maternal diabetes, birth trauma, or asphyxia. Historically, late-onset hypocalcemia is associated with ingestion of cow's milk or formula with a high phosphate load.[41] The high phosphate level increases calcium deposition in bone and antagonizes PTH secretion and action, leading to hypocalcemia.[42] However, the widespread use of high phosphorus formula in the United States and elsewhere contrasts with the rarity of this condition, which suggests that infants who develop phosphate-induced late neonatal hypocalcemia may have an otherwise undetected renal phosphate excretion problem.

The hyperphosphatemia may also result from varying combinations of immature renal tubular phosphate excretion, transiently low levels of circulating PTH, hypomagnesemia, and inadequate maternal vitamin D intake. A relatively high dietary phosphate load coupled with a low GFR leads to an increase in serum phosphate levels and a reciprocal decline in serum calcium levels. The physiologic response to hypocalcemia is an increase in PTH secretion, leading to increased urinary phosphate excretion and tubular calcium resorption. Serum calcium levels frequently increase when these infants are placed on a low-phosphate formula and supplemental calcium. After several days to weeks, serum PTH usually increases, and the infants then can tolerate more dietary phosphate. The pathogenesis of this "transient hypoparathyroidism" in late neonatal hypocalcemia is not readily apparent. Some of these infants show a persistent or recurrent inability to mount an adequate PTH response to a hypocalcemic challenge, indicating partial hypoparathyroidism.

In other infants, maternal vitamin D deficiency can cause late (or occasionally "early") neonatal hypocalcemia. This possibility is checked by measuring maternal and neonatal serum 25(OH)D levels. Maternal vitamin D deficiency is implicated by the increased incidence of late neonatal hypocalcemia in winter due to inadequate sunlight. The high prevalence of enamel hypoplasia of incisor teeth reported in affected infants indicates that the mineralization defect begins during the third trimester of pregnancy.

Hypocalcemia and hyperphosphatemia after the first 3 to 5 days should always prompt a thorough investigation for the underlying causes (see Box 83.1). Hypocalcemia in this setting usually implies primary or secondary dysregulation of (1) the parathyroid-renal (PTH–-,25[OH]$_2$D) axis, (2) hypomagnesemia, or (3) renal insufficiency. The primary hormonal and end-organ disturbances that cause neonatal hypocalcemic syndromes are described later. As a cautionary note, observations of generally favorable neurologic outcomes in newborns with hypocalcemic or hypomagnesemic seizures may be valid for those with a nutritional cause but are less relevant to patients with associated medical conditions. In this group, the neurologic prognosis may be more closely related to the causative disorder.[43]

Hypocalcemia Caused by Hypoparathyroid Syndromes

The biochemical hallmarks of hypoparathyroidism are hypocalcemia and hyperphosphatemia in the presence of normal renal function. Serum PTH concentrations are inappropriately low or undetectable. Cytogenetic and molecular genetic diagnosis permits the characterization of several types of congenital hypoparathyroidism. Isolated hypoparathyroidism is usually sporadic but may show X-linked, autosomal recessive, or autosomal dominant inheritance.

Congenital hypoparathyroidism is a common feature of DiGeorge syndrome (DGS) in addition to CHARGE (Coloboma, Heart defects, Atresia choanae, Retarded growth and development, Genital hypoplasia, and Ear anomalies/deafness) syndrome. DGS arises from a failure of migration of neural crest cells into the third and fourth pharyngeal pouches. Fully expressed DGS comprises hypoparathyroid hypocalcemia, thymic hypoplasia with defects in T-cell immunity, conotruncal cardiac defects, palatal insufficiency, dysmorphic facial features, and neurobehavioral and psychiatric features. DGS, velocardiofacial (Shprintzen) syndrome, and conotruncal face anomaly (Takao) syndrome commonly result from contiguous gene deletions in the same chromosomal region.

Most patients having a clinical diagnosis of DGS share a common 1.5- to 3-Mb deletion (monosomy or partial monosomy) of chromosome region 22q11.2, but there is molecular heterogeneity and rearrangement within this region.[44] The size of the deletion does not correlate with the clinical phenotype. Haploinsufficiency of the TBX1 (T-box 1) transcription factor gene may be responsible for most features. Studies showed patients with point mutations in the gene encoding TBX1 manifested all major phenotypes of DGS.[45] Fluorescence in situ hybridization (FISH) using 22q11 probes had a higher detection rate than high-resolution G-band karyotyping. However, the most sensitive (and preferred) laboratory assay is array-comparative genomic hybridization. In addition, rarer identification of other cytogenetic abnormalities suggests that several distinct molecular defects can lead to disturbed cranial neural crest cell migration and DGS phenotypes.[46]

DGS affects an estimated 1 in 4000 live births. It occurs sporadically or is transmitted as a variably penetrant autosomal dominant trait. Sporadic loss in the DGS chromosomal region is more common than parental transmission. In members of the same family, DGS may be associated with different phenotypic features. It often manifests itself in the first week of life with hypocalcemic tetany or seizures. Craniofacial features include microretrognathia, mandibular hypoplasia, submucous cleft palate, low-set and abnormal pinnae, telecanthus with short palpebral fissures, short philtrum, and a relatively small mouth. The presence of cardiac outflow tract or aortic arch abnormalities (especially pulmonary atresia/tetralogy of Fallot, type B interrupted aortic arch, truncus

arteriosus, anomalies of aortic arch laterality, or abnormal branching of the brachiocephalic vessels) should prompt genetic investigation even in the absence of other DGS features. Parents of an infant with DGS should be screened for carrier status. These neonates require close anticipatory monitoring for the onset of hypocalcemia.

A normal serum PTH level obtained when an infant is relatively normocalcemic does not exclude the diagnosis of DGS. The absence of a thymic shadow on chest radiograph is not a reliable indicator. Infants with DGS may show resolution of hypoparathyroidism by early childhood, although PTH reserves may remain inadequate for defense against hypocalcemic stresses.

Hypoparathyroidism is a prominent feature of several rare skeletal dysplasias. Kenny-Caffey syndrome is a rare osteosclerotic bony dysplasia associated with hypocalcemia and ocular abnormalities. Autosomal recessive and autosomal dominant inheritance patterns have been described, in addition to an association with CATCH 22 microdeletions.[47] Features include IUGR, transient neonatal hypoparathyroidism, short stature, macrocephaly, delayed fontanel closure, dysmorphic facies, and cortical thickening of tubular bones.[47] The autosomal dominant form of Kenny-Caffey syndrome is clinically distinguished by the absence of developmental delay and is caused by a heterozygous mutation of the FAM111A gene.[48] The recessive form of Kenny-Caffey syndrome is the same disorder as hypoparathyroidism-retardation-dysmorphism (HRD) syndrome, also known as *Sanjad-Sakati syndrome*. HRD syndrome is an extremely rare disorder characterized by congenital hypoparathyroidism, growth retardation, characteristic facies (deep-set eyes, depressed nasal bridge, beaked nose, long philtrum, thin upper lip, large and floppy earlobes), small hands and feet, skeletal defects, and developmental delay.[49] The loci for HRD syndrome is on chromosome 1q42–q43 with mutations in the gene encoding tubulin-specific chaperone E *(TBCE)*.[50] Parathyroid hemorrhage ("parathyroid apoplexy") is a common event in osteogenesis imperfecta (OI) type II and may contribute to early death.[51]

Several mitochondrial syndromes are also associated with hypoparathyroidism. Pearson Marrow-Pancreas syndrome is characterized by pancreatic dysfunction, sideroblastic anemia, neutropenia, and thrombocytopenia and presents as an infant.[52] Kearns-Sayre syndrome is marked by encephalomyopathy, ophthalmoplegia, retinitis pigmentosa, and heart block and presents in childhood.[53]

Barakat syndrome, also known as hypoparathyroidism, sensorineural deafness, and renal dysplasia syndrome (HDR), has an autosomal dominant inheritance. The locus for HDR syndrome is on chromosome 10p14–15 with mutations in the GATA3 gene.[54] The GATA3 protein is a transcription factor expressed in the development of the parathyroid gland, thymus, kidney, inner ear, and central nervous system. There is a wide phenotypic variability with hypoparathyroidism being asymptomatic, resolving after infancy, or severe and permanent into adulthood. Renal involvement is the least penetrant finding, with 60% of patients having renal disease and only 9% developing end-stage renal disease.[55]

The parathyroid glands are an infrequent target for autoimmunity, the exception being autoimmune polyglandular syndrome type 1 (APS 1), also known as *autoimmune polyendocrinopathy–candidiasis–ectodermal dystrophy*.[56] APS 1 is a rare autosomal recessive disorder characterized by hypoparathyroidism, adrenal insufficiency, and chronic mucocutaneous candidiasis.[57] It results from the inheritance of mutations in an autoimmune regulator gene (AIRE). Candidiasis is usually the initial clinical manifestation, most often occurring in patients less than 5 years of age. Hypoparathyroidism occurs next, usually in patients younger than 10 years. Hypoparathyroidism most often precedes the development of hypoadrenocorticism. Affected persons often eventually develop chronic hepatitis, malabsorption, juvenile-onset pernicious anemia, alopecia, and primary hypogonadism.

There are several forms of isolated congenital hypoparathyroidism caused by isolated parathyroid aplasia or defects in PTH synthesis or secretion. Affected infants have subnormal or undetectable serum PTH levels but do not have congenital anomalies or developmental defects, DGS locus deletions, candidiasis or autoimmune polyglandular failure, or antiendocrine antibodies. Two mutations affecting embryologic development have been identified as etiologies of isolated parathyroid aplasia. The most common cause is a loss of function mutation in the GCM2 gene located at chromosome 6p23–24, which encodes an essential transcription factor.[58] These mutations can be autosomal recessive or autosomal dominant. X-linked recessive inheritance is caused by the SOX3 gene.[59]

The PTH gene is located on chromosome 11p15.3–p15.1 and produces the preproPTH protein, which undergoes two cleavages to become a biologically active PTH molecule. There have been several autosomal dominant and autosomal recessive mutations described in the PTH gene.[60]

Impaired PTH secretion has been attributed to gain-of-function mutations in *CaSR* and *GNA11* genes, resulting in autosomal dominant hypocalcemia type 1 (ADH1) and type 2 (ADH2), respectively. The *CaSR* gene is located on chromosome band 3q13.3–q21, and it encodes a cell-surface protein that is expressed in the PTH-producing chief cells of the parathyroid glands and kidney tubules. Activation of the gene increases the sensitivity of the calcium-sensing receptor to extracellular Ca^{2+}, which suppresses the secretion of PTH and renal calcium reabsorption from the cortical thick ascending limb.[61] The parathyroid and renal calcistat is reset downward, so hypocalcemia does not elicit normal compensatory PTH secretion or renal calcium reabsorption. Familial activating mutations in the GNA11 gene occur at chromosome 19p13, which encodes the alpha subunit of the G protein (Gα11) that couples to the CaSR receptor and activates intracellular signaling pathways to produce PTH.[62] A similar mechanism of increased sensitivity of Gα11 to extracellular Ca^{2+} resulting in decreased PTH secretion has been proposed. ADH2 is distinguished by short stature and lack of hypercalciuria compared with ADH1. Most patients with ADH are asymptomatic. Children, in particular, may become symptomatic with seizures and neuromuscular irritability during periods of stress such as febrile illness. Serum calcium level is usually in the range of 6 to 8 mg/dL (1.5 to 2.0 mmol/L), with normal or only slightly low serum PTH concentrations. Because both familial and de novo *CaSR* mutations producing CaSR hyperfunction have been described,[63] mutational analysis of the *CaSR* gene should be considered in the work-up of isolated hypoparathyroidism in infants, especially when hypocalcemia manifests itself with inappropriately normal urinary calcium excretion (relative hypercalciuria). Hypercalciuria and nephrocalcinosis can develop even when serum calcium concentration remains below the normal range. Therefore it is important to distinguish hypocalcemia due to an activating mutation of *CaSR* gene from isolated hypoparathyroidism because ADH1 patients can develop nephrocalcinosis and

renal impairment during treatment with calcium and vitamin D. For this reason, these patients require close monitoring of urinary calcium excretion for adjustment of therapy with $1,25(OH)_2D$ analogues.

Pseudohypoparathyroidism refers to a heterogeneous group of disorders that are usually inherited as an autosomal dominant trait characterized by hypocalcemia, hyperphosphatemia, and increased serum PTH concentrations due to PTH hormone resistance of peripheral tissues. Deletion of the PTH/PTHrP receptor is embryologically or perinatally lethal. Loss of one allele for *GNAS1*, which encodes $G_s\alpha$, the G protein α subunit required for receptor-stimulated cAMP generation, produces pseudohypoparathyroidism type 1A (PHP1A).[64] In patients with PHP1A, there is the characteristic phenotype of Albright hereditary osteodystrophy (AHO) (short stature, obesity, round face, brachymetacarpalism, and subcutaneous calcifications), and it is associated with multihormone resistance (e.g., thyroid-stimulating hormone, gonadotropins, and growth hormone). Isolated PTH resistance in the absence of a somatic phenotype is called *pseudohypoparathyroidism type 1B*. This disorder is an imprinting defect in which both *GNAS1* alleles have an unmethylated (paternal) pattern.[65] Although most patients who eventually receive a diagnosis of PTH resistance syndrome are usually not hypocalcemic during the first month of life, transient neonatal pseudohypoparathyroidism has occasionally been reported.[66] Individuals with pseudopseudohypoparathyroidism have the clinical phenotype of AHO but normal serum calcium, phosphorus, and PTH levels. Pseudohypoparathyroidism type 2 is characterized by hypocalcemia, hyperphosphatemia, and increased serum PTH level. However, individuals usually lack physical features associated with AHO.[67]

Neonatal Hypocalcemia Associated With Maternal Hyperparathyroidism

Hypocalcemia is commonly observed in newborns of hyperparathyroid mothers.[68] In maternal hyperparathyroidism the increased maternal serum calcium concentration facilitates transplacental calcium transport, producing fetal hypercalcemia greater than the moderate elevations of serum calcium concentration normally observed in the third trimester. As a result, fetal PTH secretion is suppressed.[69] The suppressed parathyroids are unable to maintain normal serum calcium levels postpartum. The reason for the hypomagnesemia observed in some infants born to hyperparathyroid mothers is uncertain. However, this derangement may be due to (1) maternal magnesium depletion as a complication of hyperparathyroidism, (2) transient neonatal hypoparathyroidism, or (3) hyperphosphatemia, which may result from transient hypoparathyroidism or high dietary phosphate intake, or both. These newborns may show increased neuromuscular irritability during the first 3 weeks of life but, occasionally, do so much later as limited PTH reserve and latent hypoparathyroidism emerge under stress or with time. The serum calcium levels usually range from 5.0 to 7.5 mg/dL, and the serum phosphate levels are often greater than 8.0 mg/dL. Hypocalcemic signs may be exacerbated by high-phosphate diets or maternal vitamin D deficiency. In some instances, signs of hypocalcemia can be quite severe and may be resistant to several weeks of calcium replacement therapy.

Maternal serum calcium and phosphorus should be assayed whenever this diagnosis is suspected. Hypocalcemic tetany occurring in the infant may lead to a diagnosis of hyperparathyroidism in an asymptomatic mother. Maternal serum calcium values in the upper normal range may be falsely reassuring if the samples were obtained during pregnancy, a time when serum calcium levels normally decline.

Hypomagnesemia causes hypocalcemia by interfering with the parathyroid cell CaSR-mediated release of PTH and by blunting end-organ PTH response. Hypomagnesemia with secondary hypocalcemia (HOMG1) can present in the first weeks of life as persistent hypocalcemia, tetany, and seizures uncontrolled by anticonvulsants or calcium therapy. Delay in establishing the diagnosis may lead to permanent neurologic impairment. This rare autosomal recessive disorder results from defective intestinal magnesium absorption and renal magnesium leak. Hypocalcemia is a secondary consequence of parathyroid failure and PTH resistance as a result of severe magnesium deficiency. HOMG1 is caused by mutations in the *TRPM6* gene, a member of the transient receptor potential channel gene family expressed in intestinal epithelia and renal tubules.[70] Treatment includes immediate administration of magnesium, usually intravenously, followed by high-dose oral magnesium.

Several forms of primary renal hypomagnesemia have been described, including autosomal recessive familial hypomagnesemia with hypercalciuria and nephrocalcinosis caused by mutations in the paracellin 1 gene on chromosome 3q27[71] and a genetically heterogeneous autosomal dominant isolated renal magnesium wasting. The initial symptoms may include recurrent urinary tract infections, polyuria and polydipsia, moderate metabolic acidosis with an inappropriately high urine pH, muscle weakness, persistent tetany, failure to thrive, sensorineural hearing loss, and distal tubular acidosis. All patients exhibit hypercalciuria and nephrocalcinosis, and 50% of cases usually require renal replacement therapy in the second decade of life. The distal acidification defect is probably secondary to a medullary interstitial nephropathy. The serum magnesium level is frequently less than 0.8 mg/dL (normal range 1.6 to 2.8 mg/dL). High-dose enteral magnesium administration leads to increases in serum PTH and calcium levels and renal phosphate clearance. Kidney transplant normalizes serum magnesium and urinary calcium.

Transient hypomagnesemia in newborns often occurs in association with hypocalcemia. Less commonly, the serum calcium level may be normal. In transient hypomagnesemia, the decrease in serum magnesium level typically is less severe (0.8 to 1.4 mg/dL) than in magnesium transport defects. In many infants with transient hypomagnesemia, the serum magnesium level increases spontaneously as the serum calcium level normalizes following the administration of calcium supplements. However, in other cases, hypocalcemia responds poorly to calcium therapy, but when magnesium salts are given, serum calcium and magnesium levels both rise.

Secondary hypomagnesemia from renal magnesium wasting can result from drug administration (e.g., loop diuretics, aminoglycosides, amphotericin B) or urinary tract obstruction. It also may occur during the diuretic phase of acute renal failure. This disorder may be mistaken for neonatal hypoparathyroidism because of tetany and hypocalcemia or Bartter syndrome (hypokalemic alkalosis with hypercalciuria) because of secondary potassium wasting. An index of suspicion should be raised whenever hypomagnesemia occurs in one of these situations. The finding of low serum magnesium levels with inappropriately high urinary magnesium excretion confirms a diagnosis of renal magnesium wasting. Hypokalemia is a common laboratory feature of magnesium depletion. Attempts to replace the potassium deficit with potassium alone are usually unsuccessful unless magnesium is given concurrently.

Distal renal tubular acidosis type 1 (RTA1) is characterized by hypocalcemia, hypercalciuria, various degrees of hypomagnesemia, hyperchloremia, low serum bicarbonate level, and a fixed urinary specific gravity and urinary pH (about 5.0). The mineral excretion defect leads to nephrocalcinosis and MBD. RTA1 sometimes manifests itself during early infancy, when hypocalcemia may precede the renal tubular acidosis.

Hypocalcemia Resulting From Vitamin D Disorders

Vitamin D increases the intestinal absorption of calcium. In older children and adults, disorders of vitamin D intake or metabolism rarely present as isolated hypocalcemia. Instead, most patients with abnormalities in either production or action of $1,25(OH)_2D$ have rickets or osteomalacia. In sharp contrast, young infants may exhibit hypocalcemic tetany before rachitic features become conspicuous. Abnormalities in vitamin D metabolism can be divided into three broad categories: vitamin D deficiency, acquired or inherited disorders of vitamin D metabolism, and resistance to vitamin D actions.

Maternal vitamin D deficiency is the major risk factor for neonatal vitamin D deficiency manifesting itself as hypocalcemia. Maternal vitamin D deficiency is becoming less common in countries where dairy products and other foods are supplemented with vitamin D. It is still a common and serious health problem of women of reproductive age and their infants in developing countries. Female immigrants from the Middle East or South Asia who wear traditional concealing dress, have inadequate dietary vitamin D intake, or are dark-skinned are at particularly high risk, especially during pregnancy.[72,73] Breastfed infants of lactovegetarian mothers are also susceptible to early-onset hypocalcemic rickets. Nutritional rickets in newborns can be prevented by daily supplementation of 400 international units (IU) for infants and 400 IU daily for mothers during pregnancy and lactation or 1000 IU daily if supplementation is begun in the third trimester.

Intestinal absorption of fat-soluble vitamin D requires a functioning exocrine pancreas, biliary tract, and bowel mucosa. Consequently, pregnant women with malabsorption syndromes are likely to be vitamin D deficient.

Anticonvulsant therapy (e.g., with phenobarbital or diphenylhydantoin) during pregnancy, which increases hepatic catabolism of 25(OH)D, can also induce maternal and fetal vitamin D deficiency. Pregnant women who take anticonvulsants should receive vitamin D supplementation (800 to 1000 IU/day).

Phosphate-Induced Hypocalcemia

Phosphate directly precipitates calcium in bone or soft tissues by inhibiting bone resorption and blocking the renal synthesis of $1,25(OH)_2D$. Conditions leading to phosphate-induced neonatal hypocalcemia include excessive phosphate intake, rhabdomyolysis-induced acute renal failure, and hyperphosphatemic renal insufficiency. Phosphate-containing enemas can produce significant phosphate absorption.[74] Their use is hazardous and contraindicated for infants. Chronic renal failure is the primary cause of secondary hyperparathyroidism. Patients with mineral metabolism disorders commonly have low serum calcium levels, hyperphosphatemia, and calcitriol deficiency. In uremic conditions, however, the parathyroid glands become hyperplastic. In recent years, fibroblast growth factor 23 (FGF23) has been identified.[75] FGF23 is a bone-derived hormone that regulates systemic phosphate hemostasis and vitamin D metabolism. FGF23 inhibits renal tubular reabsorption of phosphate independently of PTH.[76]

Other Causes of Neonatal Hypocalcemia

It is important to recognize that hypocalcemia may occur whenever skeletal mineralization significantly outpaces the rate of osteoclastic bone resorption. Examples of this type of hypocalcemia occur with overzealous vitamin D replacement in infants with rickets or hypoparathyroidism.[77] Pancreatitis can cause hypocalcemia and tetany through the action of pancreatic lipase on retroperitoneal and omental fat to release free fatty acids (FFAs). FFAs avidly chelate calcium and remove it from the ECF. Pancreatitis may also result in the release of pancreatic calcium-lowering factors.[78,79] Hypocalcemia is commonly seen in critically ill patients with sepsis. It is believed to be due to parathyroid gland suppression, failure to activate vitamin D, and calcium chelation.[80] Neonatal or infantile hypocalcemia and hypocalcemic seizures may accompany long-chain 3-hydroxyacyl-coenzyme A dehydrogenase deficiency and severe cases of rotavirus gastroenteritis.[81] Hypocalcemic jitteriness or seizures can be the presenting sign of infantile osteopetrosis, likely due to the failure of osteoclasts to resorb immature bone.[82] Prompt recognition permits early referral for bone marrow or hematopoietic stem cell transplantation.

Several common therapeutic interventions can induce hypocalcemia. Bicarbonate therapy, as well as any form of metabolic or respiratory alkalinization, decreases both Ca^{2+} levels and bone turnover. Rapid blood transfusion or plasmapheresis can promote calcium complexes with the infused citrate, decreasing Ca^{2+} levels. Hypocalcemia after initiation of ECMO is related to the composition of the circuit-priming solution and to acute citrate loading and may lead to hemodynamic instability.[83] Large doses of ethylenediaminetetraacetic acid (EDTA)-containing contrast dyes have also been reported to cause hypocalcemia.[84] Furosemide therapy for hypercalcemia promotes calciuresis and nephrolithiasis. Phototherapy for hyperbilirubinemia may be associated with mild hypocalcemia. This effect has been attributed to decreased melatonin secretion, which potentiates glucocorticoid actions on bone metabolism. Lipid infusions may elevate serum levels of FFAs, which form insoluble complexes with calcium. Most of these effects are transient, and cessation of therapy is followed by a return to normal serum calcium levels. The major exception is aggressive furosemide therapy, which, when prolonged, may lead to bone demineralization and renal dysfunction.

Management of Hypocalcemia

The decision to treat hypocalcemia in an infant depends on the severity of the hypocalcemia and the presence of clinical signs and symptoms. The morbidity associated with calcium treatment must be weighed against the potential benefits. Hypocalcemic preterm infants with no symptoms and are not ill from any other cause probably do not need specific treatment. Early neonatal hypocalcemia should resolve by day 3. Some clinicians begin treatment in preterm newborns once serum calcium levels have dropped to 6.0 to 6.5 mg/dL or after Ca^{2+} concentration has decreased to 2.5 to 3.0 mg/dL. Some advocate initiation of prophylactic calcium infusions (or calcium-containing parenteral nutrition) for all extremely low birthweight (ELBW) infants within the first 24 hours. There is no role for prophylaxis or treatment with pharmacologic doses of vitamin D. For newborns who exhibit cardiovascular compromise (e.g., severe respiratory distress, pulmonary hypertension, asphyxia, sepsis) or who require cardiotoxic drugs or blood pressure support, monitoring of blood Ca^{2+} concentration is particularly helpful, to prevent the onset of significant hypocalcemia.

The mainstay of treatment for neonatal hypocalcemia is intravenous (IV) administration of calcium salts. Calcium gluconate is

preferred over calcium chloride (which, in sufficient doses, produces hyperchloremic acidosis) or calcium lactate. A 10% solution of calcium gluconate contains 9.4 mg elemental calcium per mL. A constant infusion of approximately 45 to 75 mg/kg/day of elemental calcium usually produces a sustained increase in serum calcium level (7 to 8 mg/dL). Bolus infusions are hazardous and only transiently effective.

The risks associated with calcium infusions are minimized by attention to detail. Rapid IV infusion of calcium can cause a sudden elevation in serum calcium level, leading to bradyarrhythmias. Bolus infusion of calcium should be reserved for the treatment of hypocalcemic tetany and seizures. Extravasation of calcium solutions into subcutaneous tissues may cause necrosis and subcutaneous calcification. Therefore meticulous care of peripheral IV catheter sites is particularly important when calcium-containing solutions are infused. Inadvertent intrahepatic infusion of calcium through an umbilical vein catheter due to failure to reach the inferior vena cava can cause hepatic necrosis. Rapid intra-aortic infusion via an umbilical artery can cause arterial spasm and, at least experimentally, intestinal necrosis.

Hypocalcemic Crisis

Acute hypocalcemia constitutes an emergency that requires prompt attention. For emergency treatment of a hypocalcemic crisis with seizures or tetany, 100 to 200 mg/kg calcium gluconate should be given within 5 to 10 minutes. The dose can be repeated in 10 minutes if no response occurs. Calcium should not be administered rapidly intravenously because hypercalcemia may adversely affect cardiac conduction; monitoring the electrocardiogram QT interval is useful in guiding therapy. Alternatively, 20 mg/kg calcium chloride can be given. If necessary, IV calcium therapy may be repeated 3 or 4 times in 24 hours to help control acute symptoms. After short-term treatment, maintenance calcium gluconate should be added to IV fluid. Careful observation of the infant and infusion site is essential, and the infusion should be discontinued if there is bradycardia or when the desired clinical result is obtained. Serum calcium must be frequently monitored during infusion, and calcium should not be mixed with fluids containing phosphate or bicarbonate to avoid precipitation.[85]

Nonemergency Treatment

After acute symptoms have been controlled, calcium therapy should be continued as needed to maintain a serum calcium concentration above 7.0 mg/dL. In part, the level of serum calcium to be achieved depends on serum total protein, particularly albumin. In hypoalbuminemic infants, lower levels of total serum calcium are normally present. The dose varies with age. In preterm and sick infants for whom oral intake is limited, 45 to 75 mg/kg elemental calcium gluconate may be infused with IV fluids in a 24-hour period. For older infants and children, a starting dose of 20 mg/kg elemental calcium gluconate every 24 hours should be provided. The lower dose range is preferred whenever there is hyperphosphatemia. If oral feedings are tolerated, a dose range of 50- to 150-mg/kg/day of elemental calcium gluconate in four to six divided doses should be given. Alternatively, calcium glubionate (Neo-Calglucon) may be given in a dosage of 30 to 50 mg/kg divided into feedings. Orally administered calcium gluconate is better tolerated by young infants because the high sugar content and osmolality of calcium glubionate may cause gastrointestinal irritation or diarrhea. IV or oral calcium supplements should be continued until the serum calcium level stabilizes.

Dietary factors and hypoparathyroidism are important in the pathogenesis of late neonatal hypocalcemia. Therefore therapy is often directed at reducing the phosphate load and increasing the calcium-to-phosphorus ratio of feedings to 4:1. This can be accomplished by using low-phosphorus feedings such as human milk or Similac PM 60/40 (Abbott Nutrition) in conjunction with calcium supplements. These interventions inhibit intestinal absorption of phosphorus. Phosphate binders are not generally necessary. Serum calcium and phosphorus levels should be monitored at least once to twice weekly, and the use of calcium supplements should be discontinued in a stepwise fashion after several weeks.

Magnesium Administration

When hypomagnesemia contributes to hypocalcemia, administration of magnesium salts is indicated. Magnesium may be given intramuscularly as a 50% magnesium sulfate solution (50% $MgSO_4 \cdot 7H_2O$ contains 4 mEq/mL magnesium). The suggested intramuscular or IV dose of 50% magnesium sulfate is 25 to 50 mg/kg. IV infusions should be administered slowly using electrocardiographic monitoring to detect rhythm disturbances, which may include prolonged atrioventricular conduction time and sinoatrial or atrioventricular block. The magnesium dose may be repeated every 12 to 24 hours, depending on the clinical and serum magnesium response. Many infants with transient hypomagnesemia will respond sufficiently to one or two magnesium injections. Infants with primary hypomagnesemia have permanent magnesium wasting. The low serum magnesium levels may require lifelong treatment with magnesium supplements.

Vitamin D Treatment

Infants with normal intestinal absorption who develop late hypocalcemia with vitamin D–deficiency rickets usually respond within 4 weeks to 1000 to 2000 IU/day oral vitamin D. These infants should receive at least 40 mg/kg/day of elemental calcium to prevent hypocalcemia because the unmineralized osteoid may avidly incorporate calcium once vitamin D is provided ("hungry bones" syndrome). PTH-dependent renal production of $1,25(OH)_2D$ is deficient in all hypoparathyroid states, renal failure, or vitamin D–dependent rickets. Vitamin D metabolites that do not require renal 1α-hydroxylation should be administered. Calcitriol ($1,25[OH]_2D$) is indicated, usually starting with 0.25 to 1 μg daily to twice a day. The dose may be adjusted at 2- to 4-week intervals. Most patients with hypoparathyroidism require lifelong calcium and vitamin D supplementation. The goals of therapy are to relieve symptoms and maintain the serum calcium level in the low normal range (e.g., 8.0 to 8.5 mg/dL [2.0 to 2.1 mmol/L]) to prevent hypercalciuria and nephrocalcinosis.[86] Assessment of urinary calcium excretion by urinary calcium-to-creatinine ratio should be performed within 2 weeks of initiation of therapy. The urinary calcium measurement reflects the effect of therapy and monitors the risk of nephrocalcinosis. The ratio of 0.2 mg/mg or greater usually defines hypercalciuria in older children, but the age-dependent 95th percentile can be as high as 0.7 mg/mg for infants.[87] Periodic renal sonograms are also recommended to detect nephrocalcinosis.

It is essential that patients with hypocalcemia be aware that their calcium level should be monitored more frequently during intercurrent illnesses that may affect calcium absorption to prevent the development of hypocalcemia or severe hypercalcemia.

Recombinant Parathyroid Hormone Analogue

Studies have suggested subcutaneous injections of recombinant PTH can be an alternative therapy for hypoparathyroidism. However, such treatment poses a number of challenges, particularly fluctuations in calcium levels. Further studies are needed to assess the long-term effects.[88]

Neonatal Hypercalcemia

Hypercalcemia is usually defined as total serum calcium concentration greater than 11.0 mg/dL and Ca^{2+} concentration greater than 5.0 mg/dL. Hypercalcemia in neonates may be asymptomatic if there are only mild elevations in calcium levels, but as calcium levels start to rise above 12.0 mg/dL, then symptoms may start to manifest themselves. These may include poor weight gain, poor feeding, weakness, constipation, and polyuria. As calcium levels increase (>14 mg/dL), more severe symptoms may present, such as vomiting, respiratory distress, apnea, hypotonia, lethargy, and seizures. The polyuria seen in hypercalcemic states is due to impairment in the kidney's concentration ability, leading to a state of nephrogenic diabetes insipidus.[89] This may be partly due to inhibition of sodium chloride reabsorption in the loop of Henle, which impairs the osmotic gradient needed for concentration, as well as decreased antidiuretic hormone–mediated water permeability in the collecting tubules.[90] Usually, there are no physical examination findings associated with hypercalcemia unless findings are associated with genetic syndrome dysmorphisms or SFN. Hypertension can be seen, most likely due to the effects of hypercalcemia on vasoconstriction as well as possible effects on catecholamine.[91] Shortened QT interval may also be seen on electrocardiogram.[92] When hypercalcemia is chronic, calcifications can be seen in multiple tissues and organs, including the kidney, skin, subcutaneous tissue, heart (valves, arteries, muscle), lung, brain, and gastric mucosa. Nephrocalcinosis, nephrolithiasis, and osteitis fibrosa cystica (loss of bone mass and replacement with fibrous tissue with formation of cystic lesions due to increased osteoclastic resorption) may also be seen in chronic, untreated hypercalcemia. In infants, the most clinically apparent manifestation of chronic hypercalcemia is poor growth and failure to thrive.

Neonatal hypercalcemia is associated with several clinical entities (Box 83.2). Normally calcium concentrations are tightly regulated by interplays between PTH, CaSR, calcitonin, and vitamin D analytes affecting intestinal and renal calcium absorption. Hypercalcemia may occur when there is a disruption in this system and can be due to multiple causes, which can be categorized on the basis of abnormalities of PTH, CaSR, intake, or other causes.

Neonatal Hyperparathyroid Syndromes Associated With CaSR Mutations

Inactivating mutations of CaSR can lead to a spectrum of hypercalcemic diseases from severe neonatal hyperparathyroidism (NSHPT) to mild presentations as seen in FHH. These mutations cause a shift in the dose-response curve for calcium to the right, so there is a higher setpoint for the CaSR, leading to higher serum calcium levels and an elevated or inappropriately normal PTH level. Urinary calcium-to-creatinine clearance ratios will usually be less than 0.01 (gray zone between 0.011 and 0.019), and there will often be a family history of hypercalcemia, although de novo mutations can be present. FHH is usually due to a heterozygous mutation, while NSHPT is due to a homozygous mutation; however, there have been cases of mild homozygous mutations leading to an FHH presentation and severe heterozygous mutation presenting more like NSHPT.[93–95]

FHH is an autosomal dominant disorder characterized by mild hypercalcemia, low renal excretion of calcium leading to hypocalciuria, and normal or mildly elevated PTH. Usually, FHH is asymptomatic with normal phosphorus, magnesium, vitamin D levels, and bone density. The presentation can be masked if there is concomitant vitamin D deficiency.[96] There are three types of FHH. FHH type 1 is the most common form and accounts for 65% of cases. It is due to a loss of function mutation of the *CaSR* gene on chromosome 3q21.1. Mutations lead to insensitivity of the receptor to serum calcium levels, causing a release of PTH from the parathyroid glands at higher serum calcium levels. This leads to hypercalcemia, hypocalciuria and high normal to elevated magnesium levels. More than 200 CaSR mutations have been reported.[94] FHH type 2 and type 3 account for the rest of the cases. FHH type 2 accounts for about 10% of FHH cases and is characterized by high-normal PTH and mildly elevated magnesium levels and is due to loss of function mutation of *GNA11* on chromosome 19p13.3. *GNA11* encodes the $G\alpha_{11}$ proteins, which are involved in CaSR signaling.[95,97] The mutation results in decreased CaSR-induced signal transduction and increased secretion of PTH.[94,98] FHH type 3 presents with elevated PTH, decreased phosphate, and osteomalacia. It is due to a loss of function mutation for *AP2S1* on chromosome 19q13.3, which affects the Arg15 residue.[94,99] *AP2S1* encodes for the adaptor-related protein complex 2, which is involved in the endocytosis of CaSR. Mutations result in decreased expression of CaSR at the cell membrane. FHH type 3 may have a more severe presentation with higher calcium levels. Mutations in *AP2S1* account for 20% of cases of FHH without *CaSR* mutations.[94] FHH generally does not require treatment unless there is evidence of symptoms or

• BOX 83.2 Causes of Neonatal Hypercalcemia

- Hyperparathyroidism
  - *CaSR* mutations
    - Neonatal severe hyperparathyroidism
    - Familial hypocalciuric hypercalcemia
  - Maternal hypocalcemia
  - Mucolipidosis type II
- Iatrogenic
  - Hypophosphatemia
  - Vitamin D intoxication
  - Calcium supplement excess
  - Hypervitaminosis A
  - Extracorporeal life support
- Hypercalcemia Associated With Skeletal Dysplasias
  - Infantile hypophosphatasia
  - Jansen metaphyseal chondrodysplasia (activating mutations of parathyroid hormone/parathyroid hormone–related protein receptor)
- Other Causes
  - Williams syndrome
  - Idiopathic infantile hypercalcemia
  - Subcutaneous fat necrosis
  - Blue diaper syndrome
  - Tumor-associated hypercalcemia (parathyroid hormone–related protein secretion)
  - Congenital lactase deficiency
  - Congenital sucrase-isomaltase deficiency
  - Immobilization

complications due to hypercalcemia. Parathyroid glands can be normal or hyperplastic, and surgical resection is usually not helpful. Therefore genetic testing can be very useful for both management and genetic counseling as parents who are carriers of *CaSR* mutations can have a child with NSHPT.

NSHPT is a rare autosomal recessive disorder resulting in a life-threatening disorder associated with elevated PTH, elevated serum calcium, elevated magnesium, and relatively low urinary excretion of calcium, although some may have hypercalciuria. It is diagnosed within the first 6 months of life, but severe forms will present as a critically ill newborn with symptoms of poor feeding, dehydration, lethargy, and respiratory distress. Respiratory distress can be due to hypotonia, rib cage deformities, and multiple fractures.[95] Elevated PTH may lead to bony changes, including demineralization, fractures, and rickets. Homozygous mutations of *CASR* usually lead to a more severe presentation and are inherited from parents who have FHH. Heterozygous gene mutations may have lower calcium levels and may be inherited from one parent or occur from a de novo mutation.[93,100] Typically, these patients have large, hyperplastic parathyroid glands that need to be surgically removed. However, prior to surgery, hypercalcemia needs to be medically managed. This includes the use of sodium-containing fluids and diuretics to induce sodium diuresis and therefore urine calcium excretion. Medications such as bisphosphonates and calcimimetics can decrease serum calcium as well. Pamidronate 0.5 to 1 mg/kg and cinacalcet 0.4 to 2 mg/kg/day have been used successfully in reported cases to decrease serum calcium levels as patients await total parathyroidectomy.[93,99]

Neonatal Hyperparathyroidism Not Associated With CaSR Mutations

Other forms of inherited hyperparathyroidism include those seen in syndromes such as multiple endocrine neoplasia (MEN) 1, MEN 2A, and hyperparathyroidism-jaw tumor syndrome, which typically present with hyperparathyroidism later in life.[94] Autosomal dominant forms of familial hypercalcemia have been associated with mutations in the following tumor suppressor genes: MEN1, HRPT2 (CDC73), CDKN1B, APC, SFRPs, GSK3β, RASSF1A, HIC1, RIZ1, WT1. Mutations of proto-oncogenes associated with autosomal dominant forms of familial hypercalcemia include CCND1/PRAD1, RET in MEN2, ZFX, CTNNB1, and EZH2.95.

Secondary neonatal hyperparathyroidism may also be seen as a consequence of maternal calcium-PTH abnormalities. Maternal hypocalcemia during pregnancy will lead to decreased placental transport of calcium, fetal hypocalcemia, and subsequent hyperplasia of the parathyroid glands. This condition is transient and normally resolves within the first few weeks of life. Hypercalcemia and hypophosphatemia are not seen universally in these cases, but bony changes secondary to elevated PTH are common.[101] Another condition that may lead to secondary hyperparathyroidism is mucolipidosis type II. These cases present with elevated PTH, normal calcium, bony changes and resolve with vitamin D and calcium supplementation. The etiology is hypothesized to be secondary to diminished transplacental calcium transport as this condition is associated with abnormalities of the placenta.[102]

A syndrome that has features of hyperparathyroidism but has low levels of PTH is Jansen metaphyseal chondrodysplasia. In this condition, mutations of PTHR1 lead to constitutive, ligand-independent activation of the receptor, causing hypercalcemia despite low serum PTH levels. These patients will also have irregularities of the metaphysis and rachitic changes seen on x-ray. Other distinguishing features include short-limbed dwarfism with postnatal growth failure, hypertelorism, and mandibular hypoplasia.[103,104]

Williams Syndrome and Idiopathic Infantile Hypercalcemia

Williams syndrome (WS) (or Williams-Beuren syndrome) is a genetic disorder due to the deletion of the Williams-Beuren syndrome critical region (WBSCR) of chromosome band 7q11.23, which includes the elastin gene *(ELN)*. The deletion can be detected by FISH or deletion/duplication testing. It is usually due to a de novo mutation but can be transmitted in an autosomal dominant fashion. It is characterized by distinctive facial features (broad forehead, stellate iris, short nose, broad nasal tip, malar flattening, long philtrum, wide mouth, small jaw, large earlobes), mild intellectual disability, a social personality, short stature, cardiovascular disease (elastin arteriopathy, peripheral pulmonary stenosis, supravalvular aortic stenosis, hypertension), hypotonia, joint hyperextensibility, and endocrine abnormalities (hypercalcemia, hypothyroidism, early puberty). In infancy, inguinal or umbilical hernias, hypotonia, poor feeding, failure to thrive, developmental delays, and rectal prolapse may also be present.[105]

About 30% of patients have hypercalciuria, and 15% to 45% have idiopathic hypercalcemia. The hypercalcemia is noted during infancy and typically resolves by 2 to 4 years of life, although rarely it can persist into adulthood. The cause of hypercalcemia is not well understood, but it is thought that there is increased gut absorption of calcium.[106] PTH level is normal or low, and there is no consistent pattern in vitamin D metabolism to explain the abnormality. Hypercalciuria is relatively common, and nephrocalcinosis has been reported.[107] Therefore early screening for hypercalcemia and hypercalciuria is warranted in infancy. The severity of the calcium abnormalities can range from mild to severe and can lead to dehydration, irritability, vomiting, constipation, and muscle cramps.[106] If hypercalcemia is present, the work-up should include serum blood urea nitrogen, creatinine, 25(OH)D, $1,25(OH)_2D$, PTH levels, and urine calcium/creatinine ratio. If hypercalciuria is present, renal ultrasounds will need to be monitored to evaluate for nephrocalcinosis. Treatment consists of limiting calcium and vitamin D intake and increasing fluid intake until the condition resolves.[106] More severe cases have also been treated with short courses of calcitonin, glucocorticoids, and pamidronate.[105,108]

A similar calcium profile seen in WS has been described in certain infants classified as idiopathic infantile hypercalcemia (IIH). These infants may present with failure to thrive, dehydration, and nephrocalcinosis with a biochemical profile of hypercalcemia, hypercalciuria, and suppressed PTH. Abnormal renal calcium deposition leads to a higher risk for nephrolithiasis and nephrocalcinosis.[109] They do not have the same clinical features or gene deletion as WS and can be differentiated from WS through genetic testing. In IIH, hypercalcemia can resolve in the first few years of life, although it may persist into adulthood, and treatment is similar to that of children with WS.[109,110] The cause of the hypercalcemia was a mystery for many years; however, mutations in two genes have been identified as causative, leading to differentiation into two forms of IIH.[109] IIH type 1 is inherited in an autosomal recessive manner and is due to mutations of *CYP24A1*, which encodes for the enzyme 25-hydroxyvitamin D 24-hydroxylase. This enzyme plays an important role in the metabolism and deactivation of $1,25(OH)_2D$. Mutations of the CYP24A1 gene can result in hypercalcemia in infants due

to decreased degradation of calcitriol ($1,25(OH)_2D$), leading to increased intestinal absorption of calcium and downregulation of PTH. Biochemically, this leads to hypercalcemia, hypercalciuria, suppressed PTH, increased intestinal calcium absorption, and elevated $1,25(OH)_2D$. An elevated 25-OHD/$24,25(OH)_2D$ ratio can also be seen.[95,109,111,112] Vitamin D supplementation leads to exaggerated increases of $1,25(OH)_2D$ levels and subsequent hypercalcemia. Therefore treatment is a limitation of vitamin D and calcium intake. If symptomatic hypercalcemia persists despite this intervention, then treatment with bisphosphonates, ketoconazole/fluconazole, or rifampin may be considered.[109] IIH type 2 is due to loss of function mutations of *SLC34A1*, which codes for the NaPi-IIa cotransporter. The condition has a similar biochemical profile to IIH type 1 but has a wasting of phosphate in the urine, leading to suppression of FGF23 and inappropriate synthesis of $1,25(OH)_2D$. The combination of hypercalcemia, hypercalciuria, and hyperphosphaturia leads to the development of kidney stones. Treatment for IIH type 2 includes phosphate supplementation to restore serum phosphate levels and normalize vitamin D and calcium metabolism. Molecular testing should be done for all patients suspected to have IIH.[109]

Neonatal Hypercalcemia Associated With Subcutaneous Fat Necrosis

SFN is a panniculitis that develops in term or postterm infants. Lesions are erythematous to violaceous, indurated subcutaneous nodules and plaques which appear in the first few days of life (mean 4 days) and are typically found on the back, head, and arms. The lesions may initially be painful and resolve within the first few months of life. Biopsy of the nodules shows granulomatous necrosis in the subcutis with crystal-like structures in adipocytes and giant cells. Risk factors for the development of SFN include birth trauma, asphyxia, meconium aspiration, infections, and cutaneous trauma. Maternal risk factors may include gestational diabetes, preeclampsia, cocaine or calcium blocker use during pregnancy, and high risk of thrombosis.[113] SFN has also been recognized as a complication of neuroprotective cooling with lesions occurring in areas in contact with the cooling blanket.[114]

Complications of SFN include mild to severe hypercalcemia, dyslipidemia, hypoglycemia, thrombocytopenia, anemia, and eosinophilia. Although usually asymptomatic, when symptoms are present, they are usually related to hypercalcemia (irritability, vomiting, polyuria, poor weight gain, hypotonia, hypertension, seizures, nephrocalcinosis, renal dysfunction).[115] Hypercalcemia may occur 1 to 2 months after delivery. Therefore infants with SFN should have calcium levels monitored regularly. In the majority of cases with hypercalcemia, the hypercalcemia presented less than 28 days after the appearance of skin lesions, although a minority can have hypercalcemia prior to the appearance of the lesions and >70 days after the lesions. The majority of patients have resolution of the hypercalcemia within the first 3 months of life, although some can persist longer.[115] Some of the hypercalcemia may be asymptomatic despite high levels of calcium.[113,116] Hypercalciuria is often present, and nephrocalcinosis may persist into early childhood but does not seem to result in adverse renal outcomes.[116] The etiology of hypercalcemia is not well understood, but there are a few theories. One thought is that there is uncontrolled production of $1,25(OH)_2D$ by activated macrophages that infiltrate the necrotic fat. This leads to increased intestinal absorption of calcium in the gut. Another is that elevated PTH and prostaglandin E increases bone resorption. Others hypothesize that calcium is mobilized from the resolving lesions, and another theory is that there is decreased calcium clearance by the kidneys due to early renal injury.[104,115,116] Body cooling may contribute to the presentation due to crystallization during hypothermia from the higher ratio of saturated to unsaturated fat in the skin of neonates.[115] It is recommended that infants with SFN lesions be screened for hypercalcemia at diagnosis of the lesions and again at 30, 45, and 60 days following resolution of the lesions or if a child develops symptoms of hypercalcemia.[115] Treatment includes hydration, furosemide, and a low-calcium/low vitamin D diet. However, SFN is also highly responsive to glucocorticoids. Severe, refractory cases have also been treated with bisphosphonates.[116]

Hypercalcemia Due to Iatrogenic Causes

Neonatal hypercalcemia may also be due to increased intake of calcium or vitamin D or decreased intake of phosphorus. Hypercalcemia due to phosphate depletion is most often seen in VLBW infants fed unsupplemented human milk, which is low in phosphorus, or given parenteral nutrition with inadequate phosphate. There is a high consumption of phosphorus by cells in growing newborns. If inadequate phosphorus is given, then phosphorus will be released through bone resorption, which in turn also releases calcium. This may also be associated with high alkaline phosphatase (ALP) levels. Low phosphorus will also stimulate increased renal production of $1,25(OH)_2D$ and increased calcium absorption through the gut.[117,118] The condition is preventable by anticipatory monitoring of serum calcium and phosphorus levels in high-risk infants.[119]

Excessive supplementation with vitamin D may cause neonatal hypercalcemia. The excess vitamin D may be from improperly made formula, ingestion of cow milk fortified with vitamin D, or overdose of vitamin D supplements. Infants respond to discontinuation of vitamin D supplements. These occurrences have prompted vitamin reformulation of preterm nutritional products. Laboratory studies in hypervitaminosis D typically show elevated 25(OH)D but not $1,25(OH)_2D$ and suppressed serum PTH.

Hypervitaminosis A due to renal failure or ingestion of a large amount of vitamin A has been associated with hypercalcemia. It is hypothesized that vitamin A inhibits osteoblasts and stimulates osteoclasts, leading to increased bone turnover.[120,121]

Hypercalcemia has also been reported in neonates undergoing extracorporeal life support (ECLS). The mechanism is not well understood. High PTH has been reported but does not seem to correlate well with the calcium and vitamin D levels. Hypercalcemia was associated with higher mortality and a longer duration of ECLS.[122,123]

Other Causes of Neonatal Hypercalcemia

Tumor-associated hypercalcemia in neonates is extremely rare. Congenital mesoblastic nephromas have been associated with hypercalcemia in infancy and may be due to tumor secretion of PTHrP.[124] Metabolic disorders, congenital lactase deficiency, and congenital sucrase-isomaltase deficiency are associated with hypercalcemia. They will also have symptoms of persistent diarrhea and failure to thrive. The hypercalcemia may be due to increased ileal absorption of calcium as well as effects of metabolic acidosis on the release of calcium from bone.[125,126] Immobility can also cause hypercalcemia due to increased bone turnover.[127]

Blue diaper syndrome is a rare familial disease in which hypercalcemia and nephrocalcinosis are associated with a defect in the intestinal transport of tryptophan.[128] Bacterial degradation of tryptophan in the intestine leads to excessive indole production, which is converted to indican in the liver. Oxidative conjugation of indican

in the urine forms the water-insoluble dye indigo blue (indigotin), with a consequent peculiar bluish discoloration of the diaper. The clinical course is characterized by failure to thrive, recurrent unexplained fever, infections, marked irritability, and constipation.

Hypophosphatasia is a rare autosomal recessive condition caused by loss of function mutations in the tissue nonspecific ALP gene *TNSALP*. Laboratory tests will show very low ALP levels. Depending on the age at diagnosis, six clinical forms are currently recognized: perinatal (lethal), perinatal benign, infantile, childhood, adult, and odontohypophosphatasia. Prominent features of the early-onset, severe forms are respiratory complications, increased intracranial pressure, widespread undermineralization, rickets, and hypercalcemia.[129] Recently developed enzyme replacement therapy with Asfotase Alfa has improved symptoms and prognosis for this condition.[130]

Treatment of Hypercalcemia

Treatment will depend on the cause and severity of hypercalcemia. If the hypercalcemia is mild to moderate, then restriction of calcium and vitamin D may be sufficient. In more severe cases, further steps must be taken. Part of the treatment is rehydration. Hydration with normal saline is recommended to help induce sodium diuresis and increase urine calcium excretion. Once adequate rehydration with adequate urine output has been established, a loop diuretic such as furosemide (1 mg/kg intravenously at 6- to 8-hour intervals) may be added as it inhibits calcium reabsorption. Hydrocortisone reduces calcium absorption in the intestine and can be given as 1 mg/kg every 6 hours. Calcitonin decreases skeletal reabsorption and inhibits renal reabsorption of calcium. Subcutaneous or intramuscular doses of 3 to 6 μg/kg every 6 hours can be given, but it is only effective for a few days as tachyphylaxis will occur. Bisphosphonates such as pamidronate have been used successfully in a number of conditions resulting in hypercalemia. These agents inhibit osteoclasts and bone resorption. Pamidronate doses of 0.5 to 2 mg/kg have been reported. A third-generation bisphosphonate, zolendronic acid, has also been used to treat hypercalcemia. This has been shown to decrease hypercalcemia more significantly, faster, and for longer duration than pamidronate. It should be used with caution in those with kidney disease. Neonatal dosing is 0.0125 mg/kg to 0.025 mg/kg every 3 months. Cinacalcet is a calcimimetic that works to make the CaSR more sensitive. It has been used in cases of CaSR mutations.

Neonatal Disorders of Serum Magnesium

Neonatal hypermagnesemia is usually due to maternal magnesium sulfate administration or postnatal excess administration of magnesium-containing products, including magnesium in neonatal parenteral nutrition solutions that exceeds magnesium clearance, use of magnesium hydroxide–containing antacids (e.g., milk of magnesia), or medication errors resulting in overadministration of magnesium. Magnesium inhibits acetylcholine release at neuromuscular junctions; therefore elevated levels will lead to symptoms of low tone in mild cases to paralysis in severe cases. The hypermagnesemic newborn may exhibit central nervous system depression (lethargy, hypotonia, poor suck ability), hyporeflexia, and hypotension in mild to moderate cases. Severe cases may result in muscle paralysis, hypoventilation, respiratory depression, coma, arrhythmias, complete heart block, and asystolic arrest. As hypermagnesemia decreases myocardial electrical conduction, cardiac features may include prolonged PR, QRS, and QT intervals, intraventricular conduction defects, bradycardia, and peaked T waves. Because of effects on increased bowel motility, increased fluid secretion and electrolyte abnormalities may be seen; however, delayed passage of meconium has also been reported.[131] In addition, hypocalcemia caused by inhibition of PTH by high levels of magnesium may also be seen. Treatment is mostly supportive as eventually the magnesium will be cleared by the kidney. Any administration of magnesium (oral or IV) should be discontinued. Regular monitoring for cardiac, respiratory, and electrolyte abnormalities should be done, and any abnormalities should be treated accordingly. Feedings should be deferred until normalization of bowel function occurs. Calcium infusions may be helpful in severe, symptomatic cases as calcium is an antagonist of magnesium. Hemodialysis may be considered in life-threatening situations in anuric patients.[132]

Metabolic Bone Disease in Newborns and Infants

MBD is characterized by the reduction in the mineral content and protein matrix of bone, frequently presenting with rachitic changes. MBD is especially common in premature infants and has been referred to as *osteopenia* and *rickets of prematurity*. The forms of MBD manifesting themselves in infants and children are listed in Box 83.3.

The following are terms commonly used in describing MBD:

- *Osteopenia* is generally defined as diminished bone mineral density secondary to a reduction in the thickness of bone cortex and trabeculae.

• BOX 83.3 Forms of Metabolic Bone Disease Manifesting Themselves in Newborns and Infants

Phosphate Deficiency
- Osteopathy of prematurity
- X-linked hypophosphatemic rickets
- Fanconi syndrome
- Antacid-induced osteopathy (aluminum hydroxide)
- Tumor (including hemangioma)-associated rickets

Calcium Deficiency
- Osteopathy of prematurity
- Inadequate intake of dietary calcium after weaning
- Inadequate calcium in total parenteral nutrition solution

Vitamin D Deficiency
- Maternal vitamin D deficiency (congenital rickets)
- Inadequate intake of dietary vitamin D
- Lack of adequate sunlight exposure and dietary inadequacy

Vitamin D Malabsorption
- Hepatic disease (steatorrhea)
- Short bowel syndrome
- Pancreatic insufficiency

Vitamin D Metabolic Defects
- Vitamin D 25-hydroxylation defect
- 1α-Hydroxylase deficiency
- Renal insufficiency (renal osteodystrophy)
- Anticonvulsants (increased 25(OH)D metabolism)

Vitamin D Receptor Defects
- Hereditary vitamin D resistance

Genetic/Syndromic Causes
- Osteogenesis imperfecta
- Hypophosphatasia
- Osteopetrosis

- *Osteoporosis* is defined in pediatrics as a reduction in bone mineral density with clinically significant fractures.[133] Unlike in rickets and osteomalacia, in which mineralization defects predominate, the primary abnormality in osteoporosis is either a decrease in matrix formation or an increase in matrix and mineral resorption.
- *Rickets* results from undermineralization of the bone matrix, or osteoid, in growing bone at the growth plate (physis). *Osteomalacia* is the undermineralization of established trabecular and cortical bone. Growing children can have rickets and osteomalacia concurrently; however, osteomalacia occurs after the growth plates have fused. In infancy, the most rapidly growing bones are the skull, upper limbs, and ribs. Therefore early development of rickets leads to craniotabes, characterized by softening of the skull with the "ping-pong ball" sign; widened cranial sutures; frontal bossing; swollen epiphyses of wrists; costochondral beading manifesting itself as a "rachitic rosary"; and Harrison sulcus, which is caused by diaphragmatic depression of the lower thorax on inspiration. Manifestations of muscle weakness may include dilated cardiomyopathy and ventricular dysfunction, which may respond to vitamin D therapy.[134,135] Radiographic features in rickets result from an expansion of the cartilaginous growth plate and delayed mineralization. They include lucency and widening of the gap between metaphysis and epiphysis, known as the *zone of provisional calcification*, as well as irregularity, cupping, or fraying of the metaphyseal margin. Serum phosphorus or calcium level or both are characteristically depressed, and serum ALP level is elevated. An exception is in the hyperphosphatemia of renal osteodystrophy.

Metabolic Bone Disease of Prematurity

MBD of prematurity is caused chiefly by deficiencies in dietary phosphate and calcium. Eighty percent of bone mineralization in the fetus occurs during the third trimester,[136,137] when fetal calcium and phosphorus requirements are 100 to 130 mg/kg/day and 60 to 75 mg/kg/day, respectively.

Preterm infants miss this critical period of in utero bone development and need fortified breastmilk or fortified formulas as standard infant formulas do not meet the needs for bone mineralization of preterm infants. Nevertheless, despite this improved nutritional practice, 10% to 20% of infants with birthweight <1000 g have radiographic evidence of rickets.[138] Intestinal immaturity of pre-term infants can limit the absorption of calcium and phosphorus from the diet. Many of these infants require prolonged parenteral nutrition, which may be limited in calcium and phosphate content.[139,140]

Additional, nonnutritional risk factors for MBD in ill preterm infants include the early withdrawal of placental estradiol and progesterone, lack of mobility, necrotizing enterocolitis, and therapy with a variety of medications, including dexamethasone, loop diuretics, and methylxanthines (Box 83.4), which can increase urinary calcium excretion and contribute to serum mineral imbalance, nephrocalcinosis, and osteopenia.[140] Glucocorticoids decrease bone formation by inhibiting osteoblast growth and increasing cell death of osteoblasts and osteocytes and, at least over several months, increasing osteoclastogenesis and bone resorption. Copper deficiency is a rare contributor to osteopenia in low birthweight preterm infants.[141]

• BOX 83.4 Medications Associated With Metabolic Bone Disease in Preterm Infants

Glucocorticoids
Furosemide/loop diuretics
Methylxanthines
Anticonvulsants: phenytoin and phenobarbital
Antacids: proton pump inhibitors and H_2 blockers
Anticoagulants
Cyclosporine

In preterm infants, osteopenia with or without rachitic changes at the cartilage-shaft junction usually appears between 6 and 16 weeks of age. Fractures of the ribs and long bones are reported in 10% of preterm infants, frequently presenting with pain on handling.[142] The incidence and severity increase with decreasing gestational age and birthweight, and they are more common in preterm infants with a complicated medical course and delayed nutrition. In VLBW babies, postnatal bone mineralization significantly lags behind the expected intrauterine bone mineralization rate. Gestational age and birthweight are the strongest risk factors for MBD.

Clinical Presentation

The clinical findings in VLBW infants with severe osteopathy, as in older, term infants with rickets, include a widened anterior fontanel, craniotabes, bony expansion of wrists, costochondral beading, and rib or long-bone fractures. Rib undermineralization, softening, and fractures can lead to respiratory distress (especially tachypnea), atelectasis, or pneumonia. Long-term effects of osteopathy of prematurity may include reduced height[143] and bone mineral density in adulthood,[144] although other studies have shown more contradictory results.

Pathophysiology

Phosphate depletion and osteopathy occur in rapidly growing preterm infants fed unsupplemented human milk, which has low phosphate content. Characteristically, these infants develop hypophosphatemia, hypophosphaturia, hypercalcemia, and hypercalciuria. Serum PTH level may be low or normal, 25(OH)D level is normal, and $1,25(OH)_2D$ level is elevated. The hypophosphatemia stimulates the production of $1,25(OH)_2D$, which in turn increases intestinal calcium absorption. However, in the presence of hypophosphatemia, only limited amounts of calcium can be deposited in bone, leading to hypercalcemia and hypercalciuria. The hypercalcemia inhibits PTH secretion.

MBD of prematurity does not respond to vitamin D therapy unless vitamin D deficiency also is present. Vitamin D supplementation without prior correction of the underlying dietary phosphate deficiency may aggravate hypercalcemia and hypercalciuria by enhancing intestinal calcium absorption. The bone disease in these infants responds to increased dietary phosphate, accomplished by the addition of a human milk supplement designed for preterm infants or by the switching to a preterm milk formula; both diets provide additional calcium and phosphorus. The recommendations for enteral nutrition for VLBW infants for phosphate and calcium range from 75 to 140 mg/kg/day and from 150 to 220 mg/kg/day, respectively.[138] The addition of 10 to 20 mg/kg/day of elemental phosphorus may also be required to replete deficient serum phosphorus levels. However, because phosphate

repletion promotes bone mineralization, serum calcium levels may fall to subnormal levels ("hungry bones" syndrome) unless supplemental calcium (e.g., 20 to 60 mg/kg/day elemental calcium) also is provided. The recommended intakes of calcium and phosphorus[145] have benefits of improved bone growth, less severe dolichocephaly, and avoidance of fractures. For infants with a history of osteopathy of prematurity, it is important that after hospital discharge, a mineral-enriched diet and serial laboratory monitoring be maintained for several weeks to months.

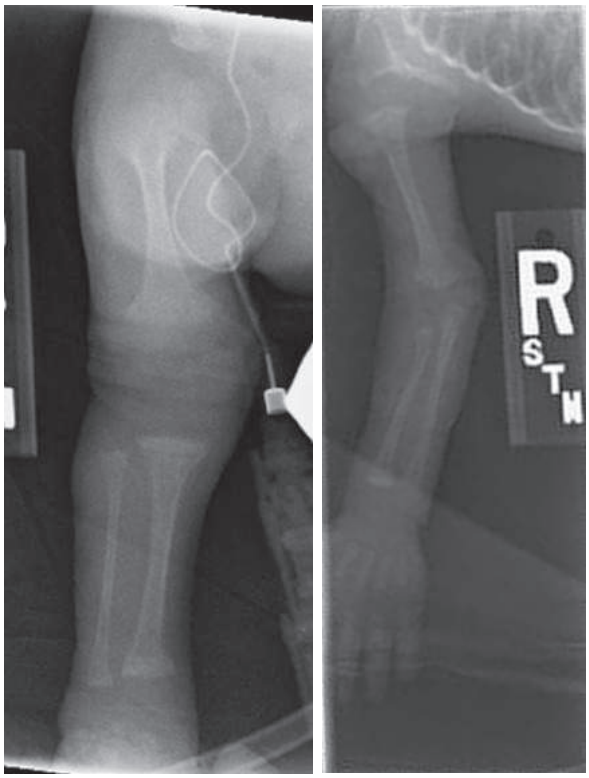
Most often, neither hyperparathyroidism nor vitamin D deficiency is present in phosphate-deficient osteopathy of prematurity. In contrast, the pathogenesis of calcium-deficiency rickets and vitamin D deficiency is similar in that hypocalcemia causes hyperparathyroidism. The elevated PTH level increases bone resorption and enhances renal $1,25(OH)_2D$ synthesis, increasing intestinal calcium and phosphorus absorption. Individual preterm babies may have predominant phosphate depletion, but mixed phosphate and calcium deficiency is more common; isolated calcium deficiency is rare. In dual-mineral deficiency, laboratory tests may show low, normal, or slightly elevated serum calcium levels and low to low-normal phosphorus levels. In cases of severe or complicated bone disease, serum 25(OH)D is a useful screen for evaluation of the sufficiency of vitamin D stores; levels less than 6 ng/mL indicate *severe* vitamin D deficiency, and levels of at least 20 ng/mL are required for sufficiency.[146]

For evaluation of bone mineral status in preterm neonates, measurement of cortical bone thickness and visual inspection of the proximal part of the humerus on a chest radiograph are a common screen for bone disease. Additional images may be required to fully assess the neonate for MBD (Fig. 83.2). However, the appearance of "osteopenia" may not be appreciated on plain film until a 20% to 40% decrease in mineralization is reached.[147] For that reason, assessment of bone mineral content by dual x-ray absorptiometry (DXA) may be used for its accuracy, reproducibility, and low radiation exposure. Normative data remain somewhat limited in preterm and former preterm infants, decreasing DXA's utility.[138] Longitudinal quantitative ultrasound measurement of the speed of sound in long bones, combined with measurement of serum bone markers, is a promising assessment tool[148] although not one commonly used clinically at this time. The use of quantitative computed tomography to assess true volumetric bone density is limited by its significantly higher radiation exposure and lack of normative data in infants.

Evaluation

Serum ALP, a marker of osteoblastic bone formation, is frequently used to monitor skeletal metabolism in preterm infants. However, the magnitude of elevations in ALP concentrations is not a good predictor of the extent of bone mineral deficits. As elevations of ALP concentrations and clinical MBD are rare in the first 4 weeks of life, the American Academy of Pediatrics Committee on Nutrition recommends ALP and serum phosphorus concentrations be measured at 4 to 6 weeks after birth in VLBW infants and biweekly thereafter. The ALP concentration usually peaks at 400 to 800 IU/L and then decreases in VLBW infants who do not develop MBD.[138]

The use of PTH has been suggested as an early marker of MBD in preterm infants.[149] Moreira et al. reported that a PTH level greater than 180 mg/dL at 3 weeks' chronologic age should alert clinicians to the possibility of MBD. Serial urinary biomarkers of bone metabolism (e.g., pyridinoline, deoxypyridinoline) have not yet been shown to be useful in predicting severe osteopathy because the levels are related to bone volume and normative data for growing preterm infants are lacking.



• **Fig. 83.2** The long bones in this 6-week-old ex-26-week infant demonstrate severe generalized osteopenia. The zones of provisional calcification are frayed and very irregular. The metaphyses are irregular, showing cupping and fraying, particularly prominent at the knees and distal part of the femurs.

Vitamin D–Deficiency Rickets

Vitamin D–deficiency rickets most often occurs in exclusively breastfed infants who also have little exposure to sunlight and are dark skinned. Unfortified human milk has a vitamin D activity ranging from 5 to 80 IU/L,[150–152] which may be insufficient for maintaining normal 25(OH)D levels in infants. Historically, a marked rise in the prevalence of nutritional rickets has accompanied industrialization and urban crowding. Clinical rickets often manifests itself at 3 months of age or later, but onset in early infancy is not uncommon. In developed countries, nutritional rickets has never been eradicated, and there is a resurgence of vitamin D deficiency in North America and Europe.[153] Maternal vitamin D deficiency during pregnancy and lactation puts the newborn at high risk. The deleterious effects of vitamin D deficiency in the mother may impact the musculoskeletal health of the offspring into adolescence.[154]

The 2008 American Academy of Pediatrics statement on prevention of rickets and vitamin D deficiency[155] doubles the previous American Academy of Pediatrics vitamin D intake recommendation. These guidelines state that breastfed and partially breastfed infants should receive supplementation with 400 IU vitamin D per day beginning in the first few days of life. All infant formulas available in the United States must contain

at least 400 IU/L vitamin D. Consequently, the guidelines also state all nonbreastfed infants who are ingesting less than 1 L of formula per day should receive a vitamin D supplement of 400 IU/day. In 2013 the American Academy of Pediatrics Committee on Nutrition recommended that VLBW infants receive 200 to 400 IU vitamin D per day. This can be increased to 400 IU/day when the weight exceeds 1500 to 2000 g, and the infant is tolerating full enteral nutrition.[138] European guidelines suggest higher intakes of vitamin D of 800 to 1000 IU/day may be used for VLBW infants.[156]

Higher doses of vitamin D may be necessary for infants with fat malabsorption or those taking anticonvulsant medications. Vitamin D status should be assessed using 25(OH)D and PTH concentrations and measures of bone mineral status.[155] Stoss therapy, which occasionally is used to treat rickets, consists of a single large oral or intramuscular dose (150,000 to 300,000 IU) of vitamin D.[157]

Nutritional rickets worldwide is due to degrees of vitamin D deficiency and calcium deficiency. Although vitamin D deficiency is thought to be the most common cause of nutritional rickets worldwide, calcium deficiency is the major cause of rickets in developing countries where dairy product consumption is low and is also recognized in other parts of the world.[158,159] In tropical populations, rickets may occur later than at higher latitudes, between 1 and 2 years of age, after weaning, and with the introduction of a low-calcium diet. One study showed, for example, that in Turkey, most rachitic children had vitamin D deficiency, whereas in Egypt, they mainly had calcium insufficiency combined with vitamin D deficiency.[160] It is likely that relative deficiencies of calcium and vitamin D interact with genetic (e.g., VDR genotypes) and/or environmental factors to stimulate the development of rickets. An international consensus statement provides guidelines for the prevention, diagnosis, and treatment of nutritional rickets due to both calcium and vitamin D deficiency.[158]

Congenital rickets, defined as biochemical and radiographic signs of rickets within the first 4 weeks of life, should always prompt an investigation for maternal vitamin D deficiency. Mothers of babies with congenital rickets typically have not taken supplemental vitamin D and are thus at risk for osteomalacia, severe vitamin D deficiency, and hypocalcemia.[158]

Renal Osteodystrophy

Because normal renal function is essential for physiologic mineral and bone metabolism, renal insufficiency induces hyperphosphatemia and bone disease. Renal osteodystrophy can be predominantly high or low bone turnover, or the two types may alternate during the clinical course in an individual infant.[161] High bone turnover or osteitis fibrosa is a manifestation of secondary hyperparathyroidism. Parathyroid hyperfunction often occurs early in the course of renal failure. Contributing factors include phosphate retention, impaired renal $1,25(OH)_2D$ synthesis, hypocalcemia, parathyroid gland hyperplasia, and skeletal resistance to PTH actions. Low-turnover osteodystrophy (adynamic bone or osteomalacia) results from a suppressed bone formation and is a major concern in the treatment of dialyzed infants.

A principal goal of therapy is to lower serum phosphate levels to prevent hypocalcemia and severe hyperparathyroidism.[162] Phosphate restriction is accomplished by the feeding of breast milk or Similac PM 60/40. Oral phosphate binders such as calcium carbonate or sevelamer may be needed to reduce the intestinal absorption of phosphate. Hypocalcemia and metabolic acidosis should be treated with appropriate supplements. If serum $1,25(OH)_2D$ level is low, $1,25(OH)_2D$ therapy will increase intestinal calcium absorption, transcriptionally suppress PTH gene expression, and decrease parathyroid hyperplasia. Serum calcium, phosphorus, and PTH levels, as well as linear growth and bone radiographs, should be serially monitored.[163] Management of severe renal osteodystrophy in neonates is particularly complicated by increased phosphate requirements for growth. Calcium supplementation and use of potent vitamin D metabolites may also produce an "oversuppression" of PTH, contributing to adynamic bone disease. As with any complex disorder, effective clinical management requires close monitoring and an integrated team approach.

Inherited Metabolic Bone Disease in Infancy

Several forms of inherited MBD or rickets have been described that can present in newborns and infants. Vitamin D–dependent rickets, type 1A (VDDR-1A), is the most prevalent form of hereditary vitamin D resistant rickets. It is caused by an autosomal recessive loss of function mutation in the gene encoding 25(OH)D 1α-hydroxylase *(CYP27B1)*[164] and is associated with the defective conversion of 25(OH)D to $1,25(OH)_2D$. Muscle weakness with severe hypocalcemia and secondary hyperparathyroidism appear shortly after birth, and rickets presents within the first year of life. Intrauterine development is not impacted as normal maternal calcium and $1,25(OH)_2D$ levels provide a sufficient calcium supply. Patients with this condition tend to have undetectable $1,25(OH)_2D$ levels. Treatment with 1α-hydroxylated vitamin D analogs induces remission.

Vitamin D–dependent rickets, type 1B (VDDR-1B) is a rare disorder with a milder phenotype than VDDR-1A. It is caused by a recessive loss of function mutation in the CYPR1 gene encoding 25-hydroxylase. This presents later in childhood (between 2 and 7 years of age) with rickets and low $25\text{-}(OH)D_3$ despite normal vitamin D intake.[164] Preparations containing $25\text{-}(OH)D_3$ (calcidiol and calcifediol) are considered the therapeutic option of choice for VDDR-1B.

Hereditary resistance to vitamin D, formerly known as *vitamin D–dependent rickets type 2*, is caused by mutations in the VDR gene. This mutation leads to end-organ resistance to $1,25(OH)_2D$. Affected infants show early-onset rickets, hypocalcemia, elevated serum $1,25(OH)_2D$ levels, secondary hyperparathyroidism, and alopecia. Depending on the genotype, there is a variable response to supraphysiologic doses of $1,25(OH)_2D$ analogues and calcium.[165]

X-linked dominant hypophosphatemia (XLH), also known as *familial hypophosphatemic rickets*, is a disorder of phosphate homeostasis.[166] Its prevalence is 1 in 20,000. XLH is characterized by hypophosphatemia, rickets, and poor linear growth and is associated with an FGF23 mediated low tubular maximum reabsorption of phosphate (TmP) and renal tubular phosphate leak. Defective regulation of vitamin D metabolism results in inappropriately normal $1,25(OH)_2D$ concentrations in the face of hypophosphatemia. XLH is caused by mutations in the phosphate-regulating endopeptidase homolog, X-linked gene *(PHEX)*. Standard treatment consists of supplemental phosphate and calcitriol, with the goal of normalizing the ALP level, keeping the PTH level normal, maximizing linear growth, and reducing rachitic changes. More recently, burosumab, a fully human monoclonal antibody against

FGF23, has been approved for management of XLH in patients ages 6 months and older.[167]

There is a similar condition of autosomal dominant hypophosphatemic rickets caused by an activating mutation in the FGF23 gene *(FGF23)* on chromosome band 12p13. Treatment is similar to that for XLH with phosphate and calcitriol. Autosomal recessive hypophosphatemic rickets type 1 and autosomal recessive hypophosphatemic rickets type 2 are caused, respectively, by mutations in the dentin matrix acidic phosphoprotein 1 gene *(DMP1)* and the ectonucleotide pyrophosphatase/phosphodiesterase 1 gene *(ENPP1)*, which interfere with bone mineralization and renal phosphate handling.[166]

OI is a heterogeneous group of disorders that cause various degrees of bone fragility and skeletal deformity.[168] OI was originally identified as an autosomal dominantly inherited abnormality of type I collagen due to mutations of *COL1A1* and *COL1A2*. Four primary types were initially described. However, in the past several years, several other types have been identified, most of which have an autosomal recessive inheritance. The most severe forms present with fractures at or shortly after birth. The affected babies may also have deformities of long bones, osteopenia of the skull, and early death due to respiratory deficiency. Administration of a bisphosphonate such as pamidronate may help reduce bone fractures and bone pain.

Hypophosphatasia is characterized by defective bone mineralization secondary to deficiency of the tissue-nonspecific isoenzyme of ALP. It is caused by a mutation of the *ALPL* gene on chromosome band 1p36. Perinatal lethal, infantile, childhood, and adult-onset forms have all been described, with the most severe forms being the perinatal and infantile forms. Clinical manifestations in infants include osteomalacia and rickets with respiratory insufficiency due to progressive chest deformity. Hypercalcemia and hyperphosphatemia can be seen in severely affected infants. Enzyme replacement with recombinant ALP can improve survival, skeletal findings, and pulmonary and overall physical function in infants with the perinatal and infantile forms of hypophosphatasia.[169]

Neonatal bone fragility with increased bone density rather than osteopenia can occur in infantile osteopetrosis, a rare autosomal recessive disorder of osteoclast formation. Children may present with failure to thrive, skull abnormalities including frontal bossing, rachitic changes, hypocalcemia, and delayed dentition. This condition is usually fatal within the first 10 years of life unless treated with hematopoietic stem cell transplantation.[170]

Suggested Readings

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84

Disorders of the Adrenal Gland

PATRICIA Y. FECHNER

KEY POINTS

- Infants born with ambiguous genitalia or nonpalpable testes need to be evaluated for congenital adrenal hyperplasia as it can be life threatening.
- Adrenal steroid levels vary with gestational age.
- Adrenal insufficiency should be treated with hydrocortisone to avoid adrenal crisis. High doses of hydrocortisone contain mineralocorticoid activity.

The Adrenal Gland

Embryology

Normal adrenal function is critically important for maintenance of intrauterine homeostasis, promotion of organ maturation, and adaptation to extrauterine life. The dual embryologic origin of the human adrenal gland results in an outer adrenal cortex and an inner adrenal medulla; each part secretes different vital hormones critical to fetal development. Embryologically, the adrenal cortex develops from the coelomic mesoderm of the urogenital ridge, whereas the medulla arises from neural crest tissue in the adjacent sympathetic ganglion at celiac plexus level. During the fifth week of fetal development, mesothelial cells from the posterior abdominal wall between the root of the bowel mesentery and developing mesonephros proliferate and form the primitive adrenal cortex. In the sixth week, a second wave of mesothelial cells surrounds the primitive cortex and later forms the adult or definitive cortex. By 8 weeks of gestation, the cortical mass separates from the rest of mesothelial tissue and becomes surrounded by connective tissue.[1] This separation divides adrenocortical and gonadal primordium.[2] Chromaffin cells, which originate from neural crest, migrate toward the adrenal cortex around this time and gradually invade the medial aspect of the cortical tissue along its central vein to gain central position, forming the adrenal medulla. Fig. 84.1 summarizes the embryologic origin of the adrenal gland.[3] By 8 to 9 weeks of gestation, the adrenal gland is encapsulated and contains an outer "definitive" zone where glucocorticoids and mineralocorticoids are synthesized and a larger inner "fetal" zone where dehydroepiandrosterone (DHEA) and dehydroepiandrosterone sulfate (DHEAS) are produced and then subsequently converted to estriol by the placenta.

Postnatally, the fetal or primitive zone of the adrenal gland rapidly involutes to disappear by approximately 6 months of age.[4] Zonation of the cortex, zona glomerulosa, and fasciculata is present at birth, but full differentiation into three separate zones occurs much later at approximately 3 years of age when zona reticularis development takes place.[5] Fig. 84.2 depicts these changes in the adrenal gland from 9 weeks of gestation through childhood.

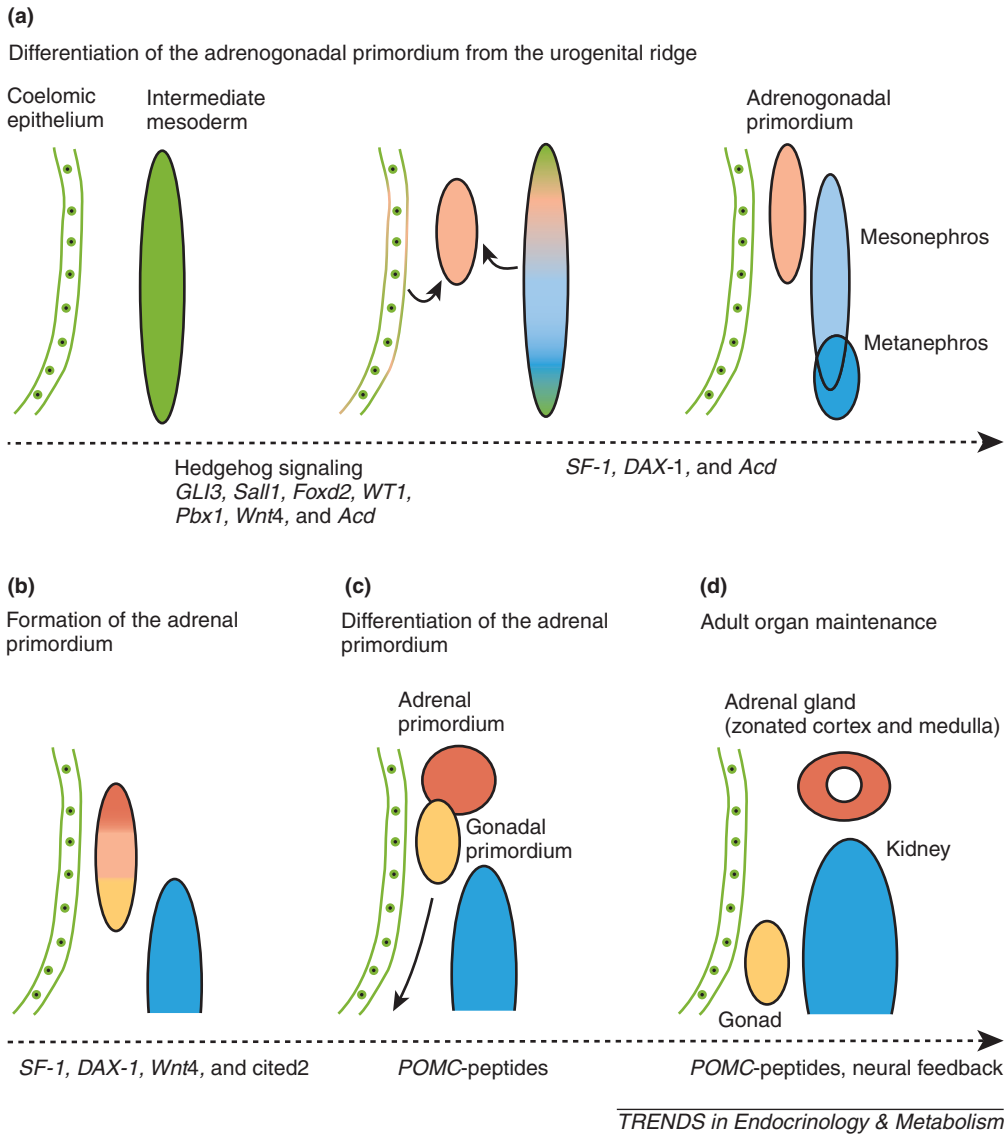
Morphology

The adrenal glands are bilateral structures located above the kidneys in the retroperitoneum area. At birth, the adrenals are approximately one-third the size of neonatal kidneys, weighing 8 to 9 g, and are 10- to 20-fold larger than the adult glands relative to body weight (0.4% vs. 0.01%).[6] In the third trimester and the first 3 months after birth, the glands predominantly consist of cortex, where active production of glucocorticoids, steroid precursors, estrogens, and progesterone takes place. Ultrasonographically, the neonatal adrenal gland characteristically has a thin reflective core surrounded by a thick transonic zone. The gland subsequently decreases in size as the active fetal cortex regresses to reach approximately 8% of the kidney size in adulthood.[5]

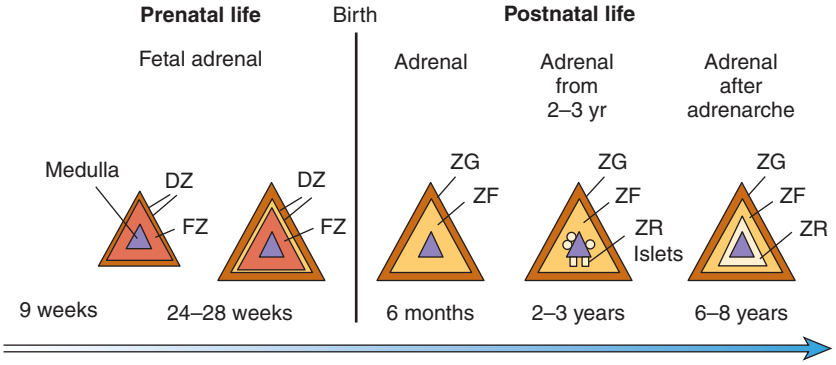
Histologically, the fetal adrenal cortex consists of a small outer definitive zone, which appears to produce few adrenal steroid hormones until late gestation, and a larger inner fetal zone that produces adrenal steroid hormones throughout gestation. In addition, there is a transitional zone where cortisol production takes place toward the end of fetal development.[2] At birth, the large fetal zone of the fetal adrenal involutes and disappears by 6 months of age. Concurrently, the definitive zone together with the transitional zone develops into the fully differentiated zona glomerulosa and fasciculata by the age of 3 years. The zona reticularis begins to develop only after 4 years of age and may not be fully differentiated before the age of 15 years. In an adult adrenal gland, these three distinctive zones lie adjacent to one another. The zona glomerulosa is located immediately below the capsule, the zona fasciculata is in the middle, and the zona reticularis is the innermost zone next to the medulla.

Adrenal Functions

A cascade of adrenal steroidogenesis in the adult is shown in Fig. 84.3. Three major pathways of mineralocorticoid, glucocorticoid, and androgen synthesis take place mainly in the glomerulosa, fasciculata, and reticularis zones of the cortex, respectively. Aldosterone is the main mineralocorticoid regulating sodium and fluid volume homeostasis. Aldosterone is under the control of the rennin–angiotensin system and blood potassium concentrations.[7] The principal glucocorticoid in humans is cortisol and has a wide range of roles in regulating body functions, from carbohydrate metabolism, immune system, and acute and chronic stress



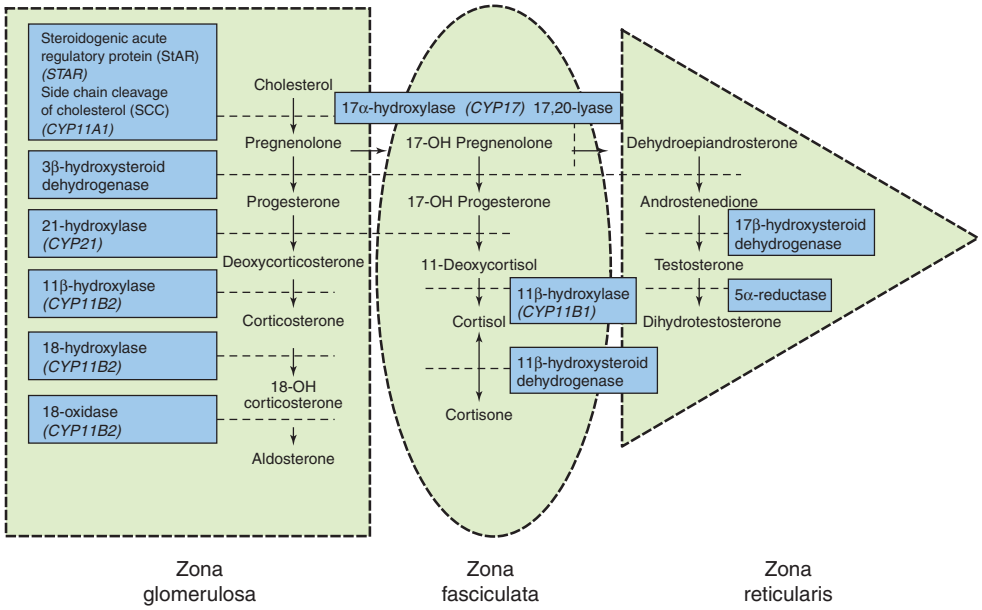
• **Fig. 84.1** Adrenocortical Development from Urogenital Ridge to Adrenal Gland. *DAX-1*, Dosage-sensitive sex reversal-adrenal hypoplasia congenita critical region on the X chromosome gene-1; *POMC*, proopiomelanocortin; *SF-1*, steroidogenic factor-1. (From Else T, Hammer GD. Genetic analysis of adrenal absence, agenesis and aplasia. *Trends Endocrinol Metab*. 2005;16:458–468.)



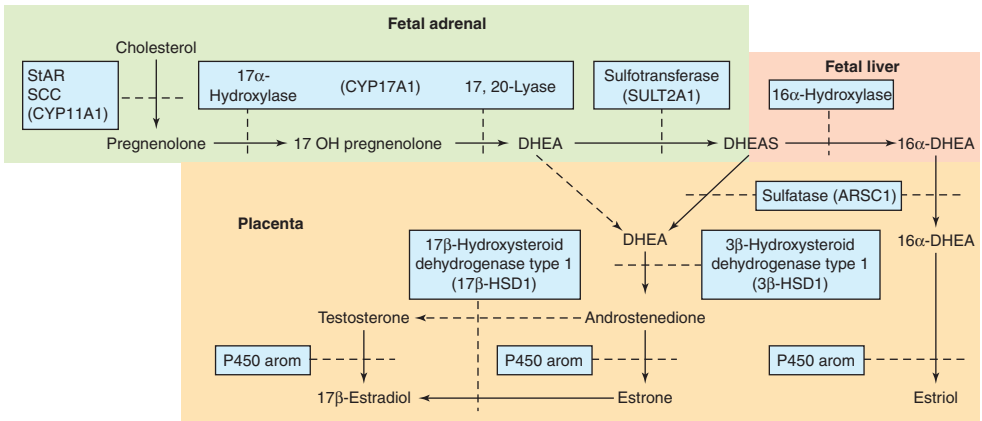
• **Fig. 84.2** Development of the Human Adrenal Cortex During Prenatal and Postnatal Life. *DZ*, Definitive zone; *FZ*, fetal zone; *ZG*, zona glomerulosa; *ZR*, zona reticularis; *ZF*, zona fasciculata. (Adapted from Stewart PM, Newell-Price JDC. The adrenal cortex. In: Melmed S, Polonsky KS, Larsen PR, et al. (eds). *Williams Textbook of Endocrinology*. 13th ed. Philadelphia: Elsevier; 2016: 489–555.)

response to musculoskeletal metabolism. Cortisol production is regulated through a negative feedback loop involving hypothalamic corticotropin-releasing hormone (CRH) and pituitary adrenocorticotropic hormone (ACTH).[8] Adrenal androgens have an age-specific secretion profile with an increase at adrenarche, which occurs 2 years before the time of puberty, and then a gradual decrease with aging until andropause.[9] The regulatory mechanism behind normal adrenal androgen production is largely unknown but involves ACTH to some extent.[10]

In the fetal adrenal gland, steroidogenic enzymes are found as early as 7 weeks' gestation.[10,11] At 8 weeks' gestation, the fetal adrenal gland produces cortisol under ACTH control. A transient expression of 3β-hydroxysteroid dehydrogenase type 2 (3β-HSD2) during this critical time from 7 to 12 weeks' gestation allows the fetal adrenal gland to produce cortisol. Activation of 3-HSD2 serves principally to prevent virilization of the female genital anlage that would otherwise result from overwhelming amounts of DHEAS and its downstream androgen metabolites. It is believed that a transient peak of cortisol during this time suppresses the fetal hypothalamic–pituitary–adrenal (HPA) axis, keeping DHEAS production at a low level.[11,12] By the end of the first trimester, cortisol secretion from the fetal adrenal gland begins to wane as a result of a decrease in 3β-HSD2 expression, thus decreasing HPA axis suppression with resultant increased DHEAS secretion. During the second and third trimesters, the fetal adrenal gland secretes abundant amounts of DHEA and its sulfated derivative DHEAS, earning the term *androgen factory*.[4] The rate of steroid secretion by the fetal adrenal glands may be fivefold that of the adult adrenal glands at rest.[13] The placenta can also convert DHEAS back to DHEA by a sulfatase enzyme (i.e., arylsulfatase). These adrenal steroids serve as precursors for 17,20 lyase activity of the P450C17 enzyme for androgen production and subsequent estrogen production (Fig. 84.4). In addition, DHEAS is oxidized in the fetal liver to a 16α-hydroxylated derivative, which



• **Fig. 84.3** Steroidogenesis of the Adult Adrenal Gland. Each of the three biosynthetic pathways takes place in different zones: aldosterone biosynthesis in the zona glomerulosa, cortisol biosynthesis in the zona fasciculata, and androgen production in the zona reticularis. The enzymes that catalyze the reactions are indicated in *boxes*.



• **Fig. 84.4** Steroidogenesis of the Fetal Adrenal Gland and Fetoplacental Unit. Predominant dehydroepiandrosterone and dehydroepiandrosterone sulfate production occurs after 12 weeks' gestation. *DHEA*, Dehydroepiandrosterone; *DHEAS*, dehydroepiandrosterone sulfate; *SCC*, side chain cleavage of cholesterol; *StAR*, steroidogenic acute regulatory protein.

is converted by the placenta to estriol by the same set of enzymes as in estradiol synthesis.

The physiology of human pregnancy involves a continuous supply of relatively increased amount of estrogens. In near-term human pregnancy, the rate of estrogen production increases strikingly, reaching concentrations 1000-fold greater than that of nonpregnant women.[13] During early gestation, the estradiol required to maintain pregnancy is provided by the corpus luteum of the maternal ovary. But after 8 weeks' gestation, the fetoplacental unit synthesizes most of the estradiol required to maintain pregnancy.[14]

DHEAS is the main steroid secreted by the fetal adrenal cortex from mid-gestation onward. The activity of 3β-HSD2 controls fetal cortisol synthesis. By the end of pregnancy, fetal cortisol is required in preparation for parturition (i.e., lung maturation or surfactant production) and could have a role in triggering parturition, as shown in other species.[15] Maternal cortisol cannot normally reach the fetus because it is oxidized to cortisone, an inactive steroid, by placental 11β-hydroxysteroid dehydrogenase type 2.[16] When pregnancy approaches term, 3β-HSD2 expression increases again and remains high, allowing increased cortisol secretion. In a child, 3β-HSD2 secretion is high in the adrenal gland until adrenarche, when 3β-HSD2 activity decreases again to allow an increase in DHEA[17] and its downstream androgen metabolite secretion, which gives rise to the development of pubic and axillary hair.

Control of Glucocorticoid and Mineralocorticoid Production

Two distinct regulatory circuits control adrenal glucocorticoid and mineralocorticoid secretion. The HPA axis determines the set point for circulating glucocorticoid (cortisol) concentration. The neuropeptide CRH (or factor) and arginine vasopressin (AVP) are synthesized in the hypothalamic paraventricular nucleus and released into the hypophysial portal circulation at the median eminence in response to stress[18] and, beginning at approximately 6 months of age, to circadian cues.[19] These neuropeptides stimulate the release of ACTH from the anterior pituitary corticotrophs. CRH is the primary stimulator of ACTH, while AVP amplifies the effect of CRH. ACTH released into the systemic circulation augments adrenocortical secretion of cortisol and DHEA by acting on the ACTH receptor,[20] a member of the melanocortin receptor family. The ACTH receptor is present on steroidogenic cells of the fetal zone and transitional zone of the fetal adrenal as well as the adult adrenal cortex. The resulting increase in plasma cortisol concentration limits further the release of hypothalamic neuropeptides and ACTH by negative feedback through glucocorticoid receptors at the central nervous system and pituitary sites. As a corollary, if glucocorticoid production is impaired by intrinsic adrenal dysfunction, then neuropeptide CRH and ACTH release are augmented.

The components of the HPA axis are present early in human development.[2] As detailed earlier, the fetal adrenal gland begins to develop at 4 weeks' gestation, when initial evagination of the pituitary primordium occurs.[21] ACTH-producing pituitary cells can be detected at 7 weeks' gestation, and an intact hypophysial portal vascular system is present by 12 weeks' gestation.[22,23] Nerve terminals containing CRH can be detected in the hypothalamus by approximately 16 weeks' gestation. Virilization caused by increased production of adrenal androgens in females with congenital adrenal hyperplasia (CAH) occurs before 12 weeks' gestation; therefore, ACTH-producing corticotrophs must undergo cortisol-mediated feedback modulation at the initial stages of hypothalamic–pituitary development.[12]

Mineralocorticoid (aldosterone) release by the zona glomerulosa of the adrenal cortex is determined by the renin-angiotensin system with acute modulation to a lower extent by ACTH as well.[24] Decreases in vascular volume result in increased secretion of renin by the renal juxtaglomerular apparatus. Renin, a proteolytic enzyme, cleaves angiotensinogen to angiotensin I, a biologically inactive decapeptide. Angiotensin I is then cleaved and activated by angiotensin-converting enzyme in the lung and other peripheral sites to angiotensin II. Angiotensin II and its metabolite angiotensin III possess vasopressor and potent aldosterone secretory activity. Although angiotensin II receptors are present on cells of the definitive zone at 16 weeks' gestation, significant aldosterone production by the fetal adrenal gland does not occur until the third trimester of pregnancy.[2]

Molecular Basis of Adrenal Development

Several transcription factors are critically important for normal adrenal development. Two related transcription factors have emerged as key regulators of adrenal development: the nuclear receptor DAX-1 (dosage-sensitive sex reversal-adrenal hypoplasia congenita [AHC] critical region on the X chromosome gene-1, encoded by *NROB1*/*AHC*) and steroidogenic factor-1 (SF-1, encoded by *NR5A1*, also known as AD4BP). Sf-1 (*Nr5a1*) knockout mice lack adrenal glands and gonads, and subsequent identification of *NR5A1* mutations in humans with adrenal insufficiency confirms the essential role of SF-1 in development.[25] *Nr5a*1 expression is found in the early urogenital ridge of the mouse in cells that give rise to both the bipotential gonad and adrenal cortex. Expression of *Nr5a1* remains high throughout embryogenesis, the postnatal period, and adult life.[25]

DAX-1 is an orphan nuclear receptor that colocalizes with SF-1 in the cells of adrenal glands, gonads, gonadotropes, and ventral–medial lateral nucleus of the hypothalamus. Deletion of *NROB1* results in AHC. Although the exact role of DAX-1 in adrenal development is not known, it has been shown to interact with SF-1.[26] Normally, DAX-1 recruits the nuclear corepressor N-CoR to SF-1 and represses SF-1.[27] Similarly, the Wilms tumor suppressor gene (*WT1*) protein has been shown to interact with SF-1. *WT1* encodes 24 different protein isoforms that act as transcription factors. *Wt1* is detected in the urogenital ridge of the mouse embryo but is not detected in adult or fetal adrenals. Mutations in *Wt1* have resulted in abnormal development of the adrenal in the mouse but have not been clearly correlated with abnormal human adrenal development.[28] *WT1* has been found though to be expressed in the fetal adrenal gland.[29]

Assessing Adrenal Function in the Newborn

The adrenal cortex plays a major role in the newborn postnatal adaptation, and significant evolution of adrenal steroid production occurs over the first days and months of life. During interpretation of the newborn adrenal steroidogenic function, special attention must be paid to age-related changes in adrenal steroid intermediates, circulating cortisol, and aldosterone concentrations that reflect ongoing adrenal maturation.[30–32] For example, until at least 1 month postnatally, a large proportion of cortisol and its metabolites is excreted as sulfate esters. This sulfation may serve to

inactivate a number of circulating cortisol metabolites during fetal and neonatal life.[33]

Immediately after birth the third trimester fetal zone that once was the predominant component of the adrenal cortex in the fetus and preferentially produced DHEA and DHEAS starts to reduce in size. This rapid loss of the fetal zone during this period results in a dramatic fall of the circulating DHEA concentration over the first week to 1 month postnatally. The variable pattern of decline in the ensuing weeks probably reflects variation in remodeling of the fetal zone and emergence of the zona fasciculata of the definitive zone, the latter being a feature of an adult cortex. In addition to diminished 3β-HSD2 activity, preterm infants have sustained elevations in 17-hydroxyprogesterone and the 17-hydroxyprogesterone-to-cortisol ratio, suggesting a reduction in 21-hydroxylase activity.[34,35] Because blood-spot 17-hydroxyprogesterone concentration is used for newborn screening of CAH in many states,[36] many preterm infants initially have an abnormal test result. Subsequent follow-up testing is then required to determine whether CAH is present. Plasma aldosterone concentrations tend to be higher in preterm infants than in term infants, both of which in turn are higher than in older children and adults.[37,38]

Cortisol has a critical role in maintaining homeostasis in response to stress. Relative adrenal insufficiency occurs when the HPA axis produces less than adequate cortisol for the degree of illness or stress. Immaturity of the adrenal gland and the HPA axis of the premature newborn infant suggest a rationale for why preterm infants are at increased risk of cortisol insufficiency.[39] Clinicians are commonly faced with critically ill infants who have cardiovascular insufficiency with hypotension, a condition that has been associated with adverse consequences. The question often arises as to whether these manifestations reflect underlying glucocorticoid insufficiency. There is increasing evidence that *relative* adrenal insufficiency may be a cause of hemodynamic instability and hypotension in the critically ill newborn, but there is definitely a paucity of data in this population.

Random plasma cortisol measurement is often inadequate to answer this question because the majority of critically ill newborns have low cortisol and ACTH values without the expected increase in response to critical illness. Infants also do not exhibit diurnal variation of ACTH and cortisol until 6 months of age. Thus, obtaining two or more samples of cortisol may be informative. Response to exogenous ACTH (cosyntropin) is usually normal, suggesting that the inadequate response to critical illness in these newborns does not result from adrenal dysfunction but arises from some other components of the HPA axis.[40] Interestingly, in extremely low birthweight infants (500 to 999 g) low cortisol concentrations were not predictive of adverse short-term mortality and morbidity. In contrast, high basal cortisol levels were associated with severe intraventricular hemorrhage, and extremely elevated values were associated with morbidity and death.[41]

Data associating treatment of adrenal insufficiency with outcomes in the term newborn are limited, and there have been no studies on outcomes beyond the immediate neonatal period. Nonetheless, no adverse events have been attributed to glucocorticoid treatment based on a relatively small number of study subjects. Currently there is insufficient evidence to support the routine use of glucocorticoids in critically ill newborns. On encountering an infant with vasopressor-resistant hypotension accompanied by signs of cardiac hypofunction, the clinician must consider the risk-to-benefit ratio before arriving at the appropriate management. Therapeutic trials with hydrocortisone at the dose of 1 mg/kg of body weight have been suggested[39] and can be discontinued if there is no clinical improvement or if the pretreatment cortisol level is later observed to be greater than 15 μg/dL. A meta-analysis of the use of hydrocortisone for hypotension and vasopressor dependence in preterm infants showed improvement in blood pressure and less need for vasopressor, but the clinical benefit is unknown, and long-term effects of hydrocortisone use are not known.[42] Special attention should be paid to the premature newborn concurrently receiving indomethacin because the combination of hydrocortisone and indomethacin is associated with spontaneous gastrointestinal perforation.[43]

Primary Adrenal Disorders

Steroidogenic Defects Caused by Adrenal Enzyme Deficiency

CAH refers to a family of inherited adrenal gland disorders in which defects occur in one of the enzymatic steps required to synthesize cortisol from cholesterol; therefore, impaired cortisol synthesis is the cornerstone shared by all forms of CAH. The pathway of steroidogenesis in the adrenal cortex is illustrated in Fig. 84.3. Five forms of CAH with autosomal recessive mode of inheritance are summarized in this section.

Disorders That Lead to Virilization in Females

21-Hydroxylase Deficiency

Epidemiology

21-Hydroxylase deficiency (21-OHD) is responsible for 90% to 95% of all CAH cases. Newborn screening has demonstrated an overall incidence of 1:15,000 live births for the classic form of 21-OHD.[44–46] The incidence of classic CAH in either homogeneous or heterogeneous general populations is as high as 1 case per 7500 live births.[47]

Pathophysiology

In 21-hydroxylase deficiency (21-OHD), the conversion of 17-hydroxyprogesterone (17-OHP), the main substrate of the 21-hydroxylase enzyme, to 11-deoxycortisol in the pathway of cortisol synthesis is impaired, and precursors are shunted through the androgen pathway. The enzyme defect also impairs the conversion of progesterone to aldosterone, causing abnormal salt loss.[48,49] There are two forms of classic 21-OHD: (1) simple virilizing and (2) salt-wasting. The forms are distinguished by the adrenal gland's ability to produce adequate aldosterone. In both forms, severe 21-OHD results in elevated levels of adrenal androgens that cause ambiguous genitalia in the genetic female fetus.

Diagnosis of 21-OHD is made by the detection of extremely high concentrations of 17-OHP. In some cases, a stimulated 17-OHP level is checked after performing an ACTH stimulation test. Table 84.1 lists steroid levels at baseline and following 250 mcg of ACTH given as an intravenous (IV) bolus in infants at various ages. Cosyntropin (synthetic ACTH) IV or IM is dosed at 15 mcg/kg up to 125 mcg in children less than 2 years and at 250 mcg for children greater than 2 years. Samples should be drawn at baseline and 60 minutes. The diagnosis is confirmed by molecular genetic analysis of the *CYP21A2* gene.

Hormonally and clinically defined forms of 21-OHD CAH are associated with distinct genotypes characterized by varying enzyme activity demonstrated by in vitro expression studies.

TABLE 84.1 Adrenal Steroid Levels in Response to Adrenocorticotropic Hormone for Various Ages

Steroid		Premature 26–28 Weeks' Gestation	Premature 34–36 Weeks' Gestation	1–6 Months	<1 Year
Androstenedione ng/dL	Baseline	92–892	90–837	6–78	6–78
	Stimulated	145–1248	183–1367	21–114	21–139
	Change	40–718	13–1084	9–76	10–75
Cortisol mcg/dL	Baseline	1–11	3–34	3–22	3–23
	Stimulated	6–52	16–76	27–50	32–60
	Change	4–41	6–44	19–41	17–40
Deoxycorticosterone ng/dL	Baseline	20–105	28–78	7–48	7–57
	Stimulated	44–320	28–95	40–158	20–157
	Change	17–215	1–67	13–144	26–110
11-Deoxycortisol ng/dL	Baseline	110–1376	70–455	10–200	10–200
	Stimulated	206–2504	81–645	101–392	80–390
	Change	15–1128	40–190	5–366	5–350
17-OH progesterone ng/dL	Baseline	124–841	186–472	13–173	11–173
	Stimulated	285–1310	334–1725	85–250	85–466
	Change	50–596	18–1253	52–193	50–275

Data was obtained by extraction, chromatography, and RIA (radioimmunoassay) method and may not be applicable to other methods. These values should be treated as approximate and not exact cutoffs. In the preterm infants, testing was done on postnatal days 2 to 4. Adapted with permission from Nakamoto JM, Mason PW (eds). *The Quest Diagnostics Manual. Endocrinology.* 5th ed. Madison, NJ: Quest Diagnostics Incorporated; 2012.

The gene encoding 21-hydroxylase is a microsomal cytochrome P450, family 21, subfamily A, polypeptide 21 (*CYP21A2*) located on the short arm of chromosome 6 within the human leukocyte antigen complex.[50,51] *CYP21A2* and its homolog pseudogene *CYP21A1P* alternate with two genes, *C4B* and *C4A*, which encode two isoforms of the fourth component of the serum complement system.[52] More than 100 mutations have been described to date. These mutations include point mutations, small deletions, small insertions, and complex rearrangements of the gene.[53] The most common mutations appear to be the result of two types of meiotic recombination between *CYP21A2* and *CYP21A1P*: (1) misalignment and unequal crossing over, resulting in large-scale DNA deletions, and (2) apparent gene conversion events that result in the transfer to *CYP21A2* of smaller-scale deleterious mutations present in *CYP21A1P*. It is not always possible to accurately predict the phenotype on the basis of the genotype; such predictions have been shown to be 79% to 88% accurate[54–56] with some non-concordance. Studies have demonstrated that there is often a divergence in phenotypes within mutation-identical groups; the reason for this requires further investigation.[57,58]

Clinical Presentation

Females with simple virilizing CAH can be diagnosed at birth because of the apparent genital ambiguity.[59] For newborn males, however, differentiation of the external genitalia is not affected because the main source of testosterone is the testes and not the adrenal gland. Postnatally, genitalia may continue to virilize because of an excess of adrenal androgens, and pseudo-precocious puberty can occur. In affected females, signs of hyperandrogenism include facial, axillary, and pubic hair; adult body odor; temporal balding; severe acne; irregular menses; and reduced fertility. Poor control of adrenal androgens in males has been associated with small testes, infertility, and short stature. Infertility occurs because the excess androgens are aromatized peripherally to estrogens, which suppress pituitary gonadotropins and function of the gonads, and due to the development of testicular adrenal rest tumors. The high estrogens also advance bone age. The high levels of androgens can also accelerate growth in early childhood, producing an unusually tall and muscular child. Thus, untreated patients with 21-OHD CAH are tall as children but short as adults. The salt-wasting phenotype, which occurs in approximately 75% of CAH cases,[60] is biochemically distinct from the simple virilizing form because of a deficiency of aldosterone, the salt-retaining hormone.[48,61] Resulting hyponatremia, hyperkalemia, high plasma renin activity, and fluid volume depletion that occur at days 5 to 15 of life are potentially fatal.

Management

Hormone replacement therapy with corticosteroids is used to correct the deficiency in cortisol secretion, which will in turn suppress ACTH overproduction and subsequent stimulation of the androgen pathway.[8] Hormone replacement prevents further virilization, allowing normal growth and onset of puberty. The initial dose of hydrocortisone required is usually 15 to 20 mg/m^2 per day divided into three doses per day[62,63] but may be even higher in the newborn. The dose can then be decreased to 10 to 15 mg/m^2 per day after there has been initial suppression of the ACTH–adrenal axis. It is important that the appropriate balance be maintained to avoid hypercortisolism, which can result in Cushing syndrome and suppression of linear growth. Attempts to suppress 17-OHP

levels to normal will inevitably result in iatrogenic Cushing syndrome. Hormonal control can be difficult to achieve in some cases, and adrenalectomy in the past had been offered as an extreme alternative therapeutic option in select patients.[64,65] Patients with salt-losing CAH have elevated plasma renin activity and require mineralocorticoid replacement with 0.05 to 0.4 mg/day of 9α-fludrocortisone. Neonates have mineralocorticoid resistance as evidenced by higher levels of aldosterone present in normal infants and thus the required dose of 9α-fludrocortisone is higher in infants than in adults. In infancy, most patients also require an oral salt supplement as in other forms of primary adrenal insufficiency. There is no parenteral formulation for mineralocorticoid. Therefore, if 9α-fludrocortisone cannot be given orally, hydrocortisone at higher doses can be given IV along with sodium chloride. A dose of 20 mg of hydrocortisone will provide the equivalent of 0.1 mg 9α-fludrocortisone.

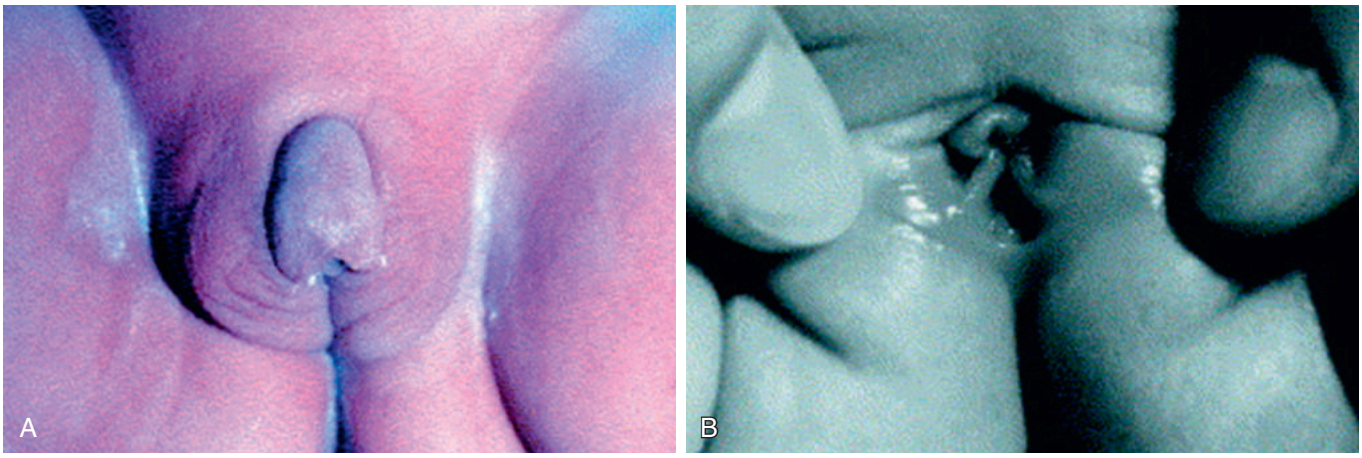
With the advent of newborn screening, infants are diagnosed with CAH prior to a salt-wasting crisis, and thus it is not possible to distinguish the infant with the 75% chance of having salt-wasting CAH without genetic testing. Because females with classic 21-OHD CAH are born with ambiguous genitalia caused by the production of excess androgens in utero, corrective surgery is contemplated. However, before surgical restoration is considered as a form of treatment in patients with ambiguous genitalia, consultation with the patient's parents, psychologist, pediatric endocrinologist, and pediatric urologist is essential.

Prevention of prenatal virilization in affected females is possible with a prenatal diagnosis and treatment program. The disease can be diagnosed prenatally through molecular genetic analysis of fetal DNA. Prenatal treatment by dexamethasone administration to the pregnant mother carrying an at-risk fetus before 6 weeks post conception is effective in reducing virilization in the genetic female, making postnatal female restorative surgery less likely to be necessary and thereby avoiding potential impairment of sexual function (Fig. 84.5). At 7 to 10 weeks, cell-free DNA testing may allow for discontinuation of the dexamethasone if an XY fetus is identified or, if using massive parallel sequencing (MPS) of cell-free fetal DNA, an XX fetus is found to not have CAH.[66] However, the technique of MPS for CAH is not readily available as routine care. Dexamethasone may also be discontinued after diagnosis is made by fetal DNA analysis obtained from chorionic villus sampling at 10 to 12 weeks' gestation in unaffected female or male fetuses.[67] Some believe there are accurate, compelling data from the largest human studies[68–70] indicating the benefit of prenatal treatment and that it is safe in the short term for both the fetus and the mother. Some preliminary data from long-term studies also support these results,[71] although other long-term follow-up studies are still under way. In contrast, studies from Sweden found that children without CAH treated prenatally with dexamethasone had decreased verbal working memory and decreased self-perception of scholastic ability. Those with CAH had decreased verbal processing speed that normalized though when adjusted for intelligence quotient.[72,73] A more recent review found that there is a negative effect on executive function and on social behavior.[74] Long-term cardiovascular, renal, and metabolic risk have yet to be determined. Thus, the ethics of prenatal dexamethasone therapy remains controversial because of unknown long-term outcomes and because seven fetuses were treated unnecessarily to prevent virilization in one fetus. Dexamethasone therapy also incurs maternal risk. Consequently, all endocrine societies consider prenatal dexamethasone therapy for 21-OHD to be experimental and that it should only be performed in a research environment with long-term outcome studies, as the risk for long-term effect is not known.[75]

11β-Hydroxylase Deficiency

Epidemiology

Classic 11β-OHD CAH occurs in approximately 1 in 100,000 births in the general white population.[76] Many cases have been reported in Israel, where the incidence was estimated to be 1 in 5000 to 1 in 7000 births with a gene frequency of 1 in 71 to 1 in 83. A subsequent study showed that 11β-OHD CAH occurred in a lower frequency, yet it remains more common in this population than in others.[77] This unexpected clustering of cases was traced to Jewish families of North African origin, particularly from Morocco and Tunisia.[78] In Turkey, 11β-OHD accounts for 13.5% of the CAH cases.[79]



• **Fig. 84.5** Two Sisters with Congenital Adrenal Hyperplasia. (A) Female infant with congenital adrenal hyperplasia. There is enlargement of the clitoris with fusion and rugation of labial scrotal folds. (B) Sister of the female infant in (A) who also has congenital adrenal hyperplasia but whose mother received dexamethasone therapy prenatally to prevent virilization of external genitalia. (From Forest MG, Morel Y, David M. Prenatal treatment of congenital adrenal hyperplasia. *Trends Endocrinol Metab*. 1998;9:284–289.)

Pathophysiology

The 11β-hydroxylase deficiency (11β-OHD) form of CAH represents 5% to 8% of all cases in the general population.[80] Deficiency of this enzyme results in an accumulation of 11-deoxysteroid precursors, which are shunted into the androgen pathway. Excess adrenal androgen secretion results in ambiguous genitalia in the affected female fetus. Hypertension in patients with this disorder is commonly attributed to deoxycorticosterone (DOC)-induced sodium retention.[81] The hallmark serum abnormality in patients with 11β-OHD is normal or suppressed renin, because hypokalemia is not uniformly present in all cases. Infants may also present with hyponatremia due to the physiologic newborn resistance to mineralocorticoids. 17-OHP may also be elevated due to the high 11-deoxycortisol levels and be detected by the newborn screen for 21-OHD CAH. Diagnosis is made by the determination of extremely high basal and stimulated levels of DOC and 11-deoxycortisol after performing an ACTH stimulation test. The diagnosis can be confirmed by molecular genetic analysis of the CYP11B1 gene.

Two 11β-hydroxylase genes have been identified within the human adrenal cortex, each encoding for a different enzyme with distinct enzymatic ability. The two genes *CYP11B1* and *CYP11B2* are located 30 to 40 kilobases apart on chromosome 8q.[82,83] Although gene conversions occur between *CYP11B1* and *CYP11B2*,[84,85] the majority of the mutations found in *CYP11B1* are random point mutations,[76,86–88] unlike what occurs in 21-OHD CAH. By 2003, approximately 41 mutations in *CYP11B1* from individuals of diverse ethnic backgrounds had been identified.[53] There are now over 100 mutations found in CYP11B1.[89,90]

Clinical Presentation

Hypertension occurs in approximately two-thirds of patients with 11β-OHD and distinguishes 11β-OHD from the more common 21-OHD in cases of virilizing CAH.[81,87] However, hypertension correlates variably with the presence of hypokalemia or with the extent of virilization.[87] Patients can present with or without hypokalemic alkalosis. Hypertension is usually not identified until later in childhood or in adolescence, although its appearance in early childhood has been documented. A patient was positively identified by the newborn screening program aiming for 21-OHD CAH.[91]

Management

Like 21-OHD CAH, glucocorticoid therapy is the most effective means of regaining hormonal control in patients with 11β-OHD. Corticosteroids at the same dose range as for 21-OHD CAH provide feedback inhibition of ACTH, reduce stimulation of the androgen pathway, and allow normal growth and the onset of puberty. Treatment with corticosteroids also contributes to the reduction of DOC and thus controls hypertension. Through careful clinical monitoring, doses can be continuously adjusted to match patients' needs while avoiding suppression of linear growth caused by overdosing. In addition to hormonal therapy, reduced salt intake is often used to reduce fluid volume and hypertension. Maintaining fluid balance in children is often difficult, however, and poses an ongoing challenge to treatment. Affected females suffer from genital ambiguity and may require genital restorative surgery. To prevent prenatal virilization, a similar experimental protocol to 21-OHD CAH for prenatal diagnosis and treatment can be performed.[92]

Disorders That Lead to Males With Undervirilization

17α-Hydroxylase/17,20-Lyase Deficiency

Epidemiology

17α-hydroxylase/17,20-lyase deficiency has an estimated frequency in most countries of approximately 1 case per 50,000 newborns and accounts for approximately 1% of all cases of CAH worldwide.[93] However, 17-hydroxylase (CYP17) deficiency is the second most common cause of CAH in Brazil.[94,95] This frequency is the result of two founder effects in areas with high coefficients of consanguinity such that two mutations account for more than 80% of cases in that country.[94]

Pathophysiology

17α-hydroxylase/17,20-lyase deficiency involves an enzyme that catalyzes more than one reaction—namely both the 17α-hydroxylation and 17,20-lyase reactions—with both reactions commonly being impaired in the disorder. Affected individuals cannot produce cortisol but synthesize large amounts of corticosterone (a weak glucocorticoid that mitigates the adrenal insufficiency) and deoxycorticosteroid, which causes hypertension and hypokalemia. Deficiency of 17,20-lyase impairs the ability to synthesize androgens and estrogens and causes male 46,XY differences of sex development (DSD) at birth and results in failure to virilize at puberty. Affected females have primary amenorrhea and clinical hypogonadism.[93,96]

Since the cloning of the gene,[97] over 100 different mutations have been described in *CYP17*.[98,99] Founder effects probably explain the high incidence of the disease in other patient populations in the Netherlands and Japan.[94] Severity of disease tends to be milder with mutations that retain partial catalytic activity, but the nature of the variability in hypertension and hypokalemia is unclear.

Clinical Presentation

The typical features of complete deficiency include hypertension and hypokalemia, which occur due to increased ACTH stimulation of the mineralocorticoid pathway, absent adrenarche and puberty in genetic females, and undervirilization in genetic males. Nevertheless, there is considerable variability in the clinical and biochemical features, including a few mutations that cause isolated 17,20-lyase deficiency.[96] The age of onset of hypertension and the severity of hypokalemia are highly variable, even among individuals with the same mutations.[94]

Management

Adequate glucocorticoid administration suppresses ACTH and the excessive mineralocorticoid secretion and generally normalizes the blood pressure. Adult females and undervirilized males reared as females require estrogen therapy. Abdominal testes should be relocated or removed because of the risk of malignancy. Adult genetic males reared as males need surgical correction of the external genitalia and androgen replacement.

3β-Hydroxysteroid Dehydrogenase Type 2 Deficiency

Epidemiology

The exact frequency of this rare disorder remains unknown.

Pathophysiology

3β-Hydroxysteroid dehydrogenase converts 3β-hydroxy $^{5}\Delta$ steroids (pregnenolone, 17-hydroxypregnenolone, and DHEA) to 3-keto $^{4}\Delta$ steroids (progesterone, 17-OHP, and androstenedione) and is essential for the biosynthesis of mineralocorticoids, glucocorticoids, and sex steroids.[100] Two forms of the 3β-hydroxysteroid dehydrogenase enzyme have been described in humans: type 1 enzyme expressed in placenta and skin and type 2 expressed in adrenal glands and gonads. The type 1 and 2 genes are closely linked on chromosomal region 1p13.1. The two forms are closely related in structure and substrate specificity, although the type 1 isoenzyme has higher substrate affinity and a fivefold greater enzymatic activity than type 2.[101] The type 1 isoenzyme can lead to elevated 17-OHP and androstenedione levels in addition to the expected elevated levels of pregnenolone, 17-hydroxypregnenolone, and DHEA.

3β-Hydroxysteroid dehydrogenase type 2 deficiency has an autosomal recessive inheritance. *HSD3B2* is the gene responsible for 3β-HSD2 deficiency CAH. There are approximately 40 mutations in the *HSD3B2* gene already described.[53] Mutations that lead to the abolition of 3β-HSD2 activity lead to the salt-wasting form.[102–105] Mutations that reduce but do not abolish type II activity lead to CAH with mild or no salt loss, which in males is associated with 46,XY DSD as a result of the reduction in androgen synthesis.[104] Mild mutations were also associated with hyperandrogenic symptoms of premature pubic hair development and hirsutism.[106,107]

Clinical Presentation

3β-HSD2 isoenzyme is essential for the formation of progesterone, the precursor for aldosterone; 17-OHP, the precursor for cortisol in the adrenal cortex; androstenedione; testosterone; and estrogen. Simultaneous 3β-HSD2 deficiency in both gonads and adrenal glands results in incomplete virilization of the external genitalia in males. Male patients with 3β-HSD2 deficiency present with ambiguous external genitalia, characterized by micropenis, perineal hypospadias, bifid scrotum, and blind vaginal pouch[108] with or without salt loss.[100] Gynecomastia is common at pubertal stage in affected males. In females, virilization of external genitalia occurs as a result of the androgen effect from the peripheral conversion of circulating $^{5}\Delta$ precursors to active $^{4}\Delta$ steroids; therefore, genital ambiguity can result in both sexes. Newborns can present with adrenal insufficiency due to deficiency of glucocorticoids, mineralocorticoids, and sex steroids. Later clinical presentations also include salt-wasting crises, premature pubic hair development, hirsutism, and menstrual disorders.[104]

Management

Like other forms of CAH, corticosteroid is the mainstay therapy. Salt-wasting phenotypes seen in some patients can be managed the same way as the salt-wasting form of 21-OHD CAH. Male patients with 3β-HSD2 deficiency have ambiguous external genitalia. Although most males are raised as males and retain the male social sex at puberty, gender identity is an important management issue. In one Brazilian family, two cousins with 46,XY DSD caused by 3β-HSD2 deficiency were reared as females; one of them had bilateral orchiectomy in childhood and retained the female social sex; the other retained their testes during childhood and changed to male social sex at puberty.[109]

Male patients may require testosterone replacement therapy during puberty and adulthood. The aim of the surgical treatment after consultation with a DSD team in this condition is to allow development of adequate external genitalia. Only skilled surgeons with specific training in the surgery of DSD should perform these procedures.[110]

Lipoid Congenital Adrenal Hyperplasia

Lipoid CAH is a severe form of congenital adrenal insufficiency. Affected patients exhibit glucocorticoid and mineralocorticoid deficiencies early in life, and males exhibit undervirilization. The reduced synthesis of steroids in patients with lipoid CAH results from an inability to transfer cholesterol to the inner mitochondrial membrane where the cholesterol side-chain cleavage complex is located.[111] It is characterized by lipid droplet accumulation in the cytoplasm of the adrenocortical cells. Most cases of lipoid CAH are caused by autosomal recessive mutations in the gene encoding steroidogenic acute regulatory protein (StAR). The *StAR* locus is in the 8p11.2 region and encodes a protein with an essential role in cholesterol transfer from the outer to the inner mitochondrial membrane, thus providing the substrate for steroid hormone biosynthesis.[112] Once in the mitochondria, cholesterol is converted to pregnenolone by the cytochrome P450 side-chain cleavage (CYP11A1) enzyme, and then steroid biosynthesis is initiated. Karyotypic 46,XY persons are phenotypically female because of Leydig cell destruction and impaired testosterone production. Sertoli cell function is intact so that anti-müllerian hormone is secreted, and thus müllerian ducts regress, leading to absence of the uterus and fallopian tubes. The ovary in XX subjects is initially spared damage because steroidogenesis is delayed until the time of puberty, after which stimulation of steroidogenesis by the tropic hormones (i.e., luteinizing and follicle-stimulating hormones) causes progressive damage to the ovary.[113] *StAR* mutations have been described most frequently in Japanese and Palestinian populations, in part because certain mutations occur repeatedly, probably reflecting a founder effect.[112,114] Although less common than mutations in *StAR*, mutations in *CYP11A1* can also cause a clinical phenotype similar to those with mutations in the *StAR* gene, but the adrenal gland does not have the adrenal hyperplasia seen in lipoid CAH.[115–117]

Cytochrome P450 Oxidoreductase (POR) Deficiency

Cytochrome P450 oxidoreductase (POR) deficiency is an autosomal recessive disorder of steroidogenesis involving P450c17, P450c21, and P450-aro with a phenotypic spectrum ranging from cortisol deficiency at the milder end to classic Antley–Bixler syndrome (ABS) at the severe end. The phenotype of cortisol deficiency can range from clinically insignificant to life threatening.[118] Manifestations of POR deficiency can include ambiguous genitalia in males and females, primary amenorrhea and enlarged cystic ovaries in females, poor masculinization during puberty in males, and maternal virilization and low estriol levels during pregnancy with an affected fetus carrying certain POR mutations. Manifestations of ABS include craniosynostosis, hydrocephalus, distinctive facies, choanal stenosis or atresia, low-set, dysplastic ears with stenotic external auditory canals, skeletal anomalies (radiohumeral synostosis, neonatal fractures, congenital bowing of the long bones, joint contractures, arachnodactyly, clubfeet), renal anomalies (ectopic kidneys, duplication of kidneys, renal hypoplasia, horseshoe kidney, hydronephrosis), and reduction of cognitive function and developmental delay. The skeletal differences have been hypothesized to be due to decreased activity

of CYP26B1, a POR-dependent enzyme that degrades retinoic acid.[119] In moderate POR deficiency, craniofacial and skeletal anomalies are less severe than in ABS.[120] Infants with ABS with normal steroidogenesis and without ambiguous genitalia have a mutation in the fibroblast growth factor receptor 2 and not in the *POR* gene.[121] As more and more patients are identified with POR deficiency, this newly discovered form of CAH may not be as rare as formerly thought.

Familial Glucocorticoid Deficiency

Familial glucocorticoid deficiency (FGD) is an autosomal recessive disorder resulting from defects in the action of ACTH to stimulate glucocorticoid synthesis in the adrenal leading to ACTH resistance. It is also known as "isolated glucocorticoid deficiency" or "hereditary unresponsiveness to ACTH." The majority of patients with FGD have episodes of hypoglycemia in the neonatal period. These episodes will often respond quickly to more frequent feeding regimens. In a few cases, excessive skin pigmentation is recognized at this early stage. Biochemically, patients with FGD have low or undetectable cortisol levels and—because of the failure of the negative feedback loop to the pituitary and hypothalamus—grossly elevated ACTH levels are found. Mineralocorticoid deficiency usually is not a presentation; therefore, aldosterone levels, plasma renin measurements, and serum electrolytes are normal. A clinical feature sometimes observed in patients with FGD is tall stature that is identified later in life.[122] Approximately half of all cases result from mutations in the ACTH receptor (*MC2R*, melanocortin 2 receptor) FGD type 1 or from mutations in the melanocortin 2 receptor accessory protein (*MRAP*), FGD type 2.[123] About 10% of FGD is due to mutations in *nicotinamide nucleotide transhydrogenase (NNT)*.[124–126] Other etiologies include MCM4, *TXNRD2*,[127] *GPX1* and *PRDS3*.[124] Milder mutations in *StAR* and CYP11A1 may also present with FGD. Other genetic causes of this potentially lethal disorder remain to be discovered in about one-third of the cases.

Triple A Syndrome

Triple A or Allgrove syndrome is a similar disorder to FGD with additional features of alacrima and achalasia. Alacrima (reduced or absent tear production) is the first symptom, followed by achalasia (difficult for food and liquid to pass through the esophagus) and then adrenal insufficiency. Presenting in the first decade of life, it is frequently associated with progressive neurologic dysfunction, polyneuropathy, deafness, mental retardation, and hyperkeratosis of palms and soles.[128] Some of these families have a defect in the alacrima–achalasia–adrenal insufficiency neurologic disorder (*AAAS*) gene encoding a protein named ALADIN.[129] ALADIN participates in the nuclear translocation of the ferritin heavy chain protein. Mutations in *AAAS* prevent ferritin heavy chain protein from entering into the nucleus, which is necessary to prevent oxidative damage of the nucleus.[130] ALADIN belongs to a WD-repeat family of regulatory proteins that shares a common motif made up of highly conserved repeating units usually ending with tryptophan-aspartate (WD).[131]

Neonatal Adrenoleukodystrophy

Neonatal adrenoleukodystrophy (NALD) is a fatal rare autosomal recessive disease of impaired peroxisome biogenesis. NALD belongs to a class of disorders involving peroxisomal biogenesis that includes Zellweger syndrome and infantile Refsum disease. NALD is the only one of the three diseases that often involves adrenal insufficiency. Mutations in seven different peroxisome biogenesis factor genes have been shown to cause NALD.[132] Mutations in peroxisome biogenesis factor 1 (*PEX1*) are the most common cause of NALD.[133] As in X-linked adrenoleukodystrophy (X-ALD), patients with NALD accumulate very long chain fatty acids and develop degenerative changes of the white matter of the nervous system and adrenal atrophy. Infants with NALD characteristically demonstrate dolichocephaly, prominent and high forehead, esotropia, epicanthic folds, broad nasal bridge, high-arched palate, low-set ears, and anteverted nostrils. Affected patients usually die in early childhood.[134] X-ALD is a recessively inherited X-linked defect of the adrenoleukodystrophy protein (ALDP).[135] It is encoded by the *ABCD1* gene on Xq28. It is also a peroxisomal defect that usually results in adrenal insufficiency and central nervous system deterioration. X-ALD can present in early childhood, and at birth there is already elevation of very long chain fatty acids. Because early diagnosis can improve outcome through the use of hematopoietic stem cell transplantation or gene therapy, some state newborn screening programs have added X-ALD to their newborn screening panel. X-ALD can also manifest later in adulthood.[136]

Defective Cholesterol Metabolism: Smith–Lemli–Opitz Syndrome

The clinical picture of adrenal insufficiency and 46,XY gonadal dysgenesis may be caused by a deficiency of 7-dehydrocholesterol C-7 reductase enzyme that catalyzes the final step in cholesterol biosynthesis leading to primary adrenal insufficiency. The syndrome results from mutations in the sterol Δ-7-reductase gene (*DHCR7*) located at 11q12-q13. Smith–Lemli–Opitz (SLO) syndrome can manifest with typical facial appearance, mental retardation, microcephaly, proximally placed thumbs, congenital cardiac abnormalities, syndactyly of the second and third toes, incomplete development of the male genitalia, and photosensitivity. The biochemical abnormalities of SLO syndrome include low cholesterol and high 7-dehydrocholesterol.[137] Thus, it is important to consider the diagnosis as an elevated 7-dehydroxycholesterol level will confirm the diagnosis.

The birth prevalence of SLO syndrome is estimated to be approximately 1:20,000 to 1:40,000 live births.[137] Among persons of northern or central European ancestry, it has been estimated to range from 1:10,000 to 1:60,000.[138] SLO syndrome is less common in those of Asian or African ancestry. As it is an autosomal recessive disorder, the recurrence risk is 25%.

In utero, the primary defect in fetal adrenal glands results in a combination of low maternal estriol levels, undervirilization of the male fetus, and large adrenal glands in the fetus with SLO syndrome. Preliminary studies suggested that cholesterol supplementation may be of benefit to patients with SLO syndrome.[139]

Unfortunately, replacement of cholesterol has not been as beneficial as once hoped. Cholesterol does not cross the blood–brain barrier and has not been shown to reduce developmental delay.[140] A more recent placebo-controlled trial using simvastatin found that the medication crossed the blood–brain barrier and was relatively safe. There was improvement in serum dehydrocholesterol-to-total-sterol ratio and improvement of irritability symptoms.[141]

Adrenal Insufficiency Associated With Other Syndromic Disorders

Lysosomal Storage Disorders

Complete deficiency of lysosomal esterase can also result in adrenal insufficiency in Wolman disease, a rare autosomal recessive disease with an incidence of 1 in 350,000 infants. Wolman disease usually is fatal in the first year of life. Affected infants exhibit mild mental retardation, hepatosplenomegaly, vomiting, diarrhea, growth failure, and adrenal calcifications. Calcifications that delineate the outline of both adrenals are pathognomonic of this condition.[142]

Mitochondrial Disorders

Adrenal insufficiency can result from mitochondrial disorders, characterized by chronic lactic acidosis, myopathy, cataracts, and nerve deafness.[143,144] Cases with the Kearns–Sayre syndrome form of mitochondrial myopathy and deafness, with large-scale deletions in mitochondrial DNA, are often associated with endocrine dysfunction, particularly short stature, hypogonadism, diabetes, hypoparathyroidism, hypothyroidism, and adrenal insufficiency.[145,146]

IMAGe Syndrome

Three patients with AHC and additional findings that represent a new syndrome known as IMAGe (i.e., intrauterine growth retardation, metaphyseal dysplasia, AHC, and genital anomalies) have been reported. Genital abnormality was described as bilateral cryptorchidism, small penis, and hypogonadotropic hypogonadism. The patients also had hypercalciuria with or without hypercalcemia, resulting in abnormal calcium deposits in vital organs.[147] As of 2016, 28 individuals in 16 families have been reported.[148] Mutations in the proliferating cell nuclear antigen (PCNA)-binding domain of the maternally expressed cyclin-dependent kinase inhibitor (*CDKN1C*) gene on the short arm of chromosome 11, which results in the loss of PCNA binding, have been identified as the etiology of IMAGe syndrome. These mutations serve as a gain of function mutation.[149] *CDKN1C* is a tumor suppressor gene, and it encodes an inhibitor of cell cycle progression. Beckwith–Wiedemann syndrome, an overgrowth syndrome, also has mutations in the *CDKN1C* gene but at a different location, resulting in a loss of function.

MIRAGE Syndrome

MIRAGE syndrome (myelodysplasia, infections, restricted growth, adrenal hypoplasia genital anomalies and enteropathy) is due to heterozygous changes in the *SAMD9* gene. Individuals affected have a poor prognosis.

Adrenal Hypoplasia Congenita

Adrenal hypoplasia congenita (AHC), a familial condition in which the adrenal cortex has arrested development, occurs in approximately 1 in 12,500 births.[150,151] The disorder can manifest as four clinical forms of primary adrenal insufficiency: (1) a sporadic form associated with pituitary hypoplasia; (2) an autosomal recessive form with a distinct miniature adult adrenal morphology characterized by small glands with a permanent cortical zone but a diminished fetal zone (the genetic basis of the recessive form of AHC is unknown); (3) an X-linked cytomegalic form associated with hypogonadotropic hypogonadism; and (4) an X-linked form associated with glycerol kinase deficiency and Duchenne muscular dystrophy.[152,153] Mutations in the *NROB1* gene encoding DAX-1 are responsible for both X-linked forms.

The X-linked or cytomegalic form of AHC is characterized by the absence or near absence of the permanent or adult zone of the adrenal cortex and by structural disorganization of the fetal cortex with abnormally large cells. It differs from the autosomal recessive miniature adult form of AHC in which the adrenal cortex has the normal adult structure but is small. X-linked AHC results in severe primary adrenal insufficiency involving glucocorticoids and mineralocorticoids and failure to respond to elevated levels of ACTH with usual age at onset in the neonatal period or during infancy. However, in some patients, age of onset is later—up to several years of age and presumably caused by residual functional cortex.[154,155] The secretion of other pituitary hormones is not impaired. Hypogonadotropic hypogonadism can manifest with cryptorchidism or delayed puberty.[156] Whereas presentation of adrenal insufficiency can occur from birth, there is great variability of presentations. Isolated adrenal insufficiency in infancy, isolated adrenal insufficiency later in life, isolated hypogonadotropic hypogonadism, adrenal insufficiency and hypogonadotropic hypogonadism, delayed-onset adrenal insufficiency from 2 to 9 years of age with incomplete hypogonadotropic hypogonadism, and delayed puberty in females all may result.[153] The phenotypic variation does not correlate well with genotype. Treatment includes both glucocorticoid and mineralocorticoid replacement.

Adrenal Hypoplasia as Part of Contiguous Gene Deletion Syndrome

An X-linked form of adrenal insufficiency associated with glycerol kinase deficiency is characterized by psychomotor retardation, muscular dystrophy, characteristic facies with hypertelorism, alternating strabismus, and drooping mouth. Additional phenotypic features can include testicular abnormalities (anorchia or cryptorchidism), short stature, and osteoporosis. Time of presentation can vary from birth through childhood. Nearly all patients reported were male. The genetic locus was mapped to Xp21.3-21.2, and variants of contiguous gene deletion syndrome (glycerol kinase deficiency, Duchenne muscular dystrophy, ornithine transcarbamylase deficiency, and mental retardation) can be seen.

Abnormalities of Development: DAX-1 and Steroidogenic Factor-1 Deficiency

The nuclear receptors DAX-1 and SF-1[157] have an important role in adrenal development and function, and mutations in the genes that encode these transcription factors have been found in patients with adrenal hypoplasia. Both SF-1 and DAX-1 belong to the family of nuclear hormone receptors. DAX-1 protein is expressed in the developing urogenital ridge, ovary, testis, all zones of the fetal adrenal cortex, hypothalamus, and anterior pituitary gland—sites in which it colocalizes with SF-1.[158,159] SF-1 is essential for the development of the adrenal cortex, gonads, and ventromedial nucleus of the hypothalamus because it interacts with the promoter of the NROB1 gene, anti-müllerian hormone gene, and the genes for the α-subunits of the pituitary glycoprotein hormones.[160] Furthermore, SF-1 is a transcription factor that regulates gene expression of the CYP steroid hydroxylases (21-hydroxylase, the aldosterone synthase isoenzyme of steroid

11β-hydroxylase, CYP11A), 3β-hydroxysteroid dehydrogenase, aromatase, and StAR in the adrenal gland; therefore, it is essential for development of the adrenal cortex.

In one large study of this relatively rare disease, *NROB1* mutations were found in 58% of 46,XY phenotypic boys referred with adrenal hypoplasia and in all boys with hypogonadotropic hypogonadism and a family history suggestive of adrenal failure in males. *NR5A1* (SF-1) mutations causing adrenal failure were found in only two patients with 46,XY gonadal dysgenesis. No *NROB1* or *NR5A1* mutations were identified in the adult-onset group.[161]

Human mutations in *NR5A1* are even less common and have been described in a few patients with primary adrenal failure. Two individuals with a 46,XY genotype, female phenotype, and müllerian structures harbored missense mutations that affected DNA binding,[162,163] whereas a 46,XX girl with an *NR5A1* mutation had primary adrenal failure and apparently normal ovarian development.[164] In addition, it is now emerging that heterozygous nonsense or frameshift mutations associated with haploinsufficiency of *NR5A1* can cause 46,XY gonadal dysgenesis in patients with normal adrenal function.[165–167] Therefore, it is possible that a range of different endocrine phenotypes are associated with mutations in different domains of *NR5A1*.

Adrenal Hemorrhage

Adrenal hemorrhage is not uncommon at birth. Birth asphyxia and sepsis are the most common causes. The incidence in the neonate is reported to be 1.7 cases per 1000 autopsied infants and as many as 3% of infants screened by abdominal ultrasound examination. The etiology of neonatal adrenal hemorrhage is largely unknown, but it has been associated with birth trauma related to difficult deliveries, sepsis, coagulopathies, traumatic shock, and ischemic disorders. Infants with minimal hemorrhage may be asymptomatic and be discovered incidentally to have adrenal calcifications, indicating an earlier hemorrhage. Adrenal insufficiency can manifest in neonates with larger hemorrhages. Major adrenal hemorrhage can manifest as an abdominal mass, anemia from blood loss, or jaundice from reabsorption of the hematoma. Hemorrhage can also lead to adrenal insufficiency, which can manifest as neonatal hypoglycemia, hypotension, hypothermia, apnea, or shock. Because of the location of the right adrenal gland between the liver and spine, it is the one most often affected by hemorrhage.[168]

In meningococcal septicemias, hemorrhage into the adrenal glands can complicate the clinical picture, leading to circulatory collapse. This disorder is called Waterhouse–Friderichsen syndrome.[169] Other infections in the neonate that have been associated with adrenal hemorrhage include herpes virus, *Pseudomonas aeruginosa, Bacteroides* spp., herpes simplex virus type 6, and echovirus types 11 and 6.[170–174] Septic shock in newborns, especially in those who are small for their age, can result in adrenal hemorrhage with rhabdomyolysis and renal insufficiency.[153]

Secondary and Tertiary Adrenal Insufficiency

Iatrogenic Adrenal Insufficiency

Secondary and tertiary forms of adrenal insufficiency result from defects in pituitary corticotroph and hypothalamic function, respectively. Supraphysiologic doses of glucocorticoids are often used for the treatment of bronchopulmonary dysplasia. With prolonged use of supraphysiologic doses of glucocorticoids, these neonates are at risk for iatrogenic suppression of corticotroph ACTH release, with secondary adrenocortical atrophy and adrenal insufficiency.[175] Evidently, even a single course of prenatal betamethasone treatment induces a suppression of stress reactivity in healthy newborns.[176] Once administration of glucocorticoids is discontinued the duration of recovery of corticotroph function from adrenal suppression is highly variable. Some reports suggest evidence of suppression of the HPA axis evident for more than 1 year.[177] Even in preterm infants, the HPA axis behaves in a similar manner as in adult subjects, and the pituitary function recovers earlier than that of the hypothalamus and the adrenals.[178]

Developmental Adrenal Insufficiency

Secondary or tertiary adrenal insufficiency in the neonate often is a consequence of abnormalities in development of the hypothalamus and pituitary. Examples include de Morsier syndrome (septooptic dysplasia; Morsier[179]), hydrencephaly or anencephaly, and pituitary hypoplasia or aplasia. If these infants have concomitant diabetes insipidus, they have an increased risk of sudden death during childhood.[180,181] Patients with developmental abnormalities of the pituitary or hypothalamus often have deficiencies of other hormones. ACTH deficiency can be part of a multiple pituitary hormone deficiency syndrome caused by abnormal expression of *HESX1*, *LHX4*, *SOX3*, or *PROP1*, which encode transcription factors.[182] Isolated ACTH insufficiency is a rare condition that can be caused by mutations in *TPIT*, a T-box factor that controls transcription of the proopiomelanocortin gene in corticotrophs only, thereby resulting in an adrenal-only phenotype.[183] However, approximately 50% of patients do not carry mutations in *TPIT*, suggesting that other unknown factors exist.[184–186] Septooptic dysplasia can be caused by mutations in *HESX1* and *SOX2*.[180,181] Signs of hypopituitarism in a neonate include hypoglycemia, prolonged jaundice, shock, and microphallus in males. Trauma to the hypothalamus, pituitary, or hypophysial portal circulation from significant head injury, cerebrovascular accident, Sheehan syndrome, or hydrocephalus may be a cause of central adrenal insufficiency. Historical factors associated with increased risk for central adrenal insufficiency include maternal drug use and traumatic delivery.

There have been rare case reports of families with inherited abnormalities of neuropeptides involved in HPA axis regulation. Adrenal insufficiency, pigmentary abnormalities, and obesity have been described in families with a defect in proopiomelanocortin (POMC).[187] One kindred has been reported with Arnold–Chiari type I malformation and suspected *CRH* deficiency. The mutation in this kindred is linked to the *CRH* locus; however, a specific mutation in the *CRH* gene has not yet been defined.[188] Mutations in *TPIT*, which encodes a highly restricted transcription factor involved in the expression of the *POMC* gene, have been found in eight individuals with autosomal recessive congenital isolated ACTH deficiency.[189]

Management

Cortisol replacement for patients with secondary or tertiary adrenal insufficiency is the same as described for patients with primary adrenal insufficiency. To minimize growth suppression, these children can be treated with doses of hydrocortisone that are slightly less than physiologic replacement doses. Furthermore, because

mineralocorticoid production is under the control of the renin-angiotensin system, patients with secondary or tertiary adrenal insufficiency do not require mineralocorticoid replacement. However, these infants require evaluation for deficiencies of other pituitary hormones.

Adrenal Crisis in the Neonate

Adrenal crisis is a potentially life-threatening disorder that can manifest with a salt-losing crisis or profound hypoglycemia in a neonate or infant. Adrenal crisis requires immediate resuscitation and appropriate steroid replacement. Determining the exact cause of this condition can be challenging once the child has started treatment, but defining a precise etiology has important implications for long-term management, for identifying associated features, and for appropriate counseling regarding inheritance and the risks of other family members being affected.[161] Detailed questioning about family history that could reveal any insight into possible adrenal disease is important.

Initial Management

In a suspected adrenal crisis, blood for determination of electrolytes, aldosterone, plasma renin activity, cortisol, and ACTH should be drawn and treatment started before the results are obtained. Fluid resuscitation with normal saline containing 5% or 10% dextrose should be given to restore cardiovascular stability. Plasma sodium should be monitored closely, as rapid correction of hyponatremia with sodium repletion of more than 0.5 to 1 milliequivalent (mEq)/L per hour increases the risk of central pontine myelinolysis. The sodium deficit may be calculated by subtracting the infant's sodium from a customarily normal sodium of 140 mEq/L and then multiplying this value by 0.6 × weight (in kg). The rate of replacement should occur over an initial rate such that the sodium increase does not exceed 0.5 mEq/L per hour. Hydrocortisone IV should be given initially at 50-100 mg/m^2 and then continued at 50-100 mg/m^2 per day, divided every 6 to 8 hours, until the infant's condition is stable.

Maintenance Therapy

Corticosteroid and mineralocorticoid replacement therapies should suppress the excessive secretion of CRH, ACTH, and resting renin levels. The normal daily cortisol production rate is 6 to 7 mg/m^2 per day in children and adolescents.[190,191] This rate translates to approximately 10 to 12 mg/m^2 per day of oral hydrocortisone to allow for step-down losses from absorption, hepatic processing, and metabolic bioavailability. Because the bioavailability of oral steroids varies from person to person,[192] infants should be monitored closely for signs of either inadequate cortisol replacement or cortisol excess.[193] Although adults and older children may be able to take hydrocortisone twice daily, most infants should be dosed three times daily to avoid hypoglycemia associated with low cortisol on a twice-daily regimen.[194,195] Hydrocortisone is the preferred steroid for treatment of infants because it has fewer growth-suppressive effects than synthetic steroids.[196–198] The United States Food and Drug Administration withdrew oral hydrocortisone suspension from the market because of poor absorption and undertreatment of children.[153] The smallest tablet for hydrocortisone is 5 mg, and it can be cut into quarters for a dose of 1.25 mg. A new formulation for lower doses of hydrocortisone—0.5 mg, 1 mg, and 2 mg—was recently FDA approved. This medication though can only be given orally and not via nasal or gastric tubes.

In primary adrenal insufficiency, aldosterone production is usually decreased. Physiologic doses of hydrocortisone do not provide enough mineralocorticoid activity to prevent salt wasting[48]; therefore, these infants often require 0.05 to 0.4 mg/day of fludrocortisone acetate (Florinef, 9α-fluorocortisol) and added salt. Because after the first month of life aldosterone production does not vary, the dose of fludrocortisone does not increase with growth and aging.[30,199] Infants with mineralocorticoid deficiency require 1 to 2 g of sodium chloride (1 g contains 17 mEq of sodium) added to their diet because formula and breast milk are low in sodium content (approximately 8 mEq/L).[200]

Stress Replacement

The normal response to surgery, trauma, or critical illness is to increase plasma ACTH and cortisol levels.[201] The secretion rate of cortisol has been found to be proportional to the degree of stress and ranges from 60 to 167 mg/day in adults after surgery.[202,203] Based on data from adults, it is recommended that infants with adrenal insufficiency receive 30 to 100 mg/m^2 per day of hydrocortisone, divided every 6 to 8 hours, when stressed. In patients at risk for adrenal crisis, stress doses of hydrocortisone should be given with the onset of fever and gastrointestinal or other significant illness and continued for 24 hours after the symptoms resolve.[204] Usually, this treatment translates to the routine steroid dose being tripled and administered over three divided daily doses. If oral steroids are not tolerated, an intramuscular or IV dose should be given. For surgery, infants should be given 30 to 100 mg/m^2 of IV hydrocortisone on call to the operating room before the administration of anesthesia. Alternatively, the 30 to 100 mg/m^2 of hydrocortisone can be given continuously during the surgery in the IV fluids. Stress dosing of hydrocortisone (30 to 100 mg/m^2 per day divided every 6 to 8 hours) should be continued postoperatively for the next 24 to 48 hours. It is unnecessary to give mineralocorticoids over such periods if the patient begins the operative period in adequate salt balance. Sodium, potassium, and glucose levels as well as blood pressure should be monitored.

The patients and their families should have instructions for such instances. Every patient should wear a medical identification (e.g., MedicAlert) bracelet or necklace and carry the emergency medical information card that is supplied with it. Both should indicate the diagnosis, the daily medications and doses, and the physician to call in the event of an emergency.

Suggested Readings

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85

Differences in Sex Development

MARGARETT SHNORHAVORIAN AND PATRICIA Y. FECHNER

KEY POINTS

- Differences in sex development (DSD) are due to a variety of etiologies, requiring expertise of the pediatric endocrinologist, pediatric urologist, geneticist, and child psychologist, as well as the adolescent gynecologist, cytogeneticist, radiologist, and ethicist in some cases, to aid in the diagnosis, treatment, and optimization of long-term outcomes.
- Understanding normal sex development in males and females is critical to determining the cause of DSD.
- Genetic testing has advanced our understanding of the origins of sex development, and specific testing may be driven by results of hormonal testing. In other situations, the differential diagnosis for a given condition such as complete gonadal dysgenesis is so large that the use of a DSD panel is necessary. Knowing the genetic diagnosis provides closure for families and identifies other risks associated with the diagnosis, such as recurrence, renal disease, or tumor risk. A genetic diagnosis may also predict outcome at puberty.

For parents of a newborn with genitalia that are not clearly male or female, the ambiguity is a distressing matter that needs to be addressed with sensitivity and some urgency. There are four general classifications that can cause differences in sex development (DSD): (1) androgenization of the 46,XX individual, (2) incomplete androgenization of the 46,XY individual, (3) sex gonadal differentiation and chromosomal disorders, and (4) syndromes associated with incomplete genital development (Box 85.1). The first category, 46,XX DSD, comprises the majority of definable cases of ambiguous genitalia, whereas in the second category, 46,XY DSD, a genetic diagnosis is seen in less than 50% of cases.

This chapter presents an overview of the pathophysiology of DSD, including a discussion of the practical aspects of diagnosis and management. An overview of the traditional surgical methods used in the treatment of infants with DSD, including the risks and benefits of these procedures, is included at the end of the chapter.

General Considerations in the Approach to the Newborn With Ambiguous Genitalia

The medical evaluation of a newborn with ambiguous genitalia is necessarily time consuming. Open and honest discussions with the parents are invaluable in allaying anxiety and establishing a trusting relationship. Full disclosure of available information is essential in this regard.

Care must be taken to avoid premature sex assignment for the infant. Proper evaluation of the infant with ambiguous genitalia requires a multidisciplinary team that should include the primary care physician, neonatologist, pediatric endocrinologist, psychologist, pediatric urologist, pediatric geneticist, and pediatric radiologist.[1] Psychological assessment and support of the family are essential in the newborn period, along with long-term psychological follow-up evaluation.[2–4] Decisions regarding the sex of rearing should be made collaboratively between the multidisciplinary team and the parents, with the recognition that cultural and psychosocial factors play a role.[5,6]

In the past, sex assignment was based largely on clitoral-phallic size, relative ease of surgical reconstruction, or the potential for fertility. This approach has come under criticism as dissatisfaction with sex assignment based on these criteria has been reported in several case studies.[7–10] The importance of prenatal androgen imprinting has been implicated as an important variable in some of these cases.[8] Studies in 46,XY males with incomplete androgenization indicate that a small phallus can be associated with a satisfying adult sex life.[11] Other studies have found that gender role tends to increasingly correspond with assigned sex as individuals with DSD proceed into adulthood[12]; therefore, female sex assignment might not be necessarily warranted for 46,XY males with partially incomplete androgenization.

The degree of genital androgenization is one determinant of sex assignment in the infant with ambiguous genitalia; however, other factors such as chromosome complement and etiology of the DSD also play an important role. Parents need to understand that only a sex of rearing is being assigned. It will be up to the individual to choose their gender identity. The formation of a healthy gender identity seems to involve a complex interplay between psychobiologic and environmental factors.[2,13–16] Today with the increased awareness of gender dysphoria, these individuals will have an easier time confirming their own gender identity, even if it is different from the chosen sex of rearing.

Embryology of Sex Determination and Differentiation

Normal and abnormal sex determination and differentiation constitute superb examples of how an understanding of embryology is critical to the approach and management of a group of complex and intriguing clinical disorders. Development of the testis and ovary is unique compared with the development of other organs in that they both derive from the same bipotential gonad. The establishment of a testis or an ovary depends on which pathway is taken based on the genetic background. In the past, the ovarian

• BOX 85.1 Differential Diagnosis for Ambiguous Genitalia

46,XX Androgenized Female

- Congenital adrenal hyperplasia
  - 21-Hydroxylase deficiency
  - 11-Hydroxylase deficiency
  - 3β-Hydroxysteroid dehydrogenase deficiency
  - P450 oxidoreductase deficiency
- Aromatase deficiency (fetal and maternal virilization)
- Virilizing maternal conditions
- Ovotesticular disorder of sex development
- Adrenal/ovarian tumors/luteoma of pregnancy
- Maternal ingestion of progestins, androgens

46,XY Underandrogenized Male

- Androgen insensitivity
  - Complete
  - Partial
- 5α-Reductase type 2 deficiency
- Testosterone biosynthetic defects
  - Steroidogenic acute regulatory (StAR) deficiency
  - P450 side chain cleavage enzyme deficiency
  - 17α-Hydroxylase/17,20-lyase deficiency
  - P450 oxidoreductase deficiency
  - 3β-Hydroxysteroid dehydrogenase deficiency
  - 17β-Hydroxysteroid hydrogenase type 3 deficiency
- Leydig cell hypoplasia
- Idiopathic, undetermined
- Drug ingestion: progestins, spironolactone, cimetidine, phenytoin
- Persistent müllerian duct syndrome

Gonadal Differentiation and Chromosomal Disorders

- 46,XY gonadal dysgenesis
  - Complete (Swyer syndrome)
  - Partial
  - Mixed (45,X/46,XY)
- Ovotesticular disorder of sex development
  - 46,XX, 46,XY, 45X/46XY, 46,XX/46,XY

Syndromes Associated With Ambiguous Genitalia

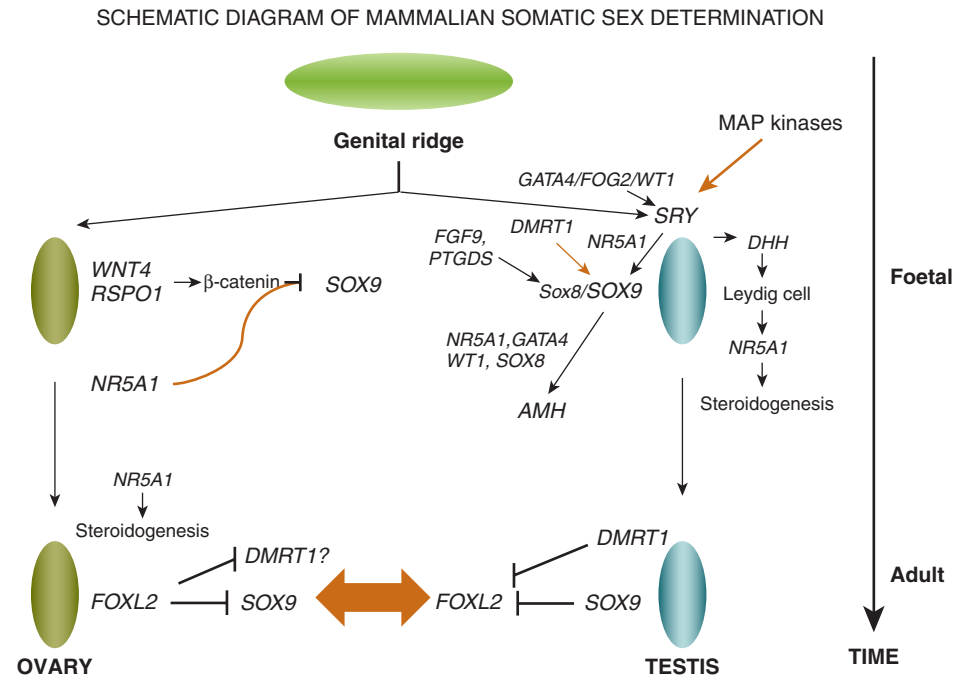
- Gonadal dysgenesis
  - 45,X/46,XY mixed gonadal dysgenesis (Turner syndrome features)
  - Campomelic dysplasia
- Renal degenerative diseases and gonadal dysgenesis
  - Denys-Drash syndrome
  - Frasier syndrome
  - WAGR (Wilms tumor, aniridia, genitourinary abnormalities, mental retardation) syndrome
- Smith-Lemli-Opitz syndrome (7-dehydrocholesterol reductase deficiency)
- Robinow syndrome

pathway was thought to be the default pathway. However, from studies of individuals with DSD, it is now known that ovarian development is an active process, and certain genes must be present for the ovary to develop.

Sex determination and differentiation are sequential processes that can be divided into three stages,[17] with the sequence as follows: chromosomal sex is determined at fertilization and dictates the differentiation of the bipotential gonad, which in turn dictates the phenotypic sex, or the differentiation of the internal ductal system and external genitalia on the basis of which hormones are produced by the gonad.[5]

Chromosomal sex is determined at the moment of conception by the sex chromosome complement of the fertilizing sperm. If this sperm carries an X chromosome, a 46,XX (normal female) complement results. If the sex chromosome is Y, a 46,XY (normal male) genotype results. The *SRY* (*s*ex-determining *r*egion of the *Y* chromosome) gene is necessary but not sufficient for testicular differentiation. Other autosomal and X chromosomal genes are also necessary for male sex development. *SRY* is, however, the only gene on the Y chromosome involved in testicular determination and differentiation. SRY, through a number of steps, instructs the medullary region of the bipotential gonad to develop into Sertoli cells and later into testicular cords and seminiferous tubules. SRY continues to be expressed at low levels in Sertoli cells until adulthood.

In addition to X chromosomal genes, autosomal genes influence sex differentiation insofar as mutations of these genes result in disorders of sex differentiation. Some of these genes include *WT1* (*W*ilms *t*umor gene *1*), associated with Denys-Drash syndrome and WAGR (*W*ilms tumor, *a*niridia, *g*enitourinary abnormalities, mental *r*etardation) syndrome; *NR5A1* (*n*uclear *r*eceptor subfamily *5*, group *A*, member *1*), which encodes steroidogenic factor 1; and *SOX9*, which has been associated with campomelic dysplasia and testicular dysgenesis in 75% of XY individuals.[18] *SOX 9* expression is upregulated by SRY. The *DMRT1* and *DMRT3* genes on chromosome arm 9p, when deleted, are associated with female development in an XY individual. More recently, a de novo missense mutation in *DMRT1* was identified in a female with 46,XY karyotype, providing evidence that *DMRT1* is the critical gene for testicular differentiation.[19] The *FOXL2* gene is expressed in fetal and adult ovarian follicular cells and is critical to ovarian development, fertility, and maintenance of the ovary. It is also expressed in eyelids, with mutations leading to blepharophimosis–ptosis–epicanthus inversus syndrome, and is located within the homologous region in the human at 3q23.[20] The *DAX1* gene is located on Xp22 and appears to be necessary for correct testis determination and, in the mouse at least, necessary for the upregulation of *Sox9* expression.[21] When the *DAX1* gene is duplicated, 46,XY individuals develop gonadal dysgenesis with a female phenotype.[22] The *WNT4* gene is critical for normal ovarian and female sexual development. A mutation in *WNT4* leads to müllerian duct regression and virilization in a 46,XX female,[23] whereas duplication of the locus containing *WNT4* leads to overexpression of DAX1 and thus a 46,XY female phenotype.[24] The desert hedgehog gene (*DHH*) is a member of a family of signaling genes with an important role in regulating morphogenesis. The follistatin gene (*Fst*) and the bone morphogenetic protein 2 gene (*Bmp2*) appear to be important for ovary organogenesis in mice.[25,26] Mutations in *Gata4* or *Fog2* can cause sex reversal in mice.[27–29] In humans, GATA4 is a transcription factor necessary for genital ridge formation, testicular and ovarian differentiation, and male and female fertility. GATA4 interacts with multiple genes, such as *SRY*, *SOX9*, and *AMH*, as well as others involved in hormonal synthesis. GATA4 acts synergistically with NR5A1 to activate the *AMH* promoter, and a mutation in *GATA4* led to the lack of this synergy with NR5A1 as well as inability for FOG2 to bind to GATA4, resulting in familial 46,XY DSD and congenital heart defects.[30] FOG2 modifies GATA4 activity by binding to GATA4's zinc finger.[31] Missense mutations in *FOG2* have been identified in two individuals with 46,XY gonadal dysgenesis.[32] The pathways to testicular and ovarian development are illustrated in Fig. 85.1.



• **Fig. 85.1** Testicular and Ovarian Development Pathways. In the XY gonad, *SRY* expression is initiated by WT1/GATA4/FOG2, which leads to the upregulation of *SOX9* expression via synergy with NR5A1. Once SOX9 levels reach a critical threshold, SOX9 autoregulates its own expression. In the XX gonad, the supporting cell precursors accumulate β-catenin in response to RSPO1/WNT4 signaling, which either directly or indirectly represses *SOX9* expression. NRA51 is also critical for steroidogenesis. *AMH*, Antimüllerian hormone; *DHH*, desert hedgehog; *DMRT1*, doublesex and mab-3 related transcription factor 1; *FGF9*, fibroblast growth factor 9; *FOG2*, aka ZFPM2 (zinc finger protein, FOG family member 2); *FOXL2*, forkhead box L2; *GATA4*, GATA binding protein 4; *MAP*, mitogen-activated protein; *MAP kinase*, mitogen-activated protein kinase; *NR5A1*, nuclear receptor subfamily 5 group A member 1; *PTGDS*, prostaglandin D2 synthase; *RSPO1*, R-spondin 1; *SOX8*, SRY-box 8; *SOX9*, SRY-box 9; *SRY*, sex determining region Y-chromosome; *WNT4*, wingless-related integration site 4; *WT1*, Wilms tumor 1. (Reproduced with permission from Bashamboo A, McElreavey K. Mechanism of sex determination in humans: insights from disorders of sex development. Sex Dev. 2016;10:313–325.)

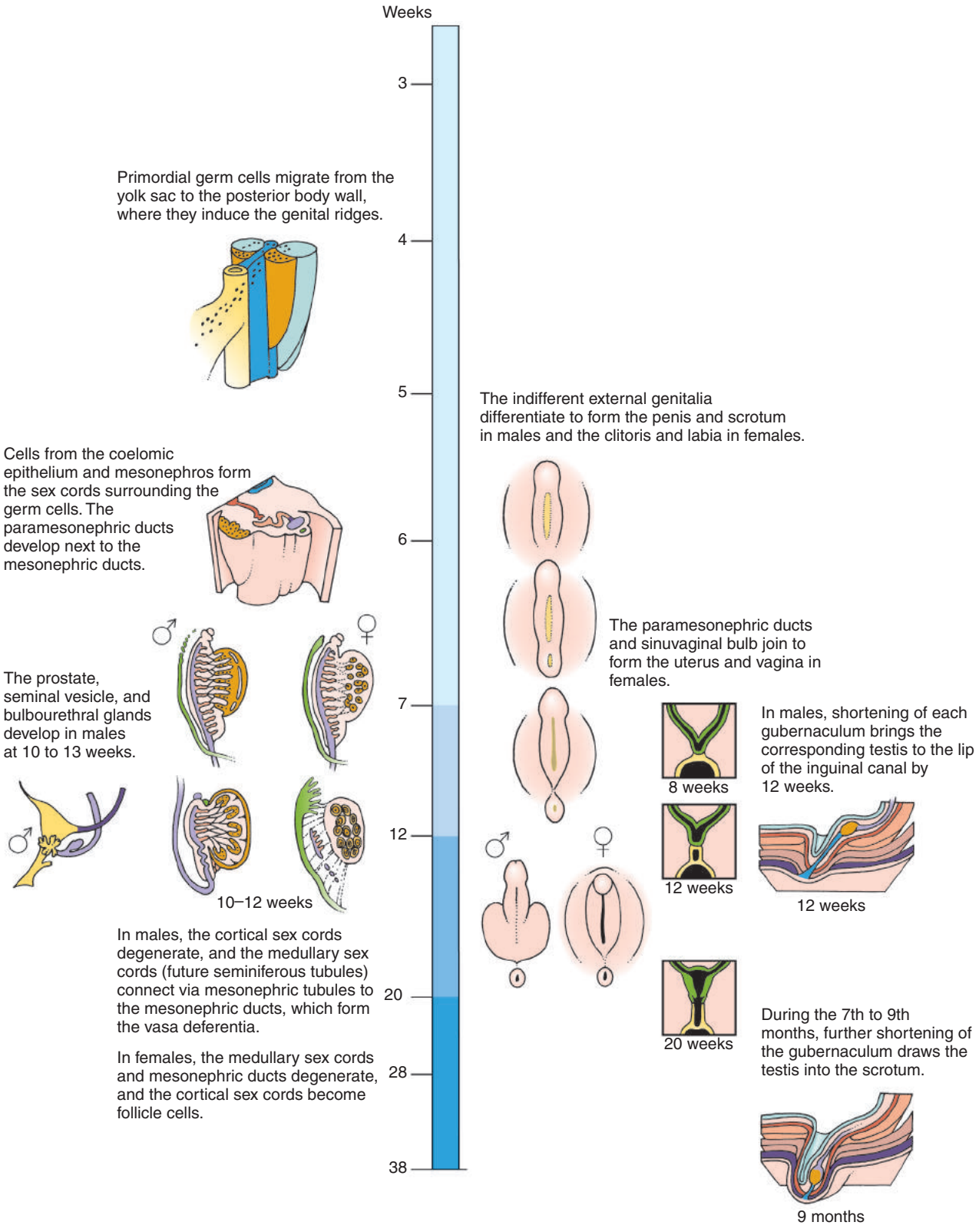
The gonad develops from both somatic and germ cells. The somatic cells are located at the ventral region of the mesonephros and arise from the mesonephric cells and the coelomic epithelium. Somatic cells become the Sertoli cells of the testis and the granulosa cells of the ovary. The germ cells migrate from a more inferior position on the yolk sac to the genital ridge, just medial to the mesonephros on each side (Fig. 85.2, upper left). This migration occurs between 4- and 6-weeks' gestation. Once the germ cells reach the gonadal ridge, they become surrounded by the somatic cells, which appear to regulate germ cell differentiation.

Gonadal sex is established by 7 weeks' gestation. At this stage the fetus contains two parallel internal ductal systems—wolffian and müllerian—and undifferentiated external genital primordia. The wolffian, or mesonephric, duct is a tubular structure that connects the capillary network of the mesonephros to the urogenital sinus. Evagination of the coelomic epithelium leads to formation of a second tubular structure adjacent to the mesonephric duct—the paramesonephric, or müllerian, duct. The distal ends of these two ducts are joined. That portion of the urogenital sinus distal to the termination of these ducts contributes to external genital development, whereas the proximal portion develops into the bladder, trigone, and posterior urethra.

Phenotypic sexual differentiation is predicated on establishing gonadal sex. If an ovary develops, the wolffian ducts involute because of a lack of local testosterone exposure, and only the terminal portion persists as a Gartner duct. In the absence of antimüllerian hormone (AMH), a glycoprotein secreted by the fetal Sertoli cells, the müllerian ducts develop into the proximal portion of the vagina, uterus, and fallopian tubes (see Fig. 85.2). The unfused cephalic portions of the müllerian ducts form the fallopian tubes, whereas the caudal ends fuse to form the ureterovaginal canal (see Fig. 85.2). The union of the fused caudal ends of the müllerian ducts and urogenital sinus forms the vagina. Thus, the proximal two-thirds of the vagina is of müllerian duct origin, and the distal third is of urogenital sinus origin. There is no fusion of the labioscrotal folds and no increase in clitoral–phallic structure in the absence of elevated levels of circulating androgens.

Male phenotypic differentiation is the result of the elaboration of two distinct testicular hormones: testosterone and AMH. These factors are produced and secreted by the 8-week stage of development, and they are essential for normal male differentiation. Involution of the müllerian ducts is caused by AMH. The remnants of the müllerian ducts persist caudally as the prostatic utricle and cephalically as the appendix testis. AMH exerts its action unilaterally and locally (exocrine secretion) rather than bilaterally via the systemic circulation.

Immediately after müllerian duct regression, the wolffian ducts develop under the local influence of testosterone secreted by the



• **Fig. 85.2** Embryologic timeline for gonadal development and development of internal and external genitalia. (From Larsen WJ. Human Embryology. New York, NY: Churchill Livingstone; 1993:237.)

fetal Leydig cells. The Leydig cells, like the Sertoli cells, differentiate from the mesenchymal cells within the gonadal ridges; this occurs at 9 to 10 weeks' gestation. Under the influence of testosterone, the wolffian ducts evolve into the epididymis, vas deferens, and seminal vesicles (see Fig. 85.2). The mesonephric tubules develop into the ductuli efferentes, which will provide continuity between the seminiferous tubules and rete testis to the vas deferens. This process occurs as a direct action of local testosterone on

the ductal structures. Elevated serum testosterone levels such as seen in congenital adrenal hyperplasia due to 21-hydroxylase deficiency in females do not cause wolffian duct stabilization.

Androgenization of the male external genitalia, fusion of labioscrotal folds, and movement of the urethral opening, starts at approximately 8 weeks' gestation (see Fig. 85.2). Androgenization relies on the ability of the tissues involved to convert testosterone into a more potent androgen: dihydrotestosterone (DHT). The target cells possess the enzyme 5α-reductase type 2, which is necessary for this conversion. In addition, an intact androgen receptor is necessary for the androgenization of the male external genitalia. By 12 to 14 weeks' gestation, formation of the male external genitalia is nearly complete. Androgen exposure after this time results in further phallic enlargement. Initially, testes are stimulated to make testosterone by maternal HCG during the first trimester but by 16 to 20 weeks' gestation, the fetus' own luteinizing hormone (LH) is driving testosterone production.

Testicular descent occurs in two stages. The initial transabdominal descent occurs at 8 to 15 weeks and depends on insulin-like 3 (INSL3) and its G protein–coupled receptor (GREAT) which are secreted by the testes. Other testicular factors likely also play a role, as most dysgenetic gonads are intraabdominal. Descent into the inguinoscrotal region occurs at 25 to 35 weeks and is androgen dependent.

Clinical Assessment of Differences of Sex Development

History

A detailed family history is important in the evaluation of ambiguous genitalia. Information on early neonatal deaths, consanguinity, or urogenital anomalies should be obtained. A family history of female infertility, amenorrhea, or müllerian duct abnormalities can be suggestive of a DSD. In one study of androgen insensitivity disorders that are X-linked, a positive family history of a sex differentiation disorder was often overlooked.[33]

The presence of maternal androgenization is suggestive of a variety of disorders that can affect the androgenization of the fetus. Features of maternal androgenization include hirsutism, severe acne, deepening of the voice, and clitoromegaly.

The ingestion of any recreational drugs, alcohol, or medications by the mother during pregnancy should be noted. Particular attention to medications with androgenic or progestational activity is indicated. Medications that affect fetal genital development include cimetidine, spironolactone, hydantoin, and progestational agents.[5] Maternal retention of progesterone-secreting intrauterine devices at the time of conception have also been associated with androgenization of a female fetus.[34] Progestational agents are used in assisted reproductive technology to support the pregnancy in the first trimester; therefore, asking if the pregnancy was conceived spontaneously or through the use of assisted reproductive technology is important.

Physical Examination

There is significant overlap of the genital anatomy among the various sex differentiation disorders. The physical examination, however, can provide the first clues to the underlying disorder. In addition, the physical examination will provide important information about the degree of androgenization of the external genitalia and the presence or absence of palpable gonads. Prader stage (Fig. 85.3A) is used to describe the androgenization of female external genitalia. The external masculinization score (see Fig. 85.3B) is used to describe the incomplete androgenization of male external genitalia.[35] The physical examination will also inform the examiner as to when the DSD occurred in fetal life.

Clitoris

Significant clitoral enlargement deserves careful evaluation. A point to keep in mind is that premature infants have relatively underdeveloped labia majora, so the clitoris may appear enlarged. A truly enlarged clitoris can be distinguished from a large clitoral hood by the presence of palpable corporal or erectile tissue.

Penis (Phallus)

Measurements of the phallic stretch length and middle shaft diameter are important in determining the degree of virilization. The phallus should be stretched and measured from the pubic ramus to the tip of the glans. Gestational age–corrected phallic stretch lengths are shown in Fig. 85.4. The presence of a chordee structure on the ventral surface of the phallus can impair measurement of the true phallic length (Fig. 85.5).[36] Chordee is the result of residual urethral tissue that continues to tether the phallus to the perineum. Measurement of the middle shaft diameter is particularly useful in this circumstance. For term male infants, a normal middle shaft diameter is approximately 1 cm.[36]

An isolated microphallus warrants careful evaluation for the presence of hypopituitarism (gonadotropin, ACTH, TSH, or growth hormone deficiency), particularly in the presence of hypoglycemia or unexplained jaundice. Microphallus and undescended testes may occasionally be the presenting phenotype for a DSD.

Labioscrotal Folds

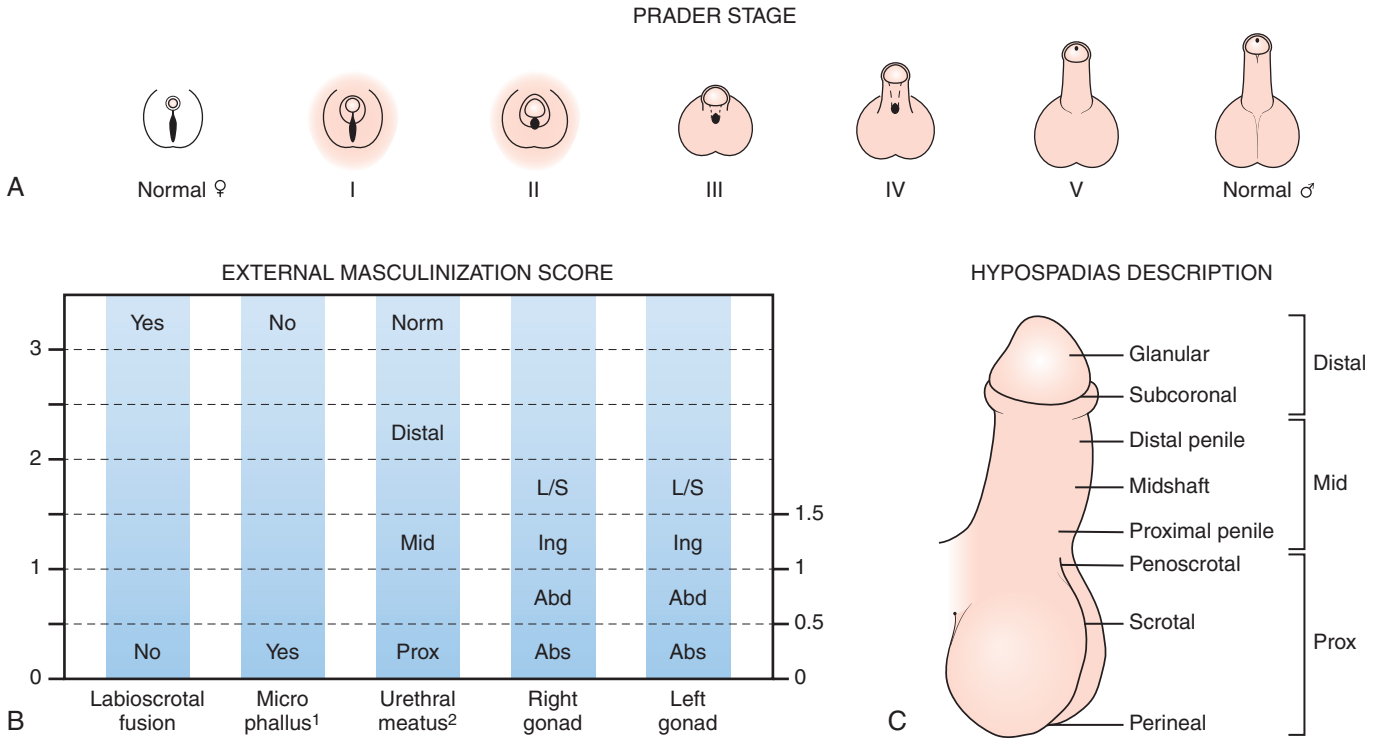
In neonates with ambiguous genitalia, assessment of the degree of fusion of the labioscrotal folds should be performed. When the infant is exposed to androgens during embryogenesis, fusion of the labioscrotal folds progresses from a posterior to an anterior direction. The spectrum of labial fusion can range from mild posterior fusion to complete labial fusion (Fig. 85.6). Fusion of the labioscrotal folds occurs in the first trimester. The examiner should note whether the folds are rugated or hyperpigmented. Is the phallus positioned in the normal superior position relative to the scrotum, or is there a shawl scrotum (penoscrotal transposition)? Is the scrotum fused normally in the midline, or is the scrotum bifid (see Fig. 85.5)?

Gonads

Careful examination for the presence of gonads should be performed in all infants with ambiguous genitalia. The presence of bilateral gonads in the labial folds is highly suggestive of a genetic male with incomplete androgenization (see Fig. 85.5). A unilaterally palpable gonad is often seen in infants with mixed gonad dysgenesis or ovotesticular DSD (Fig. 85.7), although other disorders such as androgen insensitivity can manifest themselves similarly. When cryptorchidism and hypospadias occur simultaneously, there is a greater than 25% chance of a DSD.[37,38]

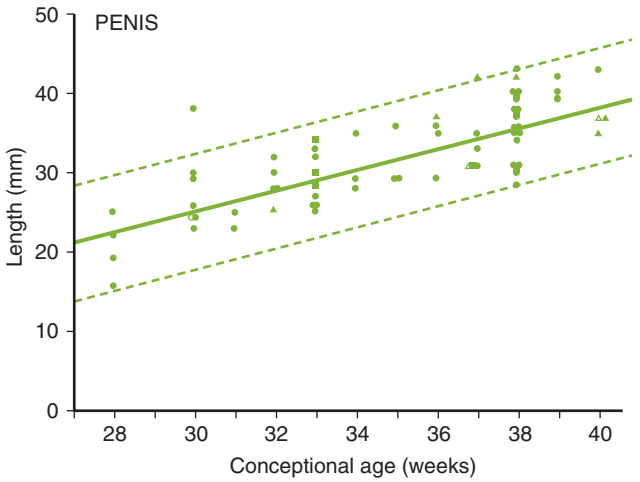
Hypospadias or Urogenital Sinus

The severity of hypospadias can differ, with the condition ranging from mild glanular hypospadias to penoscrotal hypospadias (see Fig. 85.3C), although most disorders of sexual differentiation manifest themselves with severe penoscrotal or scrotal hypospadias

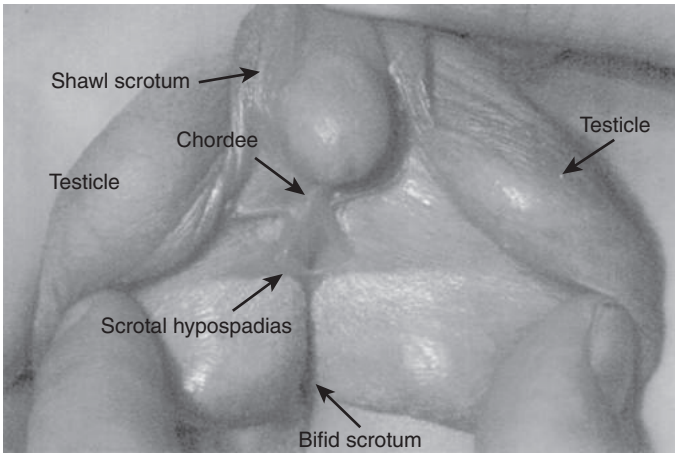


[1]Microphallus is a phallic length below the male reference range.
[2]The location of the urethral meatus is based on the location described in C.

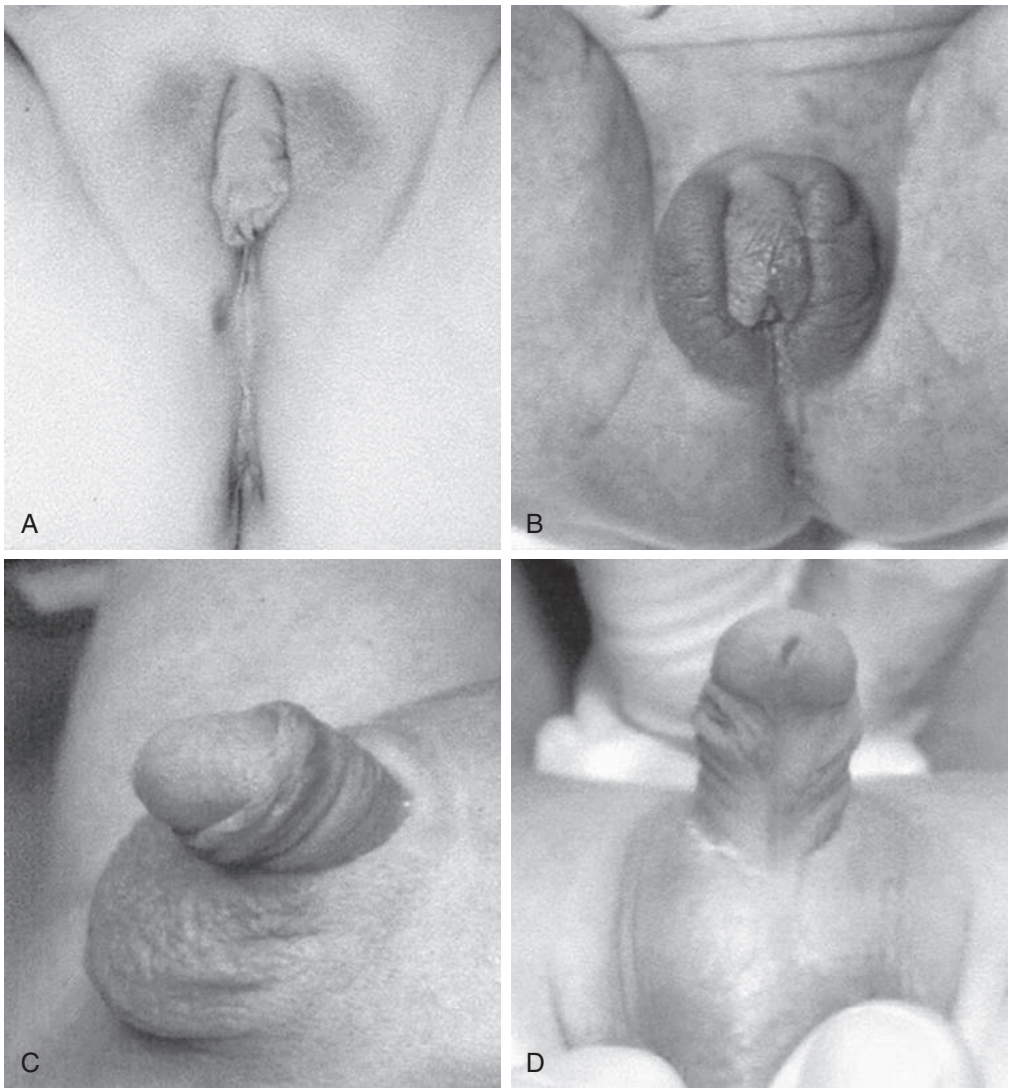
• **Fig. 85.3** (A) The stages of virilization of the female with congenital adrenal hyperplasia as developed by Prader. Stage I indicates mild clitoromegaly only; stage V indicates complete masculinization. (B) External masculinization score. A score of 0 to 3 is given for four aspects of male external genitalia. The sum of the four values is the external masculinization score. A normal male has a value of 12. (C) Hypospadias description. *Abd*, Abdomen; *Abs*, absent; *Ing*, inguinal; *L/S*, labioscrotal; *norm*, normal; *prox*, proximal. (A, from Migeon CJ, Berkovitz G, Brown T. Sexual differentiation and ambiguity. In: Kappy MS, Blizzard RM, Migeon CJ, eds. Wilkens' Diagnosis and Treatment of Endocrine Disorders in Childhood and Adolescence. 4th ed. Springfield, IL: Charles C. Thomas; 1994:573. B–C, from Ahmed SF, Achermann JC, Arlt W, et al; Society for Endocrinology UK guidance on the initial evaluation of an infant or an adolescent with a suspected disorder of sex development (Revised 2015). Clin Endocrinol. 2016;84(5):771–788.)



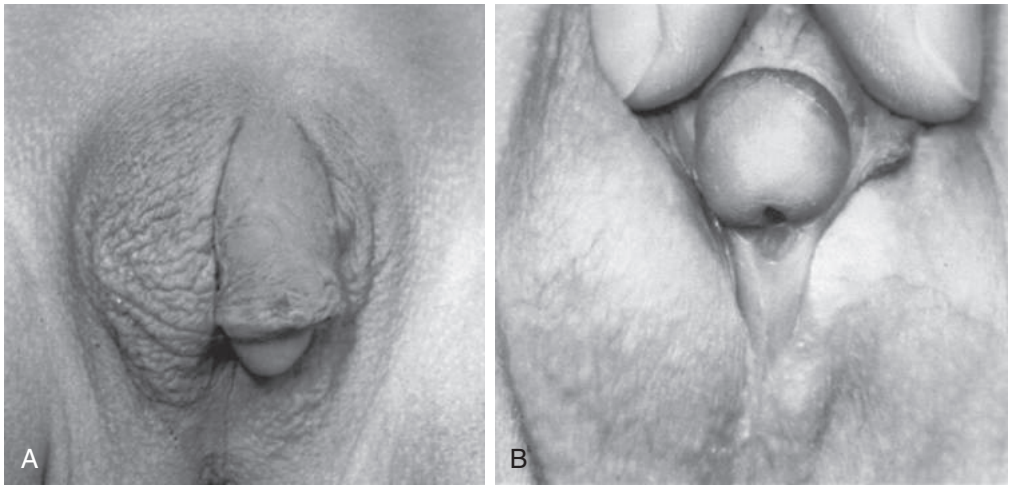
• **Fig. 85.4** Penis stretch length in 63 normal premature and full-term male infants *(circles)*, showing lines of mean ± two standard deviations. Superimposed are data for two small-for-gestational-age infants *(open triangles)*, seven large-for-gestational-age infants *(closed triangles)*, and twins *(squares)*. (Reproduced with permission from Feldman KW, Smith DW. Fetal phallic growth and penile standards for newborn male infants. J Pediatr. 1975;86:395–398.)



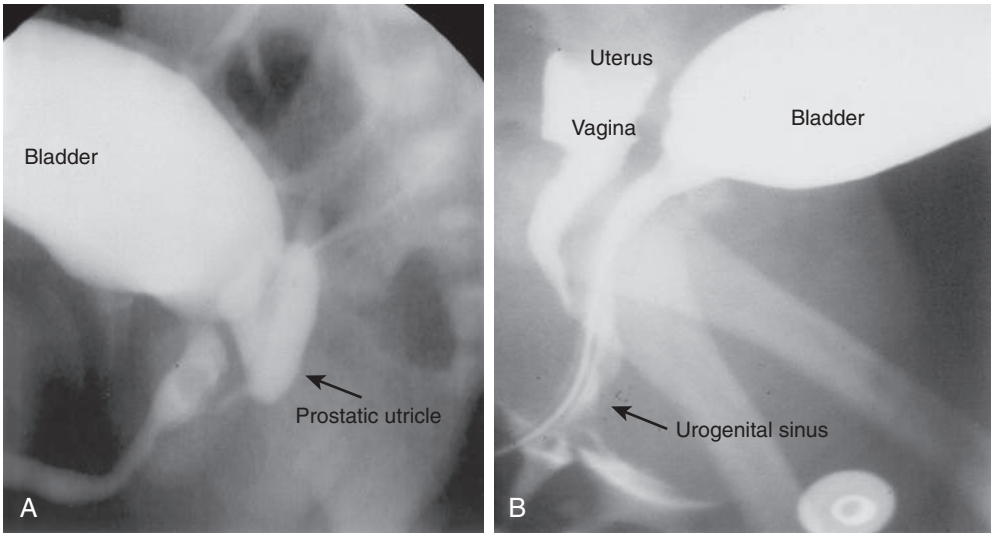
• **Fig. 85.5** Undervirilized male demonstrating bifid scrotum, scrotal hypospadias, chordee, and bilateral descended testes.



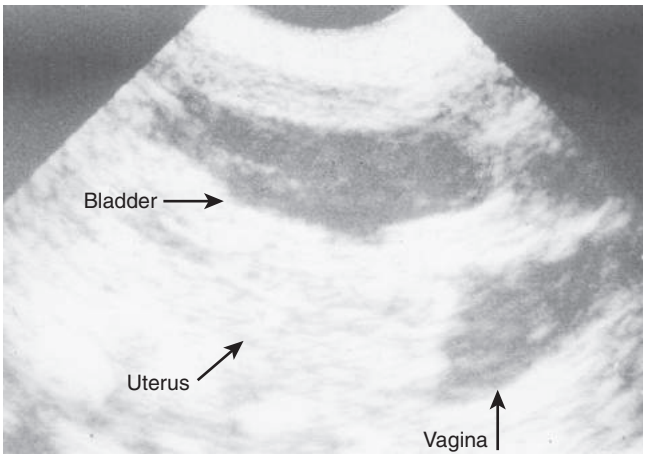
• **Fig. 85.6** Virilization of external genitalia in 46,XX congenital adrenal hyperplasia (21-hydroxylase deficiency). (A) There is a mild to moderate degree of virilization, with primarily clitoral hypertrophy and significant fusion of the labia. (B) Virilization is moderate, with clitoromegaly, labial fusion, and rugation of labial folds. (C, D) Complete masculinization is evident.



• **Fig. 85.7** Asymmetric external genitals with left unilateral descended testis (A), penoscrotal hypospadias, and chordee (B). Asymmetric external genital development or gonadal descent would be characteristic of mixed gonadal dysgenesis or ovotesticular disorder of sexual development.



• **Fig. 85.8** Genitourethrograms. (A) Genitogram from a 46,XY infant with microphallus and undescended testes with a clearly delineated prostatic utricle. (B) Genitogram from a 46,XX infant with congenital adrenal hyperplasia and severe masculinization of external genitals. The confluence of the vagina with the urogenital sinus is of intermediate severity.

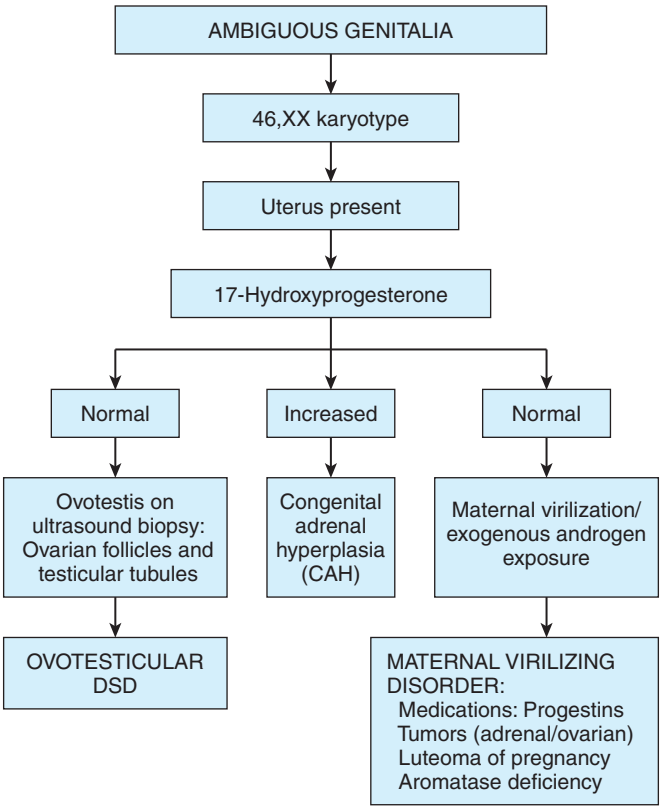


• **Fig. 85.9** Ultrasound image from a 46,XX infant with ambiguous genitalia. Note the presence of a well-developed uterus with an endometrial stripe.

(see Fig. 85.5). Examination for the presence of separate urethral and vaginal openings versus a single perineal opening (urogenital sinus) conveys important anatomic information. The vagina may be blind ending or completely formed. A urogenital sinus results from failure of the urologic and genital tracts to differentiate completely. In females exposed to androgens, the level at which the vagina enters the sinus (low-level versus high-level vaginal entry) has important implications for determining the ease of subsequent surgical exteriorization of the vagina (Fig. 85.8). In addition, when the urethra enters a urogenital sinus, there is potential for urinary stasis and therefore urinary tract infections. Excessive pigmentation of the genitals or signs of dehydration should alert the examiner to the possibility of congenital adrenal hyperplasia (CAH).

Dysmorphic Features

Dysmorphic features suggestive of Turner syndrome indicate the possibility of gonadal dysgenesis or mixed gonadal dysgenesis. Such abnormalities or multiple congenital anomalies could indicate any of a variety of syndromes associated with ambiguous genitalia (see Box 85.1).

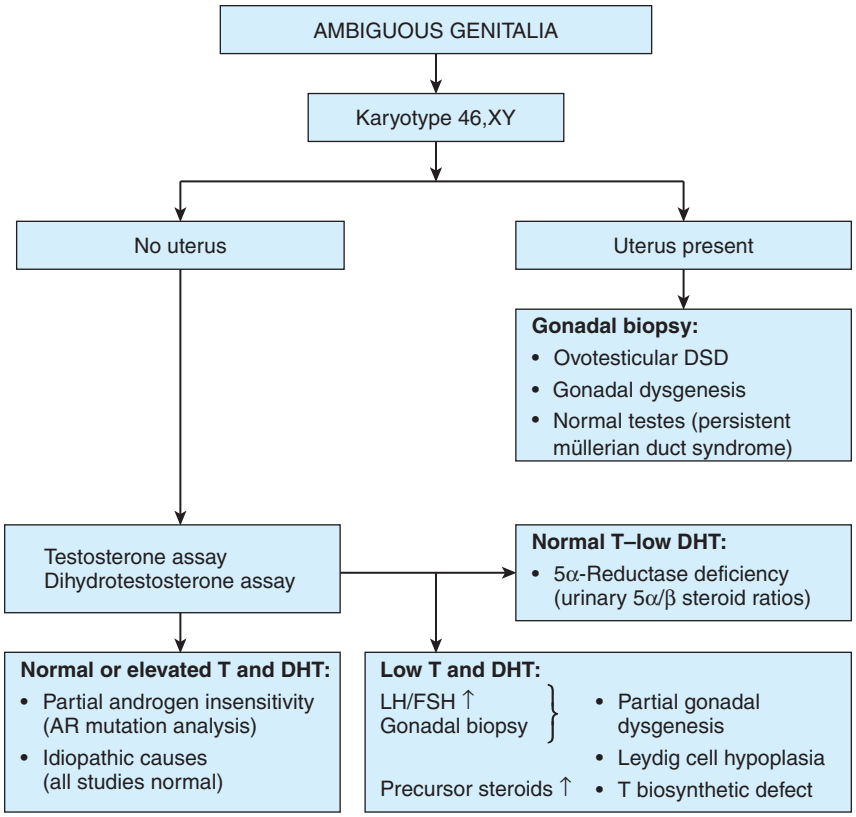


• **Fig. 85.10** Algorithm for evaluation of the 46,XX infant with ambiguous genitalia. The presence of a uterus would be determined radiographically by ultrasound examination, genitourethrogram, or magnetic resonance imaging.

Radiologic Investigations

Pelvic Ultrasonography

Pelvic ultrasonography reveals vital information in the evaluation of DSDs. The presence or absence of a uterus is a critically important determinant in the initial evaluation (Figs. 85.9–85.11). The newborn period is a time when the uterus,



• **Fig. 85.11** Algorithm for evaluation of the infant with ambiguous genitalia and 46,XY karyotype. The presence or absence of a uterus would be determined by radiographic imaging, including ultrasound examination, genitourethrogram, or magnetic resonance imaging, as appropriate. Testosterone *(T)* and dihydrotestosterone *(DHT)* levels ideally should be obtained between 2 weeks and 3 months during the mini-puberty. *AR*, Androgen receptor; *FSH*, follicle-stimulating hormone; *LH*, luteinizing hormone.

ovaries, and adrenal glands are optimally visualized.[39] The presence of a well-developed uterus will direct the differential diagnosis toward a condition leading to androgenization of a genetic female's external genitalia, 46,XY ovotesticular DSD, 46,XY complete gonadal dysgenesis, or persistent müllerian duct syndrome (PMDS); however, only a rudimentary uterus may be seen in 46,XY gonadal dysgenesis or ovotesticular DSD. Importantly, the inability to identify a uterus on ultrasound is not conclusive for lack of a uterus. Ultrasonography for testes location has sensitivity and specificity of 45% and 78%[40] and is not recommended per current American Urologic Association (AUA) guidelines.[41] Ultrasound examination can determine whether the adrenal glands appear enlarged, as in CAH; however, normal adrenal size does not rule out CAH.

Genitourethrogram

A genitourethrogram is a fluoroscopically-guided genital dye study that can provide important information on the urethra and internal genital ducts.[39] An experienced radiologist should perform this study. It is important to ensure that all perineal orifices are examined. The main features to be noted are the presence or absence of a vagina (or prostatic utricle) and the relationship between the vagina and the urethra (see Fig. 85.8). Demonstration of the level at which the vagina opens into the urogenital sinus and its relationship to the external sphincter has important surgical implications. Recognition of male or female urethral configurations may also be possible during genitourethrography.

Magnetic Resonance Imaging

Magnetic resonance imaging (MRI) has been used to assess the internal genitalia of a limited number of infants and children with genital differentiation disorders. The strength of MRI lies in its ability to image large areas in multiple planes and characterize soft tissues. Detailed information about müllerian and wolffian structures and the position of the gonads can be obtained; however, thin sections (3 to 5 mm) are required for an adequate study. Streak gonads remain difficult to visualize. MRI has the capability to differentiate between an enlarged clitoris and a penis, because the bulbospongiosus muscle and transverse perineal muscle are absent or poorly visualized in the virilized female.[39] MRI is a promising modality for the evaluation of ambiguous genitalia as it offers greater sensitivity and specificity than other studies, but it is more expensive, and anesthesia may be required.

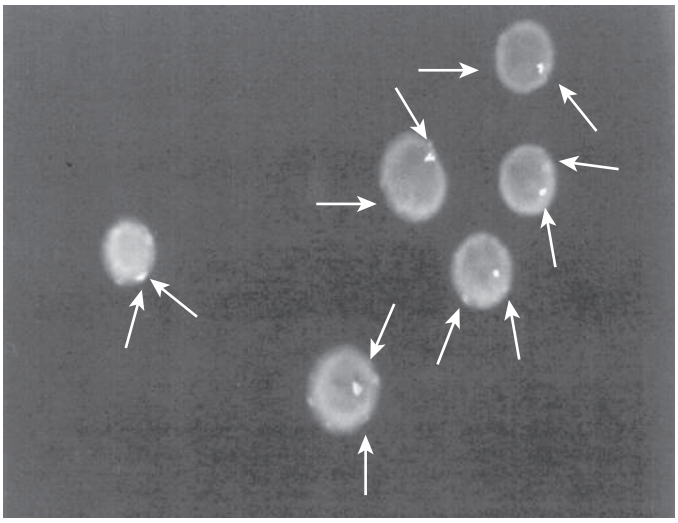
Laboratory Investigations

Endocrine and genetic laboratory studies are germane in the evaluation of ambiguous genitalia in the newborn. Day 1 of life is an ideal time to obtain serum testosterone and dihydrotestosterone (DHT) levels by liquid chromatography—tandem mass spectrometry (LC–MS/MS), as testosterone levels fall rapidly in the first several days of life.[42] It is important to use LC–MS/MS assays as other assays measure interfering substances that will falsely elevate the testosterone level.[43] Chromosomal studies should optimally be performed on day 1, as a preliminary karyotype can be available

by 24 to 48 hours with use of the fetal nucleated red blood cells. Fluorescence in situ hybridization (FISH) for *SRY* should also be performed. On day 2 or day 3 of life, determinations of serum 17-hydroxyprogesterone (17-OHP), 17-hydroxypregnenolone, 11-deoxycortisol, dehydroepiandrosterone (DHEA), androstenedione, and plasma renin activity are performed if CAH is suspected. Some clinicians perform an adrenocorticotropic hormone (ACTH) stimulation test at this time to more clearly demonstrate a block in steroid biosynthesis. However, if the ACTH level is already elevated, then additional information from an ACTH stimulation test will not be gained. Results of these studies should be sent immediately to the appropriate reference laboratory for analysis. Alerting the reference laboratory to the urgent nature of the studies performed will facilitate rapid processing. 17-OHP levels are physiologically elevated on the first day of life, and screening for CAH should preferably not be done at this time. Serum gonadotropins are often suppressed in the immediate newborn period, so they should be measured after 1 week of life. LH and follicle-stimulating hormone (FSH) levels are helpful in assessing newborns for androgen insensitivity syndrome (AIS), gonadal dysgenesis, and LH receptor abnormalities. Repeated LH, FSH, testosterone, and DHT testing should be done between 2 and 8 weeks of life in the evaluation of incomplete androgenization in males. This period coincides with the physiologic testosterone surge (mini-puberty) seen in healthy male infants.[42] AMH and inhibin B levels can be obtained at any time. When CAH is suggested, it is important to check sodium and potassium levels daily to prevent a salt-wasting crisis. An elevated renin level with a lower sodium level is concerning for impending salt wasting crisis, and therapy with hydrocortisone, fludrocortisone and sodium chloride should be initiated.

A human chorionic gonadotropin (hCG) test has been used in the past in the evaluation of suspected incomplete masculinization of the genetic male. However, if LH level is elevated, then hCG testing is not useful as it will not provide any additional information. HCG, as does LH, binds the LH receptor and stimulates the testes to synthesize sex steroids. A testosterone response greater than 200 ng/dL rules out a testosterone biosynthetic defect and is considered a normal response to hCG (a value less than 200 ng/dL may be normal in some assays as the assays have become more specific for testosterone). A normal testosterone-to-DHT ratio of less than 8:1 argues against 5α-reductase type 2 deficiency, with the caveat that the testosterone level must be high enough to adequately test the enzyme function. The gold standard for 5α-reductase type 2 deficiency is genetic testing, as the testosterone-to-DHT ratio can be misleading. In addition, the hCG test can result in phallic enlargement if there is a good testosterone response. An increase in phallic length of 0.25 to 0.75 cm in six 46,XY males with idiopathic microphallus was reported within 5 days of beginning injections.[44] A bolus of 1500 international units (IU) hCG was given intramuscularly on 3 consecutive days, with steroids and phallic length measured on the fifth day. Growth of the phallus in response to hCG suggests that the phallus will further virilize at puberty, although no longitudinal study has documented this assumption. An alternative is to give testosterone to see if there is increase in the phallus and to look at T:DHT. This bypasses the testes from producing testosterone at a nonphysiologic age, which could have negative effect on the testes.[45]

FISH can rapidly determine the sex chromosome complement of the newborn by use of X chromosome- and Y chromosome-specific centromeric probes (Fig. 85.12).[46] In addition, this method allows the detection of low levels of chromosomal mosaicism, because hundreds of cells can be analyzed rapidly. FISH is also useful in identifying an *SRY* gene translocated to an X chromosome.[47] FISH analysis for determination of sex chromosome constitution has been shown to be highly reliable, although this method has not been used extensively in the evaluation of ambiguous genitalia of the newborn. FISH will only determine the presence of the X and Y centromere and SRY if requested. It will not identify large deletions or insertions of the sex chromosomes. Therefore, results should be interpreted with some degree of caution until confirmation by karyotypic analysis is available. If there is concern for additional congenital anomalies, then a single nucleotide polymorphism (SNP) chromosome microarray may be helpful to look for microdeletions and microduplications, as well as sex chromosome complement, but the turnaround time may be longer.



• **Fig. 85.12** Fluorescence in situ hybridization technique demonstrating the sex chromosome constitution of peripheral blood leukocytes. *Arrows* point to sex chromosomes in each cell.

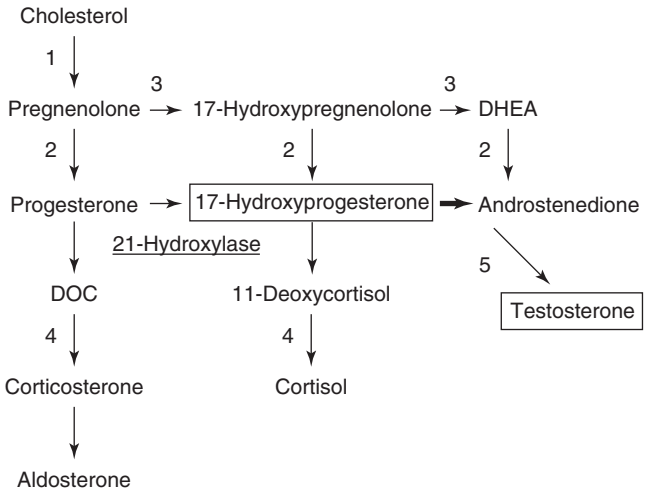
In a small percentage of ambiguous genitalia cases, laparoscopy with gonadal biopsy is necessary to confirm the diagnosis of ovotesticular DSD, gonadal dysgenesis, or Leydig cell aplasia. Obtaining a karyotype from gonadal tissue may be helpful when sex chromosome mosaicism or chimerism is suggested.

46,XX Differences in Sex Development

Androgenization of the Female

Androgenization of an XX neonate is most commonly caused by CAH, although other virilizing conditions can be involved (see Fig. 85.10). CAH encompasses a group of disorders of adrenal steroid hormone biosynthesis, of which more than 90% to 95% are due to 21-hydroxylase deficiency (OHD) (Fig. 85.13).[48]

Occasionally, the presence of excess androgens of maternal origin can result in androgenization of the female fetus.[5] A unique cause of both maternal and fetal masculinization is placental aromatase deficiency.[49] The degree of androgenization of the external genitalia of 46,XX fetuses is dependent on the timing and magnitude of androgen exposure of the fetus. Exposure of the female fetus to high androgen levels before 12 weeks' gestation results in fusion of the labial folds, with formation of a urogenital sinus.



• **Fig. 85.13** Steroid biosynthetic pathway demonstrating the defect in 21-hydroxylase deficiency *(solid bars)* and accumulated precursor 17-hydroxyprogesterone. Note the increased shunting into androgen-producing pathways. Steroidogenic enzymes are indicated as follows: (*1*) steroidogenic acute regulatory protein and side chain cleavage, (*2*) 3β-hydroxysteroid dehydrogenase, (*3*) 17α-hydroxylase/17,20-lyase, (*4*) 11β-hydroxylase, and (*5*) 17β-hydroxysteroid dehydrogenase. *DHEA*, Dehydroxyepiandrosterone; *DOC*, deoxycorticosterone.

In severely androgenized cases the external genitalia may appear completely male (see Fig. 85.6C and D). High-level androgen exposure after 12 weeks' gestation will result in mainly clitoral hypertrophy but with separate labial folds (see Fig. 85.6A). 46,XX testicular DSD occurs when the *SRY* gene is translocated from the Y chromosome to an X chromosome. Most patients with *SRY*-positive 46,XX testicular DSD have normal male external genitalia, but occasionally the genitalia are ambiguous.[47,50,51]

Congenital Adrenal Hyperplasia

21-OHD is the most common cause of ambiguous genitalia in the newborn female.[48] 21-OHD has a population frequency of approximately 1 in 15,000, and the disorder is inherited in an autosomal recessive fashion. Males and females are equally affected; however, in classic cases, females have androgenization of their external genitalia at birth, resulting in the clinical presentation of ambiguous genitalia.

Deficiency of 21-hydroxylase results in excess accumulation of the substrate 17-hydroxyprogesterone (17-OHP), which is shunted into the androgen synthesizing pathway and results in excess levels of androstenedione (see Fig. 85.13). Androstenedione is then converted peripherally to testosterone or DHT using the backdoor pathway.[52] The excess production of androgens results in androgenization of the external genitalia of the female fetus, whereas a male infant with 21-OHD is phenotypically normal. The degree of androgenization in the female is variable and can range from mild enlargement of the clitoris to complete fusion of the labioscrotal folds and urethral opening at the tip of the phallus (see Fig. 85.6). The degree of androgenization can be classified by Prader stages, ranging from I to V (see Fig. 85.3A). Stage I represents mild enlargement of the clitoris only. Stage V represents complete androgenization of the external genitalia, with intermediate stages designating lesser degrees of involvement. The internal genitalia (ovaries, uterus, and fallopian tubes) of the females with androgenization of external genitalia are normal, however, and wolffian duct–derived structures are absent, as there was no local exposure to testosterone (see Chapter 84 for more details).

The diagnosis of 21-OHD should be suspected in all newborns with ambiguous genitalia (or clitoral hypertrophy) and absent gonads in the labial or scrotal folds. A serum 17-OHP level obtained after the first day of life will often be diagnostic. 17-OHP levels are usually 50-fold to 100-fold above the normal range in classic cases, with typical random levels of 10,000 ng/dL or higher.[48] In some cases of 21-OHD, ACTH stimulation testing may be necessary to establish the diagnosis. If the ACTH level is already significantly elevated, however, an ACTH stimulation test will not provide additional information.

The levels of androstenedione, testosterone, 11-deoxycortisol, electrolytes, and plasma renin activity should also be determined. Plasma renin activity is a sensitive indicator of the intravascular volume status of the infant. Impaired sodium–potassium and sodium–hydrogen exchange owing to aldosterone deficiency in the distal tubule of the kidney results in hyponatremic dehydration, hyperkalemia, and metabolic acidosis.

Assessment for the less common forms of CAH resulting in ambiguous genitalia in an XX infant—11β-hydroxylase deficiency (11β-OHD), 3β-hydroxysteroid dehydrogenase (3β-HSD) deficiency, and P450 oxidoreductase deficiency—includes measurement of 11-deoxycortisol, deoxycorticosterone (DOC), DHEA and 17-hydroxypregnenolone in addition to the usual measurements of 17 OHP, androstenedione, cortisol and testosterone when evaluating for CAH. An elevated ACTH level may also be useful. Infants, though, who are stressed will have elevated ACTH and cortisol levels. Use of a comprehensive steroid panel from a reliable reference laboratory will limit the amount of blood required to 3 to 5 mL.

11β-OHD accounts for approximately 5% of CAH cases worldwide, and this disorder will also result in androgenization of the female fetus (see Fig. 85.13). However, the presence of volume overload and hypertension distinguishes 11β-OHD from 21-OHD.[48] Presumably, the hypertension in 11β-OHD is caused by the excess mineralocorticoid activity of the DOC metabolite. Typically, 11-deoxycortisol level is elevated and plasma renin activity is suppressed in this disorder. However, hypertension may not present in the young infant, and thus absence of hypertension does not exclude 11β-OHD.

3β-HSD deficiency may result in ambiguous genitalia in the newborn period (see Fig. 85.13). Unlike 21-hydroxylase, this enzyme is present in the gonads as well as the adrenal glands, and deficiency of 3β-HSD can result in mild androgenization of the female infant and incomplete androgenization of the male infant. This enzyme deficiency also results in excess production of the steroid DHEA, which can be converted to more potent androgens peripherally. The female infant with this disorder can have clitoromegaly, although such children are often phenotypically normal. 3β-HSD is needed for testosterone biosynthesis, leading to inadequate androgenization of the male fetus. A marked increase in the ratios of 17-hydroxypregnenolone to 17-OHP and of DHEA to androstenedione is diagnostic in mutation-positive forms of this disorder.[53]

P450 Oxidoreductase Deficiency

P450 is necessary for the function of 17,20 lyase, 21 hydroxylase, and aromatase. In POR deficiency, the hormonal picture is

quite mixed with partially deficient 17,20 lyase deficiency with or without 21 hydroxylase and aromatase deficiency. Sequencing of the *POR* gene may be necessary to determine the diagnosis. Depending on the specific mutation in *POR*, there may or may not be maternal virilization during the pregnancy. Maternal estriol levels may be low. XX infants may have ambiguous genitalia as well as skeletal defects associated with the Antley-Bixler syndrome.[54]

Sex assignment for 46,XX infants with 21-OHD has traditionally been female.[48,55–58] Gender identity of 46,XX adults with CAH is typically female, with various degrees of gender expression.[8,13,59] Prenatal androgenization affects gender-related behavior, but not gender identity, in girls with CAH aged 5 to 12 years.[60] Cases of gender identity disorder have been reported in treated females with CAH,[2] and cases of gender reassignment from female to male have been reported.[14] Individuals with undiagnosed 46,XX CAH who are profoundly virilized have functioned successfully as males[61–63] which has prompted some to suggest male sex assignment in profoundly virilized 46,XX infants. However, female sex assignment is likely to prevail in current practice until evidence is obtained to indicate otherwise.[64,65] 46,XX females with CAH are infertile.

In addition to medical therapies for CAH, efforts to normalize the appearance of the external genitalia may be pursued. It should be kept in mind that hypertrophy of the clitoris will gradually lessen after medical therapy is instituted; however, complete normalization in the more virilized cases is not likely to occur. In severe cases of clitoral enlargement, clitoral recession surgery is a treatment option, although suboptimal cosmetic results have been reported in long-term outcome studies. Atrophy or loss of the clitoris or excessive regrowth of clitoral tissue has been described in examinations of adolescent and adult patients who underwent genital surgery in early childhood.[66,67] If CAH is not well controlled with hormonal suppression of the adrenal gland, there will be clitoral enlargement in response to the elevated testosterone levels. The risk of surgery needs to be balanced against the potential detrimental effects of masculinized genitalia on the development of a poor body image[14] and of social stigmatization by family or community members.[68] Nerve-sparing ventral clitoroplasty in virilized females has been shown to preserve dorsal nerves for better sensitivity after surgery.[69]

Surgical restoration of the vagina in CAH is performed to exteriorize the vagina and to enlarge the vaginal opening so that successful intercourse can occur later in life. There is considerable debate about when to perform vaginal exteriorization surgery. A number of studies have demonstrated the development of vaginal stenosis when vaginoplasties are performed in the prepubertal period.[66,67,70] The authors advocate delaying vaginoplasty until puberty or later, when manual dilation can be undertaken by the patient, and estrogenization of the vaginal mucosa can help to prevent stricture formation. Others recommend that vaginoplasties be undertaken early in life, because the procedure is technically easier in the first several years of life, and the emotional trauma of a major surgery in adolescence is avoided.[55,71] Reports of long-term outcome of genitoplasty by patient advocate groups such as the Accord Alliance and others have called for a general moratorium on all nonessential genital surgery in infancy until affected individuals are old enough to express their wishes and give consent.[63] Problems with loss of sexual sensation and pleasure have been reported in adult patients because of genital surgery in early life.

Increased Levels of Maternal Androgens and Progestins

Androgenization of the female fetus has been reported in pregnant mothers taking various progestational agents to prevent miscarriage. These agents include norethindrone, ethisterone, and medroxyprogesterone. Danazol, which has been used in the treatment of endometriosis, has also been associated with fetal masculinization.[5] A recent report identified retained progesterone secreting intrauterine devices during the first trimester also resulted in androgenization of the XX fetus's external genitalia with labial fusion.[34]

Androgenization of the female fetus because of maternal virilizing ovarian or adrenal tumors or luteomas of pregnancy has been reported. In such cases, virilization beyond the time of birth does not occur, and the prognosis is good.[5]

Placental Aromatase Deficiency

Placental aromatase deficiency has been associated with androgenization of the female fetus. Aromatase is a cytochrome P450 enzyme responsible for the conversion of testosterone to estradiol and of androstenedione to estrone. Autosomal recessive inheritance of aromatase deficiency causes androgenization of the female external genitalia because of a failure to metabolize the large amounts of androstenedione and testosterone produced by the placenta. Placental aromatase deficiency will also cause significant virilization of the mother. The affected female infant will have androgenization of external genitalia with normal müllerian structures. The levels of gonadotropins are elevated in infancy, and ovarian cysts may develop. At puberty, females have hypergonadotropic hypogonadism with failure to feminize and progressive virilization. Plasma androstenedione and testosterone levels are elevated, whereas estrone and estradiol levels are low. Postpubertal individuals have delayed bone maturation, tall stature, and osteopenia.[49]

46,XX Ovotesticular and 46,XX Testicular Disorders of Sex Development

46,XX ovotesticular DSD is defined as the presence of both testicular tissue with distinct seminiferous tubules and ovarian tissue containing mature graafian follicles in a single individual with a 46,XX karyotype. Both testicular and ovarian elements may be found in the same gonad, or one testis and one ovary may be found in the same individual. In most cases, the karyotype is 46,XX; 46,XX/46,XY chimerism and 46,XY karyotypes can also be found. Clinically the external genitalia are often ambiguous, but also predominantly male or female phenotypes have been described.[5,72] In ambiguous cases, a relatively marked degree of virilization can be found. Almost all have some degree of hypospadias and incomplete labioscrotal fold fusion. The labioscrotal folds are asymmetric, with an appearance of a hemiscrotum on one side and labium majus on the other being seen in 10 of 22 cases.[72] At least one gonad is usually palpable. Palpation of a polarized gonad should also lead the clinician to suggest the diagnosis of ovotesticular DSD. A vagina and a uterus are present in most patients, and a genitourethrogram may provide further elucidation. Internal duct development is consistent with the associated gonad, although müllerian ducts predominate with an ovotestis. In one study, 11 of 12 individuals with 46,XX ovotesticular DSD

examined before 6 months of age had baseline testosterone levels greater than 40 ng/dL; normal levels for females of this age would be less than 15 ng/dL.[72] Breast development is common during puberty, and menses can occur in up to 50% of individuals. Virilization can also occur at puberty.

Sex assignment depends on the degree of androgenization, the capacity of testicular tissue to secrete testosterone, and the presence or absence of a uterus and fallopian tubes. In general, in less androgenized individuals with a 46,XX karyotype, a female sex assignment may be favored because there is the possibility of fertility with oocyte development in the ovarian tissue but virilization will occur from testosterone production by the testes at puberty. If the infant is raised male, there will be lack of spermatogenesis as the genes for spermatogenesis are on the Y chromosome and the testes will be small due to lack of spermatogenesis. Gynecomastia often occurs at puberty. Removal of ductal or gonadal structures not consonant with the sex of rearing is controversial as gender identity is not known in infancy. As in all cases of DSD, gender identity and behavior issues, along with general psychological well-being, should be evaluated longitudinally.

The formation of a testis or an ovary from the bipotential gonad is controlled by increased expression or repression of key pro-testis, anti-testis and pro-ovarian genes. Disruption of the expression pattern can result in a different gonad developing despite the gonads chromosomal complement. The p.Arg92Trp and p.Arg92Gln variants of the *NR5A1* gene has been found to cause ovotesticular DSD in almost 10% of the ovotesticular cases through loss of the repression of the testicular pathway in an XX individual.[73,74] The p.Arg92Trp variant has decreased ability to upregulate the expression of the anti-testis gene, DAX1, leading to decreased repression of SOX9 expression which leads to testis formation rather than ovarian formation. The p.Arg92Gln change has been associated with adrenal insufficiency with or without ovotesticular DSD.[75] *NR2F2*, encoding *COUP-TF2*, has also been associated with ovotesticular/testicular DSD and cardiac defects. Two children also had blepharophimosis-ptosis-epicanthus inversus syndrome.[76] Bashamboo et al.[76] propose that COUP-TF2 is a "pro-ovary" and "anti-testis" factor in the XX gonad. Homozygous mutations in *RSPO1*, encoding R-spondin1 which is a pro-ovarian gene can lead to ovotesticular/testicular DSD associated with hearing impairment and eye and skin abnormalities.[77]

In contrast to 46,XX ovotesticular DSD, 46,XX testicular DSD presents 90% of the time with normal male external genitalia and 10% with hypospadias. 46,XX testicular DSD and 46,XX ovotesticular DSD may be part of a continuum as there are families with different members having 46,XX testicular or 46,XX ovotesticular DSD. In the past, 46,XX testicular DSD was not often diagnosed until puberty, when a male may be noted to have small testes and/or gynecomastia, or later as an adult when he presented with infertility due to absence of the Y chromosomal genes for spermatogenesis. Unlike Klinefelter syndrome, individuals with 46,XX testicular DSD are not tall as they lack the Y chromosome and three copies of the *SHOX* gene which those with Klinefelter syndrome have. With the increased use of prenatal cell-free DNA testing, the diagnosis of 46,XX testicular DSD may be made at birth when there is discordance between genotype XX and phenotype male. Approximately 80% of the time, there is translocation of the SRY gene from the Y chromosome to the X chromosome during the obligate crossover of the Xp and Yp terminal ends during male meiosis.[50] In those who lack SRY, mutations have been found in *NR5A1* and *NR2F2*[73,76] which further support the idea that XX ovotesticular DSD and XX testicular DSD are part of a continuum. Duplications of the *SOX9* locus or three copies of SOX9 result in overexpression of SOX9 and leads to the development of XX testicular DSD.[78] Over expression of SOX3[79] on the X chromosome or SOX10[80] from chromosome 22q13 may be the etiology for 46,XX ovotesticular or 46,XX testicular DSD. Loss of function changes in *RSP01*, a pro-ovarian gene, have also been seen in 46,XX testicular DSD.[81] WNT4, which upregulates *DAX1* expression, is another pro-ovarian gene. Autosomal recessive changes in WNT4, leading to decreased function, can also lead to 46,XX ovotesticular/testicular DSD associated with IUGR, oligohydramnion, renal agenesis, adrenal hypoplasia, and lung and cardiac anomalies or SERKAL syndrome.[82]

Disorders of Ovarian Development

Heterozygous mutations in *WNT4* in 46,XX individuals have been associated with Mayer-Rokitansky-Kuster-Hauser syndrome and androgen excess.[83] Disorders of the ovary are usually not identified until the girl fails to enter puberty as the ovary is quiescent until puberty. Most often, primary ovarian failure is due to sex chromosome aneuploidy, 45,X, which is discussed later. Blepharophimosis, ptosis, epicanthus inversus syndrome is associated with premature ovarian insufficiency due to mutations in *FOXL2*.[20]

46,XY Differences in Sex Development

Disorders of Testosterone Biosynthesis and Action

Incomplete androgenization refers to absence of or incomplete androgenization of the external and internal genitalia in a person with a 46,XY karyotype and normal testes. Disorders leading to incomplete androgenization of the male include androgen receptor defects, testosterone biosynthetic defects, 5α-reductase deficiency, Leydig cell hypoplasia, and effects of maternal medications (see Fig. 85.11).

In 25% to 50% of underandrogenized males, a specific cause cannot be found.[84–86] Other factors such as medications and placental insufficiency may potentially interfere with genital androgenization. Placental insufficiency may be related to genital underdevelopment through the presumed mechanism of inadequate hCG production. Placental hCG is required for early fetal testosterone production and, therefore, early fetal genital development.

Medications such as cimetidine, spironolactone, phenytoin (Dilantin), phenobarbital, medroxyprogesterone, and cyproterone acetate have been associated with altered androgen action or metabolism. Their use during pregnancy may be detrimental to male genital development.[5,55] Furthermore, various xenobiotics can bind the androgen receptor; therefore, there has been speculation regarding the role of environmental factors in abnormal sex differentiation.[87,88]

Androgen Receptor Defects (Androgen Insensitivity)

Disorders of the androgen receptor are the most common definable cause of incomplete masculinization of the genetic male.[89] Disorders of the androgen receptor can be divided into complete

androgen insensitivity syndrome (CAIS) and partial androgen insensitivity (PAIS) syndrome. The gene for the androgen receptor (*AR*) is located on the X chromosome, and more than 800 germline mutations have been found.[90] Information regarding the *AR* mutations can be found in the McGill Androgen Receptor Gene Mutation Database (http://www.androgendb.mcgill.ca). Mutations have been found throughout the *AR* gene, with most mutations occurring in the DNA- or steroid-binding domains. Despite extensive characterization of the molecular genetics of *AR* mutations, no genotype–phenotype correlation has been found.[89]

The sex-differentiating actions of testosterone and DHT are mediated by the androgen receptor. DHT is important in the differentiation of the male external genitalia and prostate, whereas testosterone is important in the differentiation of internal wolffian ducts to epididymis, vas deferens, and seminal vesicles.

Complete Androgen Insensitivity

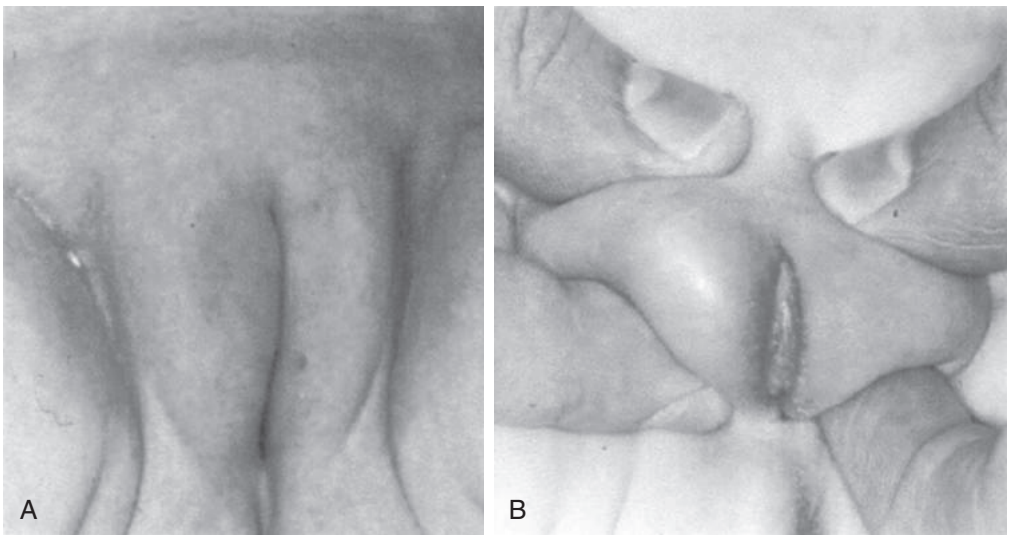
CAIS in a 46,XY is due to a mutation of the androgen receptor which is located on the long arm of chromosome X. CAIS is characterized by phenotypically normal female external genitalia in a 46,XY neonate (Fig. 85.14). Affected girls may have an inguinal hernia before puberty or primary amenorrhea with lack of pubic hair after puberty onset. With the increased use of noninvasive cell free DNA testing, many female infants are diagnosed at birth due to discordance between the cell free DNA which revealed an XY complement, and the female phenotype. Robust breast development occurs at puberty due to peripheral aromatization of testosterone to estrogen. There is usually scant or absent pubic and axillary hair, with some vulvar hair. The vagina is short and blind ending, and müllerian structures (cervix, uterus, fallopian tubes) are absent because of testicular production of AMH. There are only vestigial or no wolffian duct–derived internal structures. The testes are located in the abdomen, inguinal canal or in the labia majora. Even though there is testosterone production at puberty, in the newborn there is absence of testosterone production during the mini puberty.[91] There is no spermatogenesis due to the absence of the androgen receptor function. Gender identity and role behaviors are typically female. Removal of the testes is controversial because of low risk of development of germ cell tumors in childhood and during puberty,[92] and there is no consensus on the timing of orchidectomy.[93]

Partial Androgen Insensitivity

PAIS, also due to mutations in the androgen receptor, is characterized by various degrees of ambiguity of the external genitalia. The term *Reifenstein syndrome* was formerly used to describe partial androgen insensitivity with intermediate degrees of androgenization. Affected infants have a small phallus and a ventral chordee that tethers the phallus to the perineum. There is often a penoscrotal hypospadias and a bifid scrotum, which may or may not contain gonads (see Fig. 85.5). Cryptorchidism is a common finding. Müllerian structures are absent, and the wolffian duct–derived structures are absent or poorly developed. A genitourethrogram may demonstrate a urogenital sinus.

The diagnosis of partial androgen insensitivity is complex. A family history of ambiguous genitalia in male relatives on the maternal side is suggestive of this diagnosis since it is X-linked. However, Viner[33] reported a family history in only 25% of patients with PAIS. 5α-Reductase deficiency and testosterone biosynthetic defects should be ruled out by appropriate steroid and/or genetic analysis. This can be accomplished by measurement of intermediates in testosterone biosynthesis, especially androstenedione, to exclude 17β-hydroxysteroid dehydrogenase (17β-HSD) type 3 deficiency and by measurement of the ratio of testosterone to DHT to exclude 5α-reductase deficiency. Abnormal testosterone-to-DHT ratios can be seen in PAIS[94]; this could be caused by poor development of tissues, which express 5α-reductase type 2 in PAIS.[95] Genetic testing of the androgen receptor is the gold standard, and with the availability of DSD panels, sequencing in parallel rather than series is now possible.

Androgen levels in male newborns are highest at birth and then decline rapidly during the first week. A second testosterone surge occurs between 15 and 60 days of life,[42] which is often referred to as *mini puberty*. Androgen and LH levels should be obtained at these peak production times. Abnormally elevated levels of LH, testosterone, or both in the first several months of life are suggestive of partial androgen insensitivity. A report of five neonatal cases of PAIS showed testosterone values in the high-normal range



• **Fig. 85.14** (A) Appearance of external genitalia of a 46,XY infant with complete androgen insensitivity syndrome. (B) Note presence of bilateral palpable gonads.

on days 2 to 7 of life (mean 107 ± 27 ng/dL) and on day 30 (mean 411 ± 154 ng/dL.[91] LH levels were elevated in comparison with those of historic controls (mean LH level on days 7 to 15 of life was 5.2 IU/L ± 4.0 ng/dL; on day 30 of life the mean level was 8.7 IU/L ± 2.5 ng/dL). Testosterone response to hCG and LH response to gonadotropin-releasing hormone were exaggerated.[91]

The androgen receptor binding assay used to be considered standard for defining this disorder. However, normal ligand binding does not rule out androgen insensitivity, because there may be mutations in domains of the androgen receptor not involved in ligand binding. Direct sequencing of the androgen receptor for mutation analysis is commercially available (GeneTest) and can detect up to 95% of mutations associated with CAIS and PAIS. Androgen receptor mutations, however, are not always found in cases of possible partial androgen insensitivity, and it is speculated that defects in androgen receptor–interacting proteins may be involved[96] or, more commonly, the child may have another disorder. There have also been cases of postzygotic mutations of the androgen receptor resulting in mosaicism in the individual with PAIS.[97] In the past, individuals were often labeled as having PAIS when instead they had another disorder such as 17β-HSD deficiency type 3. This misdiagnosis has serious implications as at puberty this individual will virilize as other 17β-HSD isoenzymes convert androstenedione to testosterone.

Determining the sex of rearing in partial androgen insensitivity is a difficult task, and multiple factors must be considered. If there is a significant degree of virilization (Prader stages IV and V (see Fig. 85.3A), then male sex assignment is made.[8,63] If masculinization is severely limited (Prader stages I and II), then female sex assignment is often recommended. In intermediate forms (Prader stage III), responsiveness to exogenous testosterone may be of help in the decision-making process.[5,98] However, adult males with a small phallus have reported satisfactory sex lives.[11] These cases deemphasize the importance of phallic size in male sex assignment. Slijper[2] reported the absence of serious gender identity disorder in five undervirilized 46,XY males, although these boys were "more fearful and bothered about the smallness of their penises." These studies indicate the importance of a nurturing, supportive environment for a successful long-term outcome for these children. A recent review of the literature revealed gender identity dysphoria in 12% of females and 25% of males with PAIS which was not statistically significant.[99] More comprehensive long-term outcome studies of partial androgen insensitivity are needed.

5α-Reductase Type 2 Deficiency

5α-Reductase type 2 deficiency is an autosomal recessive disorder that results in an inability to convert testosterone to DHT. DHT is required for the development of the male external genitalia.

At birth, 46,XY infants with 5α-reductase type 2 deficiency can have a wide range of phenotype. Some have a very small phallus that appears to be a normal or slightly enlarged clitoris. However, more significant virilization of the phallus may occur, and the affected infant will then be identified as a male with hypospadias. There is usually severe penoscrotal hypospadias and a bifid scrotum. The testes are usually in the inguinal canals or labial folds. Approximately half of these patients have a penoscrotal urethra with a separate blind-ending vagina; a smaller percentage have a single urogenital sinus opening on the perineum.[95] A more recent study identified isolated microphallus (36%), microphallus with hypospadias (56%), and female external genitalia with clitoromegaly (9%) as the presenting phenotype.[100] Another study, of 55 patients with genetically confirmed 5α-reductase type 2 deficiency, found the following phenotypes: clitoromegaly (49.1%), microphallus with hypospadias (32.7%), female external genitalia (7.35%), and isolated microphallus (3.6%).[101] With the advent of genetic testing, the phenotypic range for 5α-reductase type 2 deficiency is much broader than originally described. In neonates with 5α-reductase type 2 deficiency, müllerian structures are absent, and wolffian duct–derived structures (vas deferens, epididymis, seminal vesicles) which are responsive to testosterone are well developed. The prostate is poorly developed because it requires exposure to DHT.

At the time of puberty, individuals with this disorder will characteristically virilize. The phallus will typically increase to a length of 4 to 8 cm.[56] In affected individuals who were raised as females, a change to the male gender role after puberty is commonly seen.[95,102] Unlike in partial androgen insensitivity, gynecomastia does not occur in the pubertal period.

In the past, 5α-reductase type 2 deficiency was most often diagnosed in the postpubertal period, although diagnosis in the newborn period has been reported.[103,104] The disorder is suggested by assessment of the ratio of testosterone to DHT in blood.[105] The normal testosterone-to-DHT ratio in the newborn period is 4:1, whereas the ratio in infants and children with this disorder is often greater than 14:1. An hCG stimulation test is usually needed to obtain a more definitive diagnosis in the prepubertal period but there is concern for damage to the testis when using hCG in a nonphysiologic time.[45] A positive response to hCG rules out Leydig cell aplasia or a testosterone biosynthetic defect. Measurement of normal androstenedione levels will rule out the testosterone biosynthetic defect—deficiency of 17β-HSD type 3. This enzyme is responsible for the conversion of androstenedione to testosterone in the testes, and the level of androstenedione is elevated compared with that of testosterone when 17β-HSD deficiency is present. The clinical presentation of 17β-HSD deficiency in infancy can be similar to that of 5α-reductase deficiency or PAIS. Giving exogeneous testosterone can provide a T:DHT ratio.

In addition, abnormal ratios of 5β-urinary steroids to 5α-urinary steroids can establish a definitive diagnosis of 5α-reductase type 2 deficiency,[103] but it can be challenging to perform the test. Distinguishing 5α-reductase deficiency from partial androgen insensitivity is important because androgen insensitivity can cause a secondary DHT deficiency owing to the incomplete development of tissues that express 5α-reductase activity.[95] Measurement of urinary 5β-glucocorticoids and 5α-glucocorticoids will help to make this distinction, because only 5α-reductase deficiency will also affect glucocorticoid metabolism. Finally, analysis for mutations in the *SRD5A2* gene is diagnostic, and is the gold standard. This gene test is readily available commercially as a separate test or as part of a DSD panel.

Sex assignment in cases of 5α-reductase deficiency is a complicated issue, but long-term outcome information is available. As in androgen insensitivity disorders, sex assignment is often significantly influenced by the degree of androgenization at birth, and because infants with this disorder are usually markedly undervirilized, female sex assignment has been advocated.[5,56] However, if the disorder is diagnosed early, topical DHT treatment, which is not available in the United States, has been shown to enlarge the phallus.[104] In addition, the natural history of the disorder is for masculinization of the phallus to occur at puberty. Testicular histopathologic analysis in males with this disorder has shown that, unlike in isolated bilateral cryptorchidism, their testes display type

Ad (i.e., dark) spermatogonia and a normal germ cell count. These patients are largely infertile because of defective transformation of spermatogonia into spermatocytes.[106] However, there have been case reports of men fathering children.[107,108] 46,XX females with 5α-reductase deficiency do not have a known phenotype.

There are frequent reports of reversal from female to male gender behavior after puberty.[109,110] The accumulated evidence supports male sex assignment in this disorder,[102] although female sex assignment is likely in the newborn period if the diagnosis is overlooked or if the infant with 5α-reductase deficiency has no androgenization. With the use of gonadotropin-releasing hormone (GnRH) analogs, male puberty can be suppressed and estrogen can be given to promote female secondary sexual characteristics in an individual who was raised female and has female gender identity.[111] Close follow-up with endocrinology and behavioral health is important as the child goes through puberty.

Testosterone Biosynthetic Defects

Six enzymes are necessary for the synthesis of testosterone. A defect at any step will result in inadequate testosterone synthesis (see Fig. 85.13). Defects in the first four enzymes of the testosterone synthesis pathway (cholesterol side chain cleavage enzyme, steroidogenic acute regulatory [StAR] protein, 17,20-lyase enzyme and P450 oxidoreductase) will also affect adrenal steroid production, resulting in both an underandrogenized male and CAH. POR deficiency leads to an underandrogenized male and CAH due to decreased 17,20 lyase activity. Because testosterone production is impaired in these disorders, wolffian duct structures are likely to be underdeveloped, whereas müllerian structures are absent because of normal testicular AMH production. These enzyme deficiencies are rare; therefore, these disorders are discussed only briefly here (see also Chapter 84 for more details).

StAR Deficiency or Congenital Lipoid Adrenal Hyperplasia

Lipid accumulation in both the adrenal glands and the gonads is characteristic of this disorder pathologically—hence the name *congenital lipoid adrenal hyperplasia*. Because all adrenal and gonadal steroid synthesis is affected by this disorder, infants are likely to exhibit complete adrenal insufficiency, characterized by vomiting, weight loss, and hypotension. The phenotype is likely to be female, although there is clinical variability. In an early report of 35 cases in the medical literature, only 11 patients survived beyond infancy.[57] Today, more cases are recognized early and, with glucocorticoid and fludrocortisone replacement, these infants have excellent survival rates.

Endocrine findings include elevation of ACTH level and plasma renin activity but low or immeasurable levels of all steroid hormones. The main consideration in the differential diagnosis is another form of CAH due to mutations in *CYP11A1*. In vitro studies performed on either adrenal or testicular tissue demonstrated an inability to convert cholesterol to pregnenolone in these patients. A defect in the first step of adrenal and gonadal steroid biosynthesis mediated by the cytochrome P450 side-chain cleavage enzyme was suspected. However, subsequent molecular studies demonstrated mutations in the steroidogenic acute regulatory protein (StAR).[5] StAR acts to promote sterol translocation to the cytochrome P450 side-chain cleavage enzyme in mitochondria (see Fig. 85.13).

The presence of markedly enlarged lipid-laden adrenals on ultrasound, computed tomography, or MRI studies is highly suggestive of the disorder. Successful treatment requires replacement of both glucocorticoids and mineralocorticoids. All individuals with this diagnosis have been raised as females.[5]

This disorder is due to "two hits."[112] The initial hit is the inability to make steroids, and the second hit is the destruction of the steroidogenic cell due to the high level of lipid content. The infant may not initially present with salt-losing crisis due to the initial sparing of the cells in the definitive zona, which are relatively inactive in utero. Females may have some pubertal development because the ovary is also quiescent until puberty, which spares initially the ovarian follicular cells. Eventually, though, they too are damaged, and the female develops hypergonadotropic hypogonadism. Nonclassical forms of this autosomal recessive disorder, which can present as isolated glucocorticoid deficiency have also been identified.[113]

Side Chain Cleavage Cytochrome P450

The side chain cleavage enzyme, encoded by CYP11A1, converts cholesterol to pregnenolone. Deficiencies of the side chain cleavage enzyme leads to adrenal insufficiency with a range in external genitalia phenotype from absence of androgenization in the male to hypospadias or normal male external genitalia.[114,115]

17α-Hydroxylase/17,20-Lyase Deficiency

A single enzyme encoded on the cytochrome P450c17gene (*CYP17A1*) mediates the 17-hydroxylation of pregnenolone and progesterone and the conversion of 17-hydroxypregnenolone and 17-OHP to DHEA and androstenedione. Specific mutations can cause partial loss of 17α-hydroxylase/17,20-lyase activity or dissociation between the 17α-hydroxylase and 17,20-lyase function.[116] Clinical disorders of this enzyme affect primarily either the hydroxylation or the lyase reaction, although there have been reports of combined 17α-hydroxylase and 17,20-lyase deficiency.[117]

Cases of primarily 17α-hydroxylase deficiency should be considered in underandrogenized males or females with low renin hypertension and hypokalemic alkalosis. The hypertension is presumably due to elevated levels of DOC and corticosterone (see Fig. 85.13). Although cortisol synthesis is blocked, the overproduction of DOC and corticosterone is protective against adrenal insufficiency.

Most cases are diagnosed in the pubertal period because the 46,XY phenotype is largely female. This disorder is treated with glucocorticoid, which suppresses ACTH overproduction and subsequently suppresses DOC and corticosterone overproduction if there is hypertension.[5] Sex steroid replacement is needed at puberty.

17,20-Lyase deficiency results in various degrees of undervirilization of the 46,XY infant. The phenotype ranges from complete female external genitalia to ambiguous genitalia to a mildly undervirilized male. 46,XX females will fail to enter puberty. The levels of gonadotropins will be elevated, and there will be impaired formation of DHEA and androstenedione (see Fig. 85.13). DOC and corticosterone levels are normal in this form of the disorder. ACTH stimulation tests may be helpful to reveal the steroid biosynthetic block more fully. Gene testing for *CYP17A1* is available as a single gene or as part of a DSD panel.

POR Deficiency

Phenotype of POR deficiency is similar to 17,20 lyase deficiency with or without Antley-Bixler syndrome. (See section in 46,XX Differences in Sex Development as well as Chapter 84 for more details.)

3β-Hydroxysteroid Dehydrogenase Deficiency 2

3β-HSD deficiency type 2 was first reported by Bongiovanni.[118] 3β-HSD is an important enzyme required for the conversion of Δ^5-steroids to Δ^4-steroids in the adrenal glands and gonads (see Fig. 85.13). There is marked heterogeneity in clinical presentation, and both sexes are affected.[57] With severe deficiency of 3β-HSD, salt-losing crisis can occur. Male infants may have ambiguous or completely feminine external genitalia, whereas female infants may be mildly androgenized. Severely underaandrogenized males may have normal mineralocorticoid activity; fully androgenized males may display salt loss.

Diagnosis of this disorder in the newborn period can be difficult because of relatively high levels of Δ^5-steroids physiologically. The diagnosis is based on the elevated ratio of 17-hydroxypregnenolone to 17 OHP and of DHEA to androstenedione in the basal and stimulated states.[53]

17β-Hydroxysteroid Dehydrogenase Type 3 Deficiency (17-Ketosteroid Reductase Deficiency)

The final step in testosterone biosynthesis involves the conversion of androstenedione to testosterone by the enzyme 17β-HSD type 3 in the testis. Mutations that impair the function of 17β-HSD are the cause of this relatively rare autosomal recessive disorder.[110]

The clinical presentation externally is that of a female at birth with perhaps a mild degree of clitoral enlargement. Therefore, these infants are typically raised as females unless the diagnosis is made at birth. At puberty, there is progressive androgenization, with enlargement of the phallus to 4 to 6 cm, and labial enlargement and rugation. By late puberty, the testes are found at the lower ring of the inguinal canal. They are of normal size and consistency. Internal wolffian duct–derived structures are found. In addition, a male body habitus develops, with deepening of the voice and appearance of male body hair, including a mustache and beard.[56]

In the past, patients received a diagnosis at puberty or as adults. Now with the advent of genetic testing, this diagnosis is made more frequently in infancy. Endocrine studies reveal markedly elevated androstenedione levels, whereas testosterone levels are in the low-normal range.[119] Plasma LH levels are consistently high. In infancy or childhood, the presence of inguinal hernias may bring the child to medical attention. Androstenedione levels in the prepubertal patient may be normal.

Sex of rearing is often influenced by the cultural context. In societies in which a high priority is given to the male, sex reassignment at puberty has been successful.[57] Mendonca observed changes in gender role (female to male) in 3 of 10 affected individuals.[119] Despite androgenization in some affected individuals, the female gender role was maintained. Suppression of virilization using a GnRH analog and replacement of estrogen has been used in 46,XY females with 1717β-HSD who identify as female at the time of puberty.[111]

Leydig Cell Hypoplasia

Failure of the testes to produce testosterone in response to hCG and LH is characteristic of this disorder. Histologic examination of the testes reveals absent or low numbers of Leydig cells, normal-appearing Sertoli cells, and seminiferous tubules with spermatogenic arrest.[5] LH receptor mutations have been described in this disorder.[120] Phenotypically in the 46,XY infant, the external genitalia range from those of a normal female to those of a male with microphallus. Müllerian-derived structures are absent in all patients, whereas wolffian structures may be present. LH and FSH levels are elevated in postpubertal patients, and LH levels decrease after testosterone administration. In less severe forms of the disorder, testosterone therapy augments phallic growth. In severe forms of testicular unresponsiveness to hCG/LH, sex assignment has been female. The gonads are removed, and estrogen replacement therapy is instituted at the time of expected puberty.[5] Females with LH receptor changes may present with elevated LH and amenorrhea.

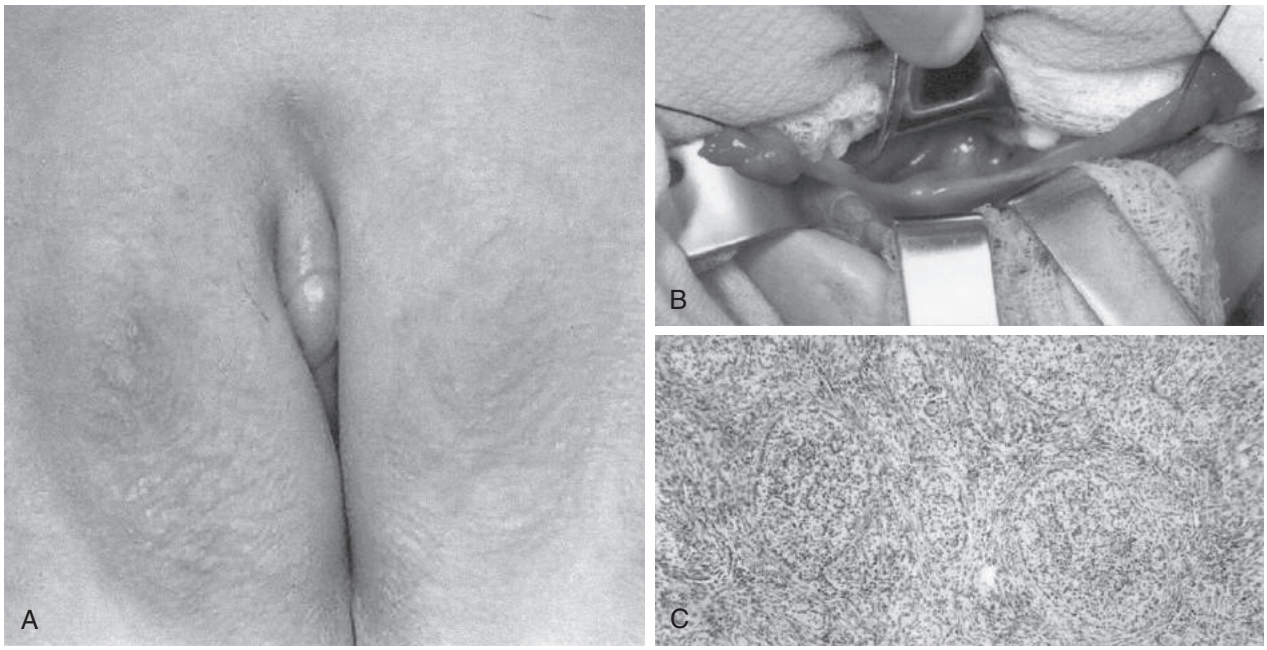
Persistent Müllerian Duct Syndrome

Diagnosis of PMDS is often made in otherwise phenotypically normal 46,XY males at the time of surgery for an inguinal hernia or orchidopexy. In the case of hernia repair, a fallopian tube and uterus are often found along with a partially descended testis. In other cases, testes, uterus, and fallopian tubes are found in the pelvis. Inheritance of PMDS is autosomal recessive, although the female phenotype is completely normal. The disorder has been found to be due to a mutation in AMH or in the AMH receptor in 85% of cases and unknown causes in 15% of cases.[121] Therapy involves orchidopexy and partial hysterectomy, with care taken to avoid injuring the vas deferens, which is embedded in the uterine wall.

Gonadal Differentiation and Chromosomal Disorders

46,XY Complete Gonadal Dysgenesis

46,XY complete gonadal dysgenesis was first described by Swyer in 1955.[5] The phenotype of the external genitalia is female, with normal development of müllerian-derived internal structures in a 46,XY individual. The gonads do not develop into testes but rather formed streak gonads. Streak gonads are completely nonfunctional; they did not produce AMH or testosterone. Therefore, müllerian structures do not regress, and wolffian structures are poorly developed or absent (Fig. 85.15). Most affected individuals are seen in the teenage years with lack of pubertal development. Serum gonadotropin levels are elevated. Mutations in the *SRY* gene account for only a small proportion of these cases.[57] Fig. 85.1 depicts other genes that when mutated can lead to complete gonadal dysgenesis. Familial cases have been reported. There has been a report of a 46,XY mother who developed as a normal woman and gave birth to a 46,XY daughter with complete gonadal dysgenesis in a family with multiple disorders of sexual development, suggesting an unidentified sex-determining gene.[122] In up to 30% of affected individuals, gonadoblastomas will develop in the streak gonad; therefore, removal of the streak gonad is recommended at the time of diagnosis (see Fig. 85.15).[56,123,124] Females with gonadoblastoma may present with breast development as



• **Fig. 85.15** (A) Appearance of external genitalia in an infant with 46,XY complete gonadal dysgenesis. (B) Uterus and fallopian tubes were found at surgery, along with a gonadoblastoma, at 1 year of age. (C) Micrograph of tumor cells.

the gonadoblastoma produces estrogen. Estrogen replacement therapy will provide appropriate feminization, and the subsequent addition of progesterone will lead to menses. Women with complete gonadal dysgenesis can successfully carry a pregnancy using donor oocytes and appropriate hormonal supplementation.

46,XY Partial Gonadal Dysgenesis

Patients with 46,XY partial gonadal dysgenesis will typically be seen in the newborn period for evaluation of ambiguous genitalia.[125] The extent of androgenization of the external genitalia depends on the extent of testicular differentiation. Gonadal tissue is usually intraabdominal, but testes can be found in the scrotum. Testosterone response to hCG is variable but usually low. In most cases, there is a mix of müllerian and wolffian structures. The presence of müllerian structures on genitourethrogram or ultrasound examination increases the index of suspicion for this disorder. The diagnosis is confirmed by gonadal biopsy. Some affected children are found to have one dysgenetic gonad on one side and a streak gonad on the other; others have bilateral dysgenetic gonads. Dysgenetic gonads are histologically defined by poorly formed and disorganized seminiferous tubules surrounded by wavy ovarian stroma. In many cases, the dysgenetic gonads resemble ovotestes, except that primordial ovarian follicles are lacking.[125]

Single Genes Important in Normal Testicular Development

SRY

SRY is the only gene on the Y chromosome necessary for normal male sex development. Mutations in the highly conserved HMG DNA binding domain account for about 15% of the cases of complete gonadal dysgenesis. Mutations have also been identified in the non-HMG domain. There is variable penetrance in that a few cases have been described in which the father and unaffected brother carry the same mutation in SRY as the female with complete gonadal dysgenesis.[126]

NR5A1

NR5A1 encodes steroidogenic factor 1 (SF1) a master regulator which controls the expression of *SRY, SOX9, AMH, AMHR, LHCGR, STaR, CYP11A1, CYP17A1*, and gonadotropins. Consequently, the range of phenotype in 46,XY infants with changes in *NR5A1* goes from complete gonadal dysgenesis to male infertility. Ovotesticular DSD can be present as well as adrenal insufficiency. Bashamboo et al. demonstrated how the p.Arg92Trp mutation acts as a switch between male and female gonadal development.[73]

SOX8

SOX8 located on chromosome 16p.13.3 shares the same HMG motif with *SRY*. Heterozygous missense mutations have been shown to cause 46,XY complete gonadal dysgenesis.[127]

NROB1

NROB1 located on the X chromosome at Xp21.2 encodes DAX1 which is an orphan nuclear receptor. Duplication of NROB1 leads to 46,XY complete gonadal dysgenesis.[22,128] Loss of function changes in NROB1 leads to adrenal hypoplasia congenita in XY individuals.[128] In addition, there can be unilateral or bilateral cryptorchidism and, at puberty, hypogonadotropic hypogonadism.

MAMLD1

MAMLDI is also located on the X chromosome and is expressed in the Leydig cells of the developing testis. Heterozygous mutations were initially found in boys with isolated severe hypospadias.[129] Changes in MAMLD1 have also been seen in other 46,XY DSD which have hypospadias as a feature,[130] but changes in other DSD genes may also be necessary.[131]

MAP3K1

Map3K1 is expressed in early testis cords and Sertoli cells in mice. Mutations in MAP3K1 have been seen in a range of phenotype from complete gonadal dysgenesis to isolated hypospadias to micropenis associated with cryptorchidism. It is inherited in an autosomal dominant fashion.[132]

GATA4

Heterozygous changes in GATA4 have been associated with congenital heart disease and disorders of sex development in 46,XY individuals.[133]

DMRT1 and DMRT2

DMRT1 and *DMRT2* are on 9p24.3, a region long associated with 46,XY gonadal dysgenesis. Haploinsufficiency of *DMRT1* has been found in gonadal dysgenesis.[134] The range of phenotype associated with 9p24.3 deletion, though, includes gonadal dysgenesis, ovotestis, hypospadias, penoscrotal transposition, and cryptorchidism.[135]

Testicular Regression Syndrome

Testicular regression syndrome refers to the spectrum of disorders affecting individuals with 46,XY karyotype who demonstrate evidence of prior testicular function, followed by usually symmetric gonadal regression.[136] Loss of testicular function between weeks 8 and 10 of gestation would result in ambiguous genitalia and variable internal genitalia. Loss of testicular function after 12 to 14 weeks' gestation would result in normal male genital differentiation with a small phallus. When the male external and internal ducts are completely normal, the term *vanishing testis syndrome* is used by some authors. Presumably the testes were lost during the second half of pregnancy. Testicular torsion or loss of blood supply has been invoked as a possible explanation in this syndrome.[137] Another potential etiology is anomalous vascular development.[138]

FSH levels are elevated in infancy, and an exaggerated response to gonadotropin-releasing hormone in the prepubertal period is typically seen.[5] AMH levels in infancy and childhood are very low in anorchia and intermediately low with abnormal testes.[64]

46,XY Ovotesticular Disorder

Ovotestes, or an ovary on one side and a testis on the contralateral side, can also develop in individuals with 46,XY karyotype. While not as commonly seen as in individuals with a 46,XX karyotype, the diagnosis should be considered when ambiguous genitalia are noted, especially if müllerian structures are present. Changes in the *NR5A1* gene have also been associated with XY ovotesticular DSD. In this case, the p.Arg92Trp mutant NR5A1 protein has decreased ability to upregulate the *SOX9* gene leading to decreased SOX9 formation and, therefore, decreased Sertoli cell and testis formation.[73] The ovotestis and testis in a 46,XY ovotesticular disorder have spermatogenesis, unlike the 46,XX ovotesticular DSD.

Syndromes Associated With Ambiguous Genitalia

Denys-Drash Syndrome

Denys-Drash syndrome is a rare syndrome consisting of the classic triad of XY ambiguous genitalia, congenital nephrotic syndrome leading to end-stage renal failure, and Wilms tumor. The external genitalia of 46,XY individuals are either ambiguous or female. Gonadal development encompasses a spectrum from streak gonads to dysgenetic testes. Nephropathy and proteinuria are noted at an early age, and renal biopsy will demonstrate mesangial sclerosis. More than 90% of cases will have a mutation in the *WT1* gene, which is a critical gene for the development of the normal genital tract. *WT1* is also associated with WAGR (Wilms tumor, aniridia, genitourinary anomalies, retardation) syndrome. Large deletions of chromosome band 11p13 that encompass the *WT1* gene are responsible for this disorder. Frasier syndrome also involves the *WT1* gene. Frasier syndrome manifests itself in 46,XY females with gonadal dysgenesis and progressive glomerulopathy. The risk of gonadal malignancy in Frasier syndrome is reported as 60% and in Denys-Drash with a Y chromosome as 40%.[123]

Campomelic Dysplasia

Campomelic dysplasia is a rare autosomal dominant disorder associated with often lethal skeletal dysplasia, in which 75% of affected 46,XY males have dysgenetic testes associated with undervirilization.[18] Manifestations of this disorder include bowing of the femora and tibiae, hypoplastic scapulae, 11 rib pairs, pelvic malformations, bilateral clubfoot, cleft palate, macrocephaly, micrognathia, hypertelorism, and a variety of cardiac and renal defects. The disorder is caused by heterozygous mutations in the *SOX9* gene.[139] Most patients with campomelic dysplasia die of respiratory distress in the neonatal period.

Smith-Lemli-Opitz Syndrome

Smith-Lemli-Opitz syndrome is an autosomal recessive disorder with an estimated frequency of 1 in 20,000 to 1 in 40,000. The disorder is caused by deficiency of 7-dehydrocholesterol reductase[57] enzyme which catalyzes the final step in cholesterol biosynthesis leading to elevated serum 7-dehydrocholesterol level that is pathognomonic for Smith-Lemli-Opitz syndrome. Cholesterol levels are not always low. Growth failure, severe developmental delay and multiple congenital anomalies (microcephaly, ptosis, anteverted nostrils, micrognathia, cleft palate, cardiac defects extra axial polydactyly, syndactyly of second and third toes) characterize this syndrome.[140] Males can present with genital anomalies ranging from severe hypospadia and micropenis to complete absence of external virilization of genitalia due to inability to synthesize cholesterol, the precursor for testosterone. Adrenal insufficiency has also been reported in this condition as cholesterol is also a precursor for cortisol and aldosterone.

Treatment with cholesterol has been used in Smith-Lemli-Opitz syndrome to try to improve growth and neurodevelopmental status. Unfortunately, cholesterol replacement has not been as beneficial as once hoped as it does not cross the blood-brain barrier and has not been shown to reduce developmental delay.[141] A more recent placebo-controlled trial using simvastatin (non–FDA-approved indication) found that the medication crossed the blood–brain barrier and was relatively safe. There was improvement in serum dehydrocholesterol-to-total sterol ratio and improvement of irritability symptoms.[142]

Robinow Syndrome

Robinow syndrome is an autosomal dominant disorder characterized by a flat facial profile, short forearms, and hypoplastic genitals.[140] Sporadic cases have been reported. Microphallus

may be severe in males, although normal virilization at the time of puberty has been reported.[140] Undescended testes have been reported in 65% of affected boys. Females have characteristic hypoplastic labia and clitoris. Other features include small size at birth, macrocephaly, frontal bossing, hypertelorism, prominent eyes, small, upturned nose, micrognathia, and posteriorly rotated ears. Short forearms are seen in 100% of described cases. Other skeletal abnormalities include thoracic hemivertebrae, fusion or absence of ribs, and scoliosis. The abnormal facial features become less pronounced as the child grows, and cognitive performance has been normal in most affected individuals.

Other Disorders of Genital Differentiation

Hypospadias

Hypospadias is one of the most common anomalies of male genital development, with an estimated incidence of 4 to 8 cases per 1000 male births.[5] Hypospadias can be classified as glanular, subcoronal, distal penile, midshaft, proximal penile, penoscrotal, scrotal, and perineal (see Fig. 85.3C). Glanular and subcoronal hypospadias account for more than 50% of hypospadias. Typically, the more severe forms of hypospadias have been associated with DSD, although the phenotypic spectrum of DSD is wide. In a study of 33 patients with severe (scrotal or penoscrotal) hypospadias, 12 were found to have a DSD, which included Denys-Drash syndrome (in 3 of the 12), partial androgen insensitivity (in 2), ovotesticular DSD (in 2), chromosomal abnormality (in 1), AMH abnormality (in 1), gonadal dysgenesis (in 1), 5α-reductase deficiency (in 1), and 46,XX *SRY*-positive karyotype (in 1).[38] The testes were undescended in 11 of the 12 patients. Aarskog[143] found an approximately 15% prevalence of DSD in association with hypospadias, in addition to a significant role for maternal progestins. Intrauterine growth retardation and prematurity also have a higher association with hypospadias and undescended testes thought to be due to placental insufficiency, with lower hCG levels leading to decreased androgen production early in the pregnancy.[144] Other factors associated with hypospadias include maternal hypertension, preeclampsia, maternal vegetarian diet, assisted reproductive techniques, multiple births, pollution, and endocrine disruptors.[145] Genetic factors also play a role, and mutations in MAMLD1, NR5A1, and AR have been found in a few cases.[130] In addition, studies of families with multiple males having hypospadias have identified other genes such as AKR1C2, AKR1C3, and AKR1C4 as having possible involvement in the development of hypospadias.[146]

Severe cases of hypospadias require a thorough evaluation, including karyotype and evaluation of testosterone biosynthesis, along with examination of the genitourinary tract, particularly if accompanied by undescended testes.

Cryptorchidism

Cryptorchidism is one of the most common genital differences at birth. The testes descend into the scrotum in two phases. The transabdominal descent occurs in the first trimester with the testes at the internal inguinal ring at 22 to 25 weeks gestational age. The second phase of descent, the inguinoscrotal phase, occurs between 25 and 30 weeks and is androgen dependent.[147] Consequently, the prevalence of cryptorchidism is higher in premature male infants (15% to 30%) compared to term male infants (1% to 3%).[148] In premature infants with cryptorchidism, if testicular descent into the scrotum does not occur by 6 months corrected age, it is unlikely to occur spontaneously. Orchiopexy is usually done after 6 months of age and prior to 12 to 18 months to decrease risk of infertility, testicular malignancy, and torsion.[41] Male newborns with bilateral, nonpalpable testes need further evaluation to exclude a 46,XX female with congenital adrenal hyperplasia. The male infant should also be evaluated for pituitary dysfunction (ACTH, TSH, and growth hormone deficiency) which could be associated with hypogonadotropic hypogonadism. Evaluation for a DSD should also occur. If the cryptorchidism is unilateral with hypospadias or micropenis or other penile abnormalities, an evaluation for a DSD should also occur.[41]

Sex Chromosome Disorders of Sex Development

Klinefelter Syndrome

Klinefelter syndrome, 47,XXY, has a prenatal cytogenetic incidence of 1 in 667. The prevalence of newborns with 47,XXY in Denmark based on a public health registry over a 53-year span was 57 per 100,000 males, yet only 38% of males with Klinefelter syndrome received a diagnosis of Klinefelter syndrome.[149] Individuals identified by prenatal diagnosis may not have as many features of Klinefelter syndrome as those ascertained postnatally because of concern for Klinefelter syndrome. Most infants have normal male phenotype. Boys with Klinefelter syndrome often come to medical attention when they are noted to have smaller testes, delayed or absent puberty, tall stature, and gynecomastia. The testes have decreased testosterone production and spermatogenesis due to progressive testicular failure. Males may have decreased language and executive function.[150] Guidelines for the care of males with Klinefelter syndrome have been developed.[151]

Turner Syndrome

Turner syndrome is a condition in which a female has complete or partial loss of one of her sex chromosomes. Mosaicism may be present with an XX or XY cell line. Turner syndrome has a prevalence of 1 in 1700 newborn females.[149] There is a high level of fetal loss as demonstrated by Gravholt, who identified a prevalence of Turner syndrome of 392/100,000 by chorionic villus sampling, of 176/100,000 by amniocentesis, and 32/100,000 by newborn karyotype.[152] With the increase in use of cell free DNA, a noninvasive prenatal testing (NIPT) screening test, there is increased recognition of possible Turner syndrome at birth. However, cell free DNA was not developed to diagnose Turner syndrome and thus the predictive value may be only 23-36%.[153] In the absence of prenatal genetic testing, the timing of diagnosis is dependent on physical features. Prenatally, an infant may be suspected as having Turner syndrome due to cystic hygroma, increased nuchal translucency, or coarctation of the aorta. Confirmation by a postnatal karyotype with 30 cells to exclude mosaicism is necessary. The presence of a Y cell line increases the risk for gonadal tumor, and gonadectomy needs to be considered. At birth, an infant may be suspected of having Turner syndrome when she presents with webbing of her neck or pedal edema or congenital heart disease.

Guidelines for the health maintenance of girls with Turner syndrome were updated in 2017.[154] At the time of diagnosis, all infants should have a cardiovascular evaluation by a cardiologist, including a cardiac echo. Girls with Turner syndrome are at

increased risk for bicuspid aortic valve, coarctation of the aorta and anomalous pulmonary veins. They should also have a renal ultrasound to look for a horseshoe kidney. They will need a formal audiologic evaluation as well as an ophthalmologic exam at 1 year due to increased risk of strabismus. They should also be screened for hip dysplasia. Long-term follow up with a pediatric endocrinologist is recommended for evaluation of short stature, ovarian function due to premature ovarian failure which can occur prior to birth, and yearly thyroid function tests. Girls with Turner syndrome have normal intelligence but may have decreased visual-spatial skills, information processing and math skills.[155] They may also have decreased social skills.[156]

Mixed Gonadal Dysgenesis

Mixed gonadal dysgenesis refers to asymmetric gonadal dysgenesis with ambiguous genitalia (see Fig. 85.7) and a mosaic karyotype with an XY cell line. The most common karyotype is 45,X/46,XY. There is a wide spectrum of phenotypes, ranging from a female with clitoral enlargement to a male with hypospadias. Asymmetric external and internal genital development has been classically described in this syndrome.[57] Considerable phallic development was reported in most patients in one study, and there was often penoscrotal hypospadias.[157] The phenotypic features of Turner syndrome are described in a significant percentage of patients, although these features may not be readily apparent in the newborn period. An incompletely formed uterus is found in almost all patients. Fallopian tubes are always found on the side of the streak gonad and often on the side with the dysgenetic gonad. Wolffian structures may be developed on the side with the dysgenetic gonad.

A genitourethrogram is likely to demonstrate internal müllerian structures that can be confirmed at laparoscopy. Demonstration of abnormal gonadal histopathologic features will confirm the diagnosis. Mixed gonadal dysgenesis shares many features with partial gonadal dysgenesis, and some authors view these disorders as representing a continuum of gonadal dysgenesis.[125] Histologic analysis will also differentiate this disorder from ovotesticular DSD. Although the characteristic karyotype is 45,X/46,XY, this genotype has been associated with normal male differentiation in most cases diagnosed by prenatal amniocentesis.[158] While these males may have normal external genitalia, they may also have short stature and cardiac features associated with Turner syndrome. While there are no formal recommendations for screening, many follow the guidelines developed for females with 45,X/46,XX Turner syndrome.[154]

Some authors advocate female sex assignment in mixed gonadal dysgenesis because surgical repair of the vagina is usually easy and a uterus or hemiuterus is present. In addition, the dysgenetic gonad is at risk of development of a tumor and should be removed, particularly if the gonad cannot be brought down into the scrotum.[57] However, sex assignment is likely to be guided by the degree of androgenization with the more virilized cases being assigned as males. The capacity for near-normal androgen production in this disorder has been described.[157] In all cases the streak gonads should be removed because of the risk of malignancy.

46,XX/46,XY Chimerism

Chimerism is the result of the fusion of two different zygotes. In cases where the zygotes have the same sex chromosomes, chimerism may not be recognized. However, if the zygotes have different sex chromosomes, then the infant may be identified by the presence of ambiguous genitalia. The gonad will develop into an ovary, testis, or ovotestis depending on the genetic complement of the cells in the gonad. Internal and external genitalia will reflect the type of gonad and the amount of testosterone produced by the gonad. Ovotesticular DSD is often seen in 46,XX/46,XY chimerism. There can also be isolated chimerism in the blood, 46,XX/46,XY hematologic chimerism, due to twin-twin transfusion.[41,159] This may only be identified if a chromosomal analysis of the blood was done as the gonad and genitalia are consistent with the donor karyotype. Chimerism is not mosaicism, which arises with a mitotic error in a single zygote.

Surgical Management of Disorders of Sexual Differentiation

Surgery for DSD conditions has been recently criticized. The criticism has focused not only on the timing of surgery but also on whether reconstructive surgery should be done at all. Some authorities have advised that surgery be postponed until the affected person is of an age to make his or her own decision regarding the advisability of surgical correction.[63] Others have found that delay in surgery may be associated with problematic outcomes.[8,14,68] It is imperative that these divergent viewpoints be discussed with the parents of an infant with a DSD condition.

The need for surgical intervention in DSD should be assessed on a case-by-case basis. The parents need to be informed about the risks and benefits of surgery. Surgery on the external genitalia should perhaps be reserved for individuals with significant discord between the sex of rearing and the appearance of the external genitalia.

The infant with a DSD and the family will have to cope with difficult psychosocial challenges throughout life. Physicians caring for these patients should ensure the integration of well-trained mental health professionals into the longitudinal care of these complex infants and children. These issues must be kept in mind in any decision regarding surgery for management of DSD. Current surgical techniques that are available for correction of ambiguous genitalia and DSD are presented here.

Feminizing Genitoplasty

Feminizing genitoplasty is one of the more common procedures done for correction of ambiguous genitalia. Feminizing genitoplasty is indicated in the genetic female who is externally virilized, most commonly as the result of CAH. The degree of virilization can be highly variable (see Fig. 85.6) and will have a significant influence on the type of procedure done, especially the vaginoplasty portion of the operation. Restorative surgery in this group of patients has three components: clitoral recession, vaginoplasty, and labial reconstruction. The timing of surgical correction also has undergone some changes over the years. The current thinking is that once a decision is made to proceed with genital reconstruction, performance of this type of surgery at a younger age will have distinct advantages, including easier mobilization of the urogenital sinus and a more benign postoperative course.

Clitoral Reduction

Attempts at managing the enlarged clitoris in genetic females with clitoral hypertrophy started with total clitorectomy.

Young[160] originally advocated this procedure. Later, Lattimer[161] suggested a recession rather than a resection of the clitoris, and he hoped to be able to preserve the arousal function of the clitoris. This led to cases in which painful clitoral erections occurred later in life; therefore, further modification was needed. Spence and Allen[162] advocated the preservation of the glans with reduction in the size of the clitoris. Since then, several reports have examined preservation of the neurovascular bundle using a clitoral reduction and recession type of approach. Kogan et al.[163] and Snyder et al.[164] separately described a similar approach in which the erectile tissue of the clitoris is removed, but preservation of the neurovascular bundle and the glands is afforded to preserve the neurologic and arousal functions of the clitoris. If the glans is unusually large, then a reduction of the glans size may be indicated as well.

Vaginoplasty

Reconstruction of the vagina in cases of virilization in females requires an understanding of the anatomy. One may consider the anatomic abnormality an embryologic arrest of maturation with a persistence of an early embryologic stage. The anatomic issue that is important to the surgical management of the common urogenital sinus is the site of confluence of the genital tract and urethra. This site varies considerably but is somewhat predictable from the appearance of the degree of external virilization. Children with severe degrees of external virilization are more likely to have a higher confluence of the urethral and vaginal channels, leading to a longer urogenital sinus or a more masculinized urogenital sinus. In the classic article on urogenital sinus abnormalities, Hendren and Crawford[165] described the variable anatomy that can be seen in these children and noted that the operative procedures needed to be tailored toward the location of the confluence of the urinary and genital tracts. One may describe the confluence anatomically as it relates to the external sphincter, with confluences distal to the external sphincter being considered *low* and those proximal to the external sphincter being referred to as *high*. One also may describe the variable anatomy according to the length of the urethra from the bladder neck to the point of confluence. If that length of urethra were long, then one would consider this a low confluence. Conversely, if the length of the urethra were short and, therefore, close to the bladder neck, then a high confluence would be present (see Fig. 85.8B).

The low confluence cases can generally be repaired either by a cutback procedure on the fused labioscrotal folds or by a flap vaginoplasty. A cutback procedure would be indicated in cases with a minor degree of fusion of the labioscrotal folds. The middle to high vaginal confluence, however, generally requires either a pull-through vaginoplasty or a total urogenital mobilization to bring the vagina down to the perineum.

Flap Vaginoplasty

Flap vaginoplasty should be used for a low confluence of the urogenital sinus. The procedure entails mobilization of a perineum-based flap with its apex at the meatus of the urogenital sinus. Dissection then proceeds along the posterior wall of the urogenital sinus until the vaginal opening is identified. The perineum-based flap is then inserted into the posterior wall of the vagina, thereby exteriorizing the vagina to the perineum.

Total Urethral Mobilization

Total urogenital mobilization can be used for the high urogenital sinus, which has been advocated by Peña[166] and subsequently substantiated by a report from Rink et al.[167] This approach has been shown to have a superior cosmetic result, compared with that obtained with a flap vaginoplasty, for a middle to high confluence. In addition, there has been a reduced incidence of urethral vaginal fistula and vaginal stenosis. The mobilization occurs in a plane both anterior to the urogenital sinus and up to the bladder neck under the pubic symphysis and posteriorly along the urogenital sinus, and then along the posterior wall of the vagina.

Pull-Through Vaginoplasty

Pull-through vaginoplasty is reserved for use in severely masculinized genetic females, whose surgical treatment continues to present a major challenge. Initially, the approach was a combined perineal and abdominal approach with complete mobilization of the vagina and uterus and separation of the vagina from the urethra at the confluence. The abdominal mobilization will then allow the vagina to be brought down to the perineum. A modification of this approach was described by Passerini-Glazel[168] in which the more distal urogenital sinus tissue was used to provide an anterior vaginal wall flap, which will then connect to the true vagina and allow a complete perineal approach to the procedure.

Vaginal Reconstruction

Vaginal reconstruction has a role in certain DSD or structural abnormalities of the genital urinary tract. The DSD in which vaginal reconstruction may be indicated are 46,XX vaginal agenesis, 46,XY male karyotype with severely inadequate virilization or CAIS, and structural urogenital defects such as cloacal exstrophy or persistent cloaca or after a pelvic exenteration for malignancy. A variety of tissues and techniques are used for vaginal reconstruction: buccal tissue, skin grafting, progressive perineal indentation, and split-thickness or fold thickness tissue grafts with expanders, myocutaneous flaps, and bowel segment. The critical point in creating a vagina is to maintain an adequate perineal opening, an adequate-length tunnel, and good fixation to pelvic structures. This area is highly controversial in terms of the best management. Overall, the most popular tissue for vaginal plate replacement has been the split-thickness skin graft as described by McIndoe.[169] The major disadvantage of this technique is the need for long-term dilatation to maintain patency and to avoid vaginal stenosis. Recently, buccal mucosa grafts have been used rather than skin grafts for vaginal replacement as an alternative to the McIndoe graft.

The use of bowel segments for vaginal replacement was first described by Baldwin.[170] Because of an extraordinarily high mortality rate associated with this approach, earlier attempts using this technique were abandoned. Since then, this approach has been adopted by many groups and has been shown to be highly successful, with minimal complication rates. Early on, intestinal mucus production can be a problem, but this lessens over time, and mucus may act as a natural lubricant. Minimal perineal scarring is associated with this approach as well, and it can be done at a very young age.[171] Because of the risks and complications with any surgical approach, vaginal dilation has been recommended, when feasible, as first-line therapy for patients with vaginal agenesis who are candidates for a nonsurgical approach.

Gonadectomy

Gonadectomy prior to the age of consent is considered in DSD when the assigned sex is different from the gonadal sex, mostly in the 46,XY DSD and 45,X/46,XY DSD, and when there is a risk of tumor related to the testicular tissue. This is most commonly seen in complete gonadal dysgenesis (46,XY karyotype) in which dysgenetic gonads are high risk for gonadoblastoma at a young age in a female with a Y chromosome. An example is shown in Fig. 85.15, in which a 1-year-old infant with complete XY gonadal dysgenesis was found to have a gonadoblastoma on gonadectomy. Other syndromes in which gonadal malignancy is a concern include mixed gonadal dysgenesis and the presence of a dysplastic testis in an XY male.

Conservative management of the gonads in CAIS until puberty is recommended since tumors in these gonads do not develop until puberty and the risk is less than 1%. Alternatives to gonadectomy include bringing the gonads down (orchiopexy), monitoring with clinical examinations, imaging studies, and biopsies, although there are limited data regarding safe and effective long-term surveillance. The importance of shared decision making is critical for these adolescent girls. In DSD where there is a risk of virilization at puberty, hormonal suppression with sex hormone replacement congruent with gender identity is an alternative to surgical management until the patient is able to consent.[111]

Removal of Müllerian Remnants

Removal of müllerian remnants in male-assigned patients is not recommended unless they are associated with symptoms such as dysuria, urinary tract infections, pain, bleeding, herniation, and stone formation. Removal of the müllerian remnant can be performed laparoscopically. In most cases, müllerian remnants are asymptomatic, and cancers have rarely been reported.[121,172]

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86

Disorders of the Thyroid Gland

GRACE KIM, DEBIKA NANDI-MUNSHI, AND CAROLINA CECILIA DI BLASI

KEY POINTS

- Appropriate thyroid hormone function is essential for normal neurodevelopment in infancy and childhood. Hypothyroidism in the first year of life can result in significant deleterious effects on growth and neurologic injury.
- Delays in treating congenital hypothyroidism (CH) is the most common preventable cause of intellectual disability.
- Neonatal screening can provide early diagnosis and can prevent delays in treatment. Newborn screening methods differ and may miss rare forms of congenital hypothyroidism.
- In preterm newborns, thyroid hormone levels may fall because of the immaturity of the thyroid gland, but these changes may be exacerbated by complications of prematurity.
- Thyroid metabolism can be affected by exogenous sources of iodine, dopamine infusions, blood transfusion, and glucocorticoid treatment.
- The clinical manifestations of Graves disease in the newborn include irritability, flushing, diarrhea, vomiting, tachycardia, hypertension, poor weight gain, thyroid enlargement, and exophthalmos.

Regulation of Thyroid Function

The hypothalamic-pituitary-thyroid (HPT) axis functions as a typical feedback loop (Fig. 86.1). Triiodothyronine (T_3) levels in the pituitary gland direct the secretion of thyroid-stimulating hormone (TSH). Also, an inverse relationship exists between thyroid hormone formation and iodide level in the thyroid. Subsequently, the rate of hormone production is not affected by rapidly changing levels of iodide, and the reservoir of thyroid hormone balances against quick changes in hormone synthesis.

TSH, produced in the anterior pituitary, is the chief player in thyroid gland function and morphology. TSH provides negative feedback by decreasing the synthesis of thyrotropin-releasing hormone (TRH) from the hypothalamus, as well as blocking the action of TRH that stimulates TSH release.[1] Release of TSH fuels uptake of iodide by the thyroid gland and accelerates many steps in thyroid hormone synthesis. It also drives the growth and vascularization of the thyroid gland itself. Both T_3 and thyroxine (T_4) provide negative feedback to TSH secretion, while TRH determines the setpoint. Given the logarithmic relationship between free T_4 (fT_4) and TSH, TSH levels are a sensitive indicator of thyroid hormone status.

Thyroid Hormone Synthesis

Synthesis of thyroid hormone is initiated by the entry of iodide molecules into the follicular cell of the thyroid gland via the sodium–iodide transporter; these are then transported into the colloid. Once they are in the thyroid gland, oxidization of iodide occurs, after which it is quickly associated with thyroglobulin (TG). Organification of the iodide forms tyrosine residues, which are then coupled to form the two active thyroid hormones: T_4 and T_3. This complexed TG, which resides in the colloid of the thyroid gland, serves as the reservoir for the production of T_3 and T_4. Thyroid hormones are then moved via endocytosis into the follicular cell. This vesicle fuses with lysosomes, after which T_4 and T_3 are disassociated from TG via hydrolysis and eventually released into the bloodstream. Residual monoiodothyronine (MIT) and diiodothyronine (DIT) are deiodinated and enter the synthesis cycle again along with iodide (I^-) and tyrosine. Important steps in thyroid hormone synthesis are summarized in Fig. 86.2.

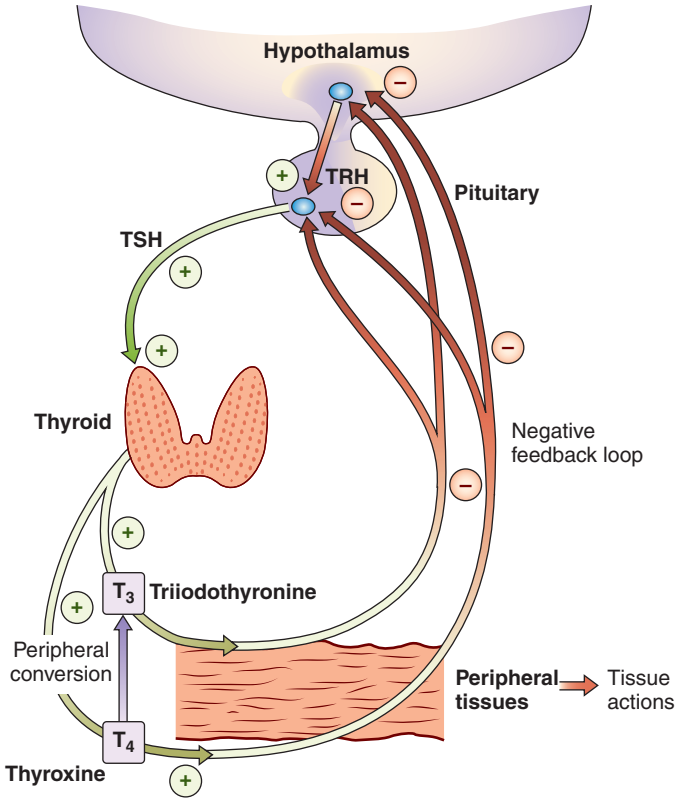
Serum Protein Binding and Transport

In the circulation, T_3 and T_4 are transported by attachment to binding proteins that include T_4-binding globulin (TBG), transthyretin (TTR; formerly termed T_4-binding prealbumin [TBPA]), and albumin. These binding transport proteins are produced in the liver. Production of these proteins increases through the latter half of gestation and is stimulated by estrogen. Free thyroid hormone levels appear to remain constant despite increasing total hormone concentrations because of an increase in binding protein production.

The serum concentration of T_4 is vastly greater than that of T_3 by a factor of 50 to 100. The primary transport protein (~75% of T_3 and T_4) is TBG. The remaining 25% of thyroid hormone is equally distributed between TBPA and albumin. The fT_4 concentration more precisely reflects the metabolic status in comparison with total T_4 or T_3. This is because only an unbound hormone can enter cells to exert its action.[2]

Embryogenesis of Hypothalamic-Pituitary-Thyroid Axis

The thyroid gland is the first endocrine organ to develop in the fetus. The development of the thyroid gland and the HPT axis occurs in two phases. The first phase is characterized by the embryogenesis of the structures involved, and the second phase is characterized by the maturation of the HPT axis, which is described later.[3] By about 3 weeks of gestation, the rudimentary beginnings of the hypothalamus have developed. Subsequently, anlagen from the floor of the forebrain and the Rathke pouch

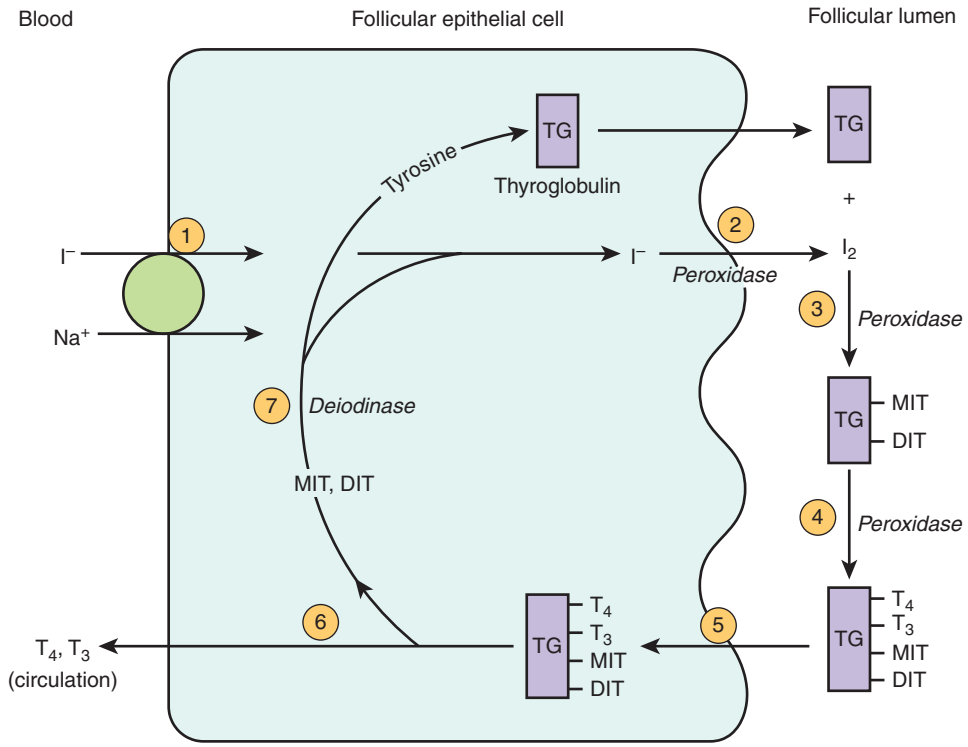


• **Fig. 86.1** The Hypothalamic–Pituitary–Thyroid Axis. Thyroid stimulating hormone *(TSH)* is secreted in response to TSH-releasing hormone *(TRH)* and stimulates secretion of thyroxine *(T_4)* and triiodothyronine *(T_3)* from the thyroid. T_3 and T_4 have actions in peripheral tissues and exert negative feedback on the hypothalamus and the pituitary.

converge to form the pituitary gland. By 14 to 15 weeks of gestation, this embryologic structure has become the mature pituitary gland.[4] Detection of TSH has been observed as early as 10 to 12 weeks of gestation.[5]

A programmed sequence of transcription and homeobox factors (e.g., thyroid transcription factors-1 and -2 [TTF-1 or TiTF-1, now designated as NKX2 homeobox 1 -NKX2.1-; and TTF-2, now Forkhead box E1 -FOXE1-] and paired box gene 8 [PAX8]) directs thyroid embryogenesis. Thyroid gland development begins with convergence of three structures: the median anlage from the floor of the pharyngeal pouch (caudal end of the foregut) and two lateral anlagen from the fourth pharyngobranchial pouch. The median anlage is visible by day 22 after conception. The lateral anlagen develop into a pair of ultimobranchial bodies from which the calcitonin-producing parafollicular C cells arise. Between day 32 and day 48 after conception, this primordial thyroid structure breaks off from the pharyngeal pouch and completes its migration to the pretracheal region. Little is known about the molecular factors involved in this migration; however, FOXE-1 has been implicated in cases of thyroid ectopy.[6]

By 4 weeks after thyroid descent is complete, functional and structural changes are mature, and the thyroid can produce thyroid hormone. Follicles fill with colloid. By 12 weeks of gestation, the thyroid gland is able to take up iodide, synthesize TBG, and produce hydrogen peroxide (H_2O_2) at the apical membrane—all steps required for thyroid hormone production. T_4 has been detected in human fetal blood as early as 11 to 12 weeks of gestation.[7] This accrual of the ability to produce thyroid hormone coincides with the maturation of the hypothalamus and pituitary gland with the production of TRH and TSH.



• **Fig. 86.2** Steps involved in the synthesis of thyroid hormones: (1) iodide transport; (2) oxidation of I^- to I_2; (3) organification of I_2 into monoiodothyronine *(MIT)* and diiodothyronine *(DIT)*; (4) coupling reaction of MIT and DIT to form triiodothyronine *(T_3)* and thyroxine *(T_4)*; (5) endocytosis of thyroglobulin *(TG)*; (6) hydrolysis of T_4 and T_3, T_4, and T_3 enter circulation; and (7) deiodination of residual MIT and DIT, recycling of I^- and tyrosine.

Fetal–Placental–Maternal Thyroid Interaction

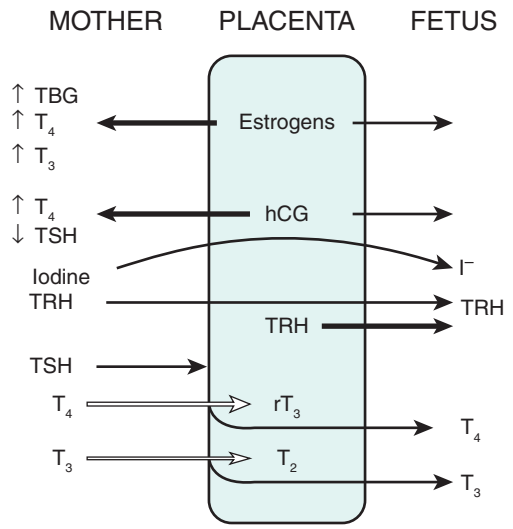
The placenta is of utmost importance in fetal thyroid physiology as it regulates the passage of maternal thyroid hormone to the fetus (Fig. 86.3). Thyroid hormone is paramount for normal neurodevelopment. Maternal-to-fetal transfer of T_4 is essential, especially in the first trimester when the fetal thyroid axis has yet to mature. With the landmark study by Vulsma et al.,[8] it was shown that T_4 does cross the placenta, contradicting previous concepts—placental transfer of T_4 in athyreotic fetuses resulted in levels that were 25% to 50% of those of normal term newborns. There are mixed reports on the effect of hypothyroidism in the mother on the neonate. In case series of women with severe hypothyroidism diagnosed during pregnancy and treated to normalize levels during the third trimester, the offspring were noted to have normal intellectual outcomes.[9,10] Additionally, similar developmental outcomes were seen in offspring of both T_4-treated versus placebo-treated women with low T_4 levels.[11,12] Multiple clinical studies have shown the deleterious effects of inadequate maternal thyroid hormone levels.[13–15]

TSH does not cross the placenta; however, TRH does. During pregnancy, maternal thyroid axis changes result in increased fT_4 levels early and maintenance of higher levels until birth (Fig. 86.4). This is a result of human chorionic gonadotropin produced by the placenta, which is homologous to TSH with some TSH-like activity.[16]

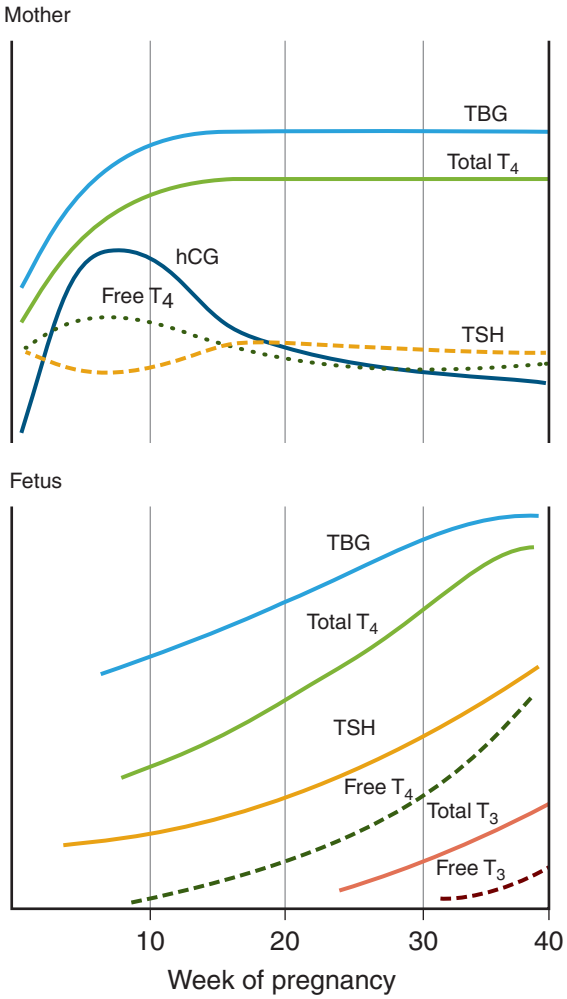
Although most of the transfer of the maternal thyroid hormone is dependent on maternal thyroid hormone concentrations, plasma membrane thyroid hormone transporters and metabolism via deiodinases (DIs) also play a role. Thyroid hormone transporters are found in the apical and basolateral membrane of the placenta. Several transporters have been discovered in the placenta (monocarboxylate transporters, L-type amino acid transporters, and organic anion–transporting polypeptides).[17] The placenta also has both type 2 and type 3 DI, which is involved in the metabolism of thyroid hormone in this milieu. The activity level of type 3 DI is 200 times higher than that of type 2 DI. T_3 is thought not to cross the placenta; however, this is not clear.[18]

Iodide is taken up by the thyroid gland for thyroid hormone synthesis. There is free passage of iodides between the mother and the fetus. Large-quantity ingestion of iodine will decrease the synthesis of T_4 transiently. This decreased synthesis is due to decreased uptake via the sodium–iodide symporter. Typically, there is an escape from this phenomenon in about 48 hours. However, cases of goiter and hypothyroidism have been reported with excessive iodine intake.[19] An adequate amount of iodine, around 250 µg/day, is still recommended for pregnant women according to the American Thyroid Association.[20]

Miscellaneous other compounds can cross the placenta, affecting fetal thyroid function. TSH receptor-blocking antibodies (TRBAbs), also known as TSH Binding Inhibitory Immunoglobulins, have been implicated in cases of transient neonatal hypothyroidism.[21] In mothers with a history of Graves disease,



• **Fig. 86.3** The Placental Role in Thyroid Metabolism. The placenta produces estrogens and human chorionic gonadotropin *(hCG)*, which increase maternal thyroxine *(T_4)*-binding globulin *(TBG)* levels and stimulate maternal thyroid hormone production, respectively. Both activities tend to increase maternal T_4 and triiodothyronine *(T_3)* concentrations and inhibit maternal thyroid-stimulating hormone *(TSH)* secretion. Iodide and TSH-releasing hormone *(TRH)* readily cross the placenta. In addition, the placenta synthesizes TRH. The placenta is impermeable to TSH and only partially permeable to T_4 and T_3. *rT_3*, Reverse triiodothyronine.



• **Fig. 86.4** Relative changes in maternal and fetal thyroid function during pregnancy. The effects of pregnancy on the mother include a marked and early increase in hepatic production of thyroxine *(T_4)*-binding globulin *(TBG)* and placental production of human chorionic gonadotropin *(hCG)*. The increase in serum TBG level, in turn, increases serum T_4 concentrations; hCG has thyroid-stimulating hormone *(TSH)*-like activity and stimulates T_4 secretion. The transient hCG induces increase in serum T_4 and inhibits maternal secretion of TSH.

thyroid-stimulating immunoglobulin (TSI) can lead to thyrotoxicosis in the neonate.[22] Antithyroid medications ingested by the mother can result in fetal goiter with or without hypothyroidism.[23]

Thyroid System Maturation

The fetal thyroid gland can synthesize and secrete thyroid hormone after 12 weeks of gestation. As the pregnancy continues, the levels of fT_4 and T_4 continue to rise in step with the gestational age of the fetus. T_3 levels, however, remain low until about 30 weeks of gestation, after which they increase slowly until birth. This delayed rise in T_3 levels is due to low rates of conversion of T_4 to T_3 by type 1 DI and high activity of type 3 DI metabolizing T_3 to diiodothyronine (DIT).[4] With an increased amount of type 1 DI produced by the liver after 30 weeks, there is increased conversion of T_4 to T_3 in the liver and decreased metabolism of T_3 by placental type 3 DI, resulting in the steady rise until delivery. TSH has also been detected at 12 weeks of gestation, with subsequent increases in its level in parallel with increases in fT_4 level.[24] The level of reverse T_3 (rT_3) is relatively high early in the third trimester because of placental type 3 DI activity converting T_4 to rT_3 and T_3 to DIT (Fig. 86.5).

Fetal Thyroid Hormone Metabolism

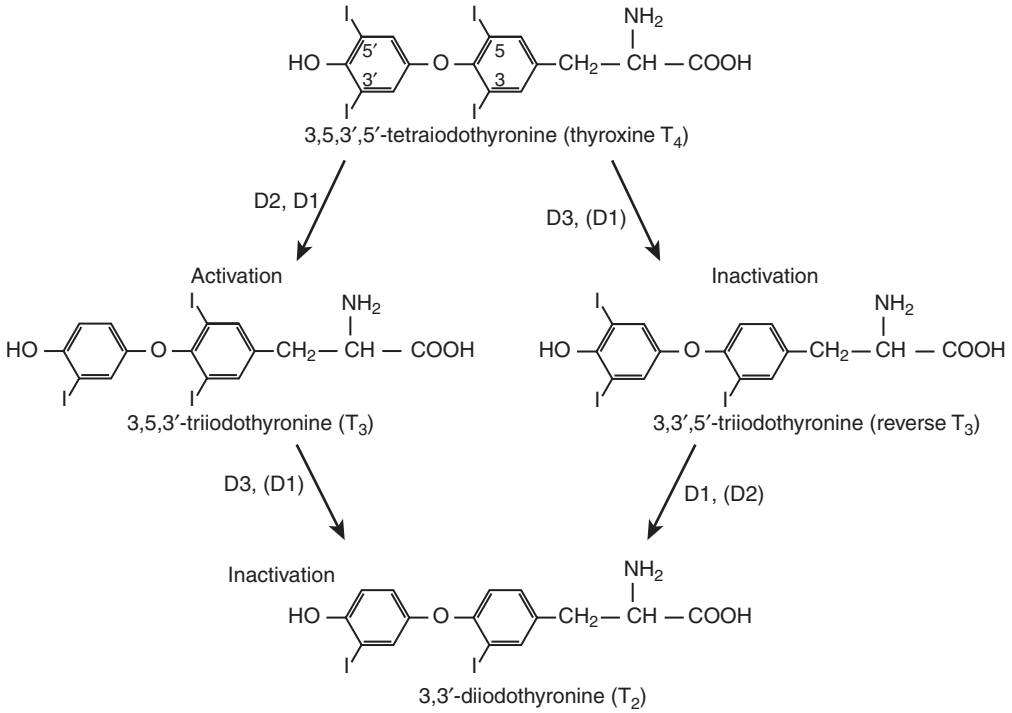
The thyroid gland is the body's only source of T_4. Conversion of T_4 to T_3 peripherally contributes to most of the circulating T_3. This conversion is accomplished by a group of three DIs, types 1, 2, and 3 (see Fig. 86.5). T_3, with its greater affinity for the thyroid hormone receptor, is the active form of thyroid hormone. T_3 is derived from the deiodination of the outer ring of T_4. Conversely, deiodination of T_4 at the inner ring produces the inactive rT_3.[25]

Type 2 and type 3 DIs are selective in their action, performing deiodination on only the outer ring and inner ring, respectively. Type 1 DI is nonselective, with deiodination at the outer ring converting T_4 to T_3 and deiodination at the inner ring producing rT_3 from T_4. Type 1 DI is produced in the liver, kidney, and thyroid. Type 1 DI is inhibited by propylthiouracil and rT_3 and stimulated by thyroid hormone. Type 2 DI is primarily found in the brain, pituitary, placenta, skeletal muscle, heart, thyroid, and brown adipose tissue, and is not sensitive to propylthiouracil and is inhibited by thyroid hormone. It converts T_4 to T_3. Type 3 DI is found primarily in fetal tissues, including the placenta, brain, and skin. Type 3 DI converts T_4 to rT_3 and T_3 to DIT.[26]

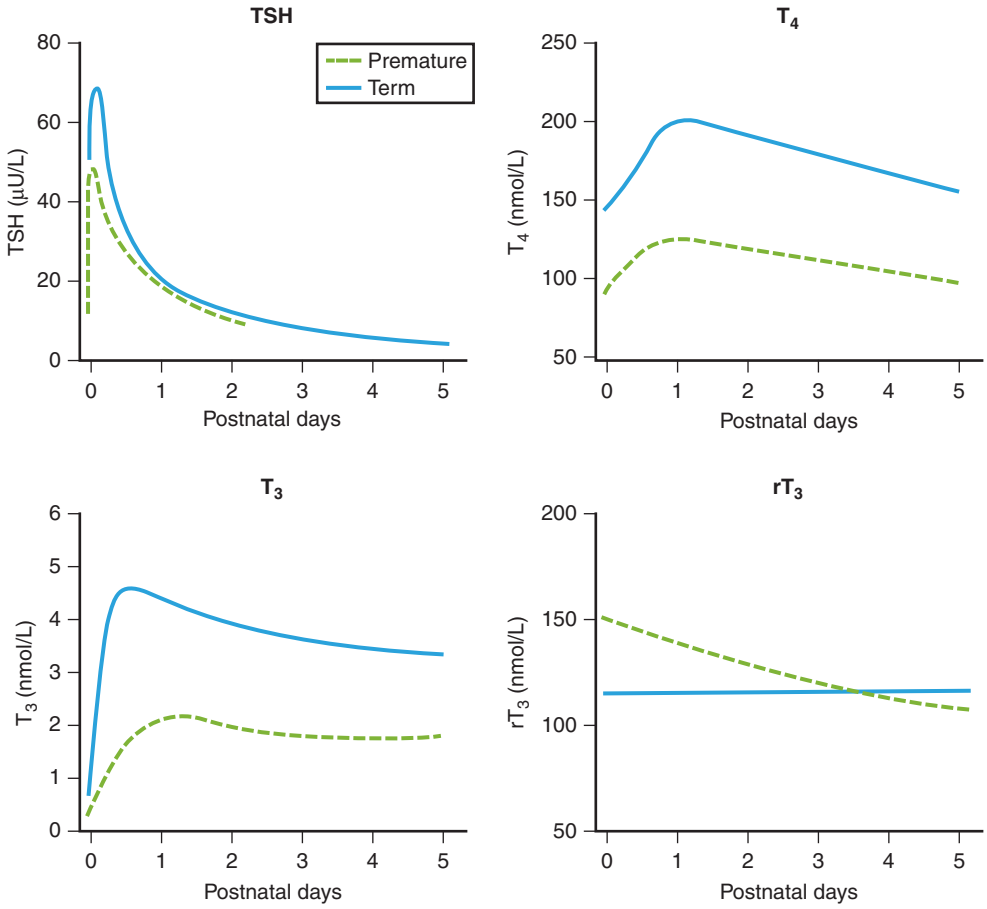
During pregnancy, fetal DI levels vary with time and are reflective of thyroid hormone concentrations. Type 1 DI in hepatic tissues remains quiescent until late in gestation, resulting in a late rise of fetal T_3 levels. Type 3 DI activity is responsible for high rT_3 levels in the fetus. The interplay of these enzymes is critical to normal brain development. Given that both type 1 and type 2 DI are sensitive to thyroid hormone levels, a hypothyroid fetus will have increasing type 2 DI activity in the brain and decreasing type 1 hepatic activity, resulting in more T_4 in the brain, at which point it is converted to T_3 by type 2 DI.[27]

Extrauterine Thyroid Adaptation

At birth, fT_4 levels in the newborn are slightly below that of the maternal concentration, and the T_3 level is relatively low in umbilical cord blood. Also, at this time, partly in response to cold and stress, there is a TSH surge that peaks at about 2 to 4 hours of life and then returns to its initial value within about 48 hours (Fig. 86.6). T_3 and T_4 levels also rise within a few hours of birth and reach their peak by 24 hours. TSH, as well as extrathyroidal conversion of T_3 to T_4 by type 1 and type 2 DIs, contributes to this surge of thyroid hormone.[28] During the next 4 to 5 weeks of life, T_4 and T_3 levels progressively decrease to levels just above the normal range of children. By 1 month of age, the TSH level has



• **Fig. 86.5** Enzymatic conversion of iodothyronines. *D1*, Type 1 deiodinase; *D2*, type 2 deiodinase; *D3*, type 3 deiodinase.



• **Fig. 86.6** Postnatal thyroid-stimulating hormone *(TSH)*, thyroxine *(T_4)*, triiodothyronine *(T_3)*, and reverse T_3 *(rT_3)* secretion in the term and premature infant in the first week of life. (Adapted from www.thyroid-manager.org, the free online thyroid textbook. In: Brown R, Larsen PR, De Groot LJ, eds. *Thyroid Gland Development and Disease in Infants and Children*. South Dartmouth, MA: Endocrine Education, Inc; 2009.)

declined to about 5 mU/L[29] and declines further to a near-adult range of 0.5 to 4 mU/L by the second month.[30]

Infants born prematurely appear to have an attenuated TSH surge. This is due to an immature HPT axis. T_3 and T_4 levels also have a blunted rise in comparison with the levels in term newborns. The TSH level can peak at about 40 mU/L by 30 minutes.[31] Many factors need to be taken into account when one is assessing thyroid function in preterm infants. As many preterm newborns have other complications, such as respiratory distress syndrome or nutritional problems, serum T_4, and especially T_3, concentration may fall to low levels as a result of the immaturity of the thyroid gland, as well as reduced TBG production. Illness also causes suppression of the hypothalamic-pituitary-thyroid axis, impairs the conversion of T_4 to T_3, and increases type 3 DI activity.[28]

Congenital Hypothyroidism

Epidemiology

Congenital hypothyroidism (CH) is defined as variable dysfunction of the hypothalamic-pituitary-thyroid axis present at birth, resulting in insufficient thyroid hormone production and thyroid hormone deficiency. CH is caused by abnormal development or function of the thyroid gland, pituitary, hypothalamus, or thyroid hormone function. The overall incidence is 1 in 2500 to 1 in 3000 newborns.[32,33] Higher incidence rates have been reported in certain populations. The prevalence rates of CH can differ with sex, ethnicity, and birthweight. Twice as many female as male infants are affected. Compared with whites, the prevalence of CH is higher in Asians and Hispanics and lower in Blacks. A study noted an increased risk of CH in macrosomic (>4500 g) and low birthweight (<2000 g) infants.[32] There is an increased risk in infants with Down syndrome. The reported incidence of central CH is 1 in 80,000 to 1 in 100,000.

Pathophysiology

In most cases, CH is permanent and results from an abnormality in thyroid gland development (dysgenesis or agenesis) or a defect in thyroid hormonogenesis. Less commonly, the altered thyroid function is transient, attributable to transplacental passage of maternal medication, maternal blocking antibodies, or iodine deficiency or excess. In rare cases, CH may result from a pituitary or hypothalamic abnormality. Potential causes are listed in Table 86.1. Regardless of the duration of the thyroid dysfunction, immediate treatment with levothyroxine (LT_4) in the newborn period is needed to prevent cognitive or neurodevelopmental decline. The developing fetal brain is protected in utero by an adequate source of T_3, supplied by local deiodination of maternal T_4 in the fetal brain.

TABLE 86.1 Thyroid Disorders and Their Approximate Incidence in the Neonatal Period

Disorder	Incidence
Thyroid Dysgenesis	1 in 2500 to 1 in 4000
Agenesis	
Hypogenesis	
Ectopia	
Thyroid Dyshormonogenesis	1 in 30,000
TSH receptor defect	
Iodide-trapping defect	
Thyroid peroxidase defect	
H_2O_2 generation defect	
Defect in thyroglobulin	
Pendred syndrome	
Dehalogenase defect	
TSH signaling defect: Albright hereditary osteodystrophy	
Transient Hypothyroidism	1 in 12,000 to 1 in 30,000
Drug induced	
Maternal antibody induced	
Idiopathic	
Hypothalamic-Pituitary Hypothyroidism	1 in 80,000 to 1 in 100,000
Hypothalamic-pituitary anomaly	
Panhypopituitarism	
Isolated TSH deficiency	

H_2O_2, Hydrogen peroxide; *TSH*, thyroid stimulating hormone.

TABLE 86.2 Clinical Signs and Symptoms of Congenital Hypothyroidism in Infancy

Age/Manifestation	Frequency (%)
0–7 Days	
Prolonged jaundice >3 days	73
Birthweight >4 kg	40
Poor feeding	40
Transient hypothermia	38
Large posterior fontanel (>5 mm)	32
1–4 Weeks	
Failure to gain weight	45
Constipation	35
Hypoactivity	33
1–3 Months	
Failure to thrive	90
Umbilical hernia	49
Macroglossia	43
Myxedema	40
Hoarse cry	30

Clinical Presentation

The diagnosis of hypothyroidism should be considered in any infant with prolonged jaundice, transient hypothermia, an enlarged (greater than 5 mm) posterior fontanel, failure to feed properly, or respiratory distress with feeding.[34] Approximately one-third of maternal T_4 crosses to the fetus at term. With a half-life of 6 days, the maternal T_4 will be metabolized and excreted by 3 to 4 weeks of age.[35] The classic signs evolve during the first few weeks after birth. Fetal growth is normal; however, there is a delay in bone maturation and a rapid reduction in growth rate after birth[36] with progressively worsening myxedema in subcutaneous tissues and tongue. The thickened tongue becomes protuberant, and increasing difficulty in nursing and handling salivary secretions is noted. The cry is hoarse because of myxedema of the vocal cords. Additional signs and symptoms include marked muscular hypotonia; constipation; thick, dry, cold skin; long, abundant, and coarse hair; abdominal distention; umbilical hernia; hyporeflexia; bradycardia; hypotension with narrow pulse pressure; anemia; and widely patent cranial sutures. Goiter can be present. The typical facies are characterized by a depressed nasal bridge, a relatively narrow forehead, and puffy eyelids.[37] The cardiac silhouette may be enlarged, and the electrocardiogram shows low voltage and a prolonged conduction time. Some of the signs and symptoms are present by 6 to 12 weeks postnatally, especially lethargy, constipation, and umbilical hernia. The characteristic facies and growth retardation become increasingly obvious during the first several months of life.

Nonspecific symptoms and signs associated with hypothyroidism are listed in Table 86.2. Because clinical manifestations of hypothyroidism may not appear until weeks after birth, even in athyreotic infants, newborn screening has enabled pediatricians to identify newborns with low thyroid hormone production and to initiate therapy within the first 2 weeks of life before the development of signs and symptoms.

Evaluation

Screening for Neonatal Hypothyroidism

Newborn screening programs for CH avoid delay in its diagnosis because signs and symptoms of CH may not manifest for several weeks.

Newborn screening programs are designed to detect abnormal thyroid hormone levels in newborn blood samples collected on filter paper. Some programs measure TSH primarily with T_4 levels as a backup, some measure T_4 primarily with TSH as a backup, and some measure both TSH and T_4 primarily. Screening programs have been established in industrialized countries. Despite the benefits of neonatal screening, 70% of infants worldwide are born in areas that do not have access to neonatal screening.[38]

Both screening methods—primary TSH/backup T_4 method and primary T_4/backup TSH method—appear to be capable of detecting almost all infants with primary CH. However, the combined TSH plus T_4 approach is the ideal screening approach.

Primary CH is associated with a low serum T_4 and fT_4 concentration and a high TSH concentration in umbilical cord blood or neonatal blood samples. It is estimated that 5% to 8% of affected infants can escape detection by newborn screening because of a delayed elevation in serum TSH concentration or because of errors in sample collection or laboratory routine. In addition, infants with TSH deficiency who have normal TSH levels are not detected because most newborn screening programs report only those infants with elevated TSH levels. Infants with central hypothyroidism may have normal or low TSH. Normal TSH would be inappropriate in the setting of low thyroid hormone levels. Newborn screens that use elevated TSH to detect congenital hypothyroidism will miss diagnosing these infants. Infants with signs or symptoms suggestive of thyroid dysfunction (see Table 86.2) should be investigated regardless of previous screening results. Determination of serum fT_4, T_4, and TSH values is necessary for any infant with suspicious clinical or laboratory findings.

Accurate screening results depend on good-quality blood samples. Blood samples should be collected on approved filter paper forms, dried at room temperature, and not subject to excessive heat. Capillary blood samples are placed in the circular areas to fill and saturate them and applied to one side only. Filter paper should not be handled or placed on wet surfaces.[39]

Every infant should be tested before discharge from the nursery, optimally by 2 to 4 days of age. The practice of early hospital discharge (before 48 hours of age) has led to an increased frequency of false-positive results because of the normal physiologic TSH surge that occurs after birth. Infants screened before 48 hours of age require the newborn screen to be rechecked by the primary care physician at 2 weeks of life.

Some infants may have a false-negative neonatal screening result or have a high risk of mild CH not detected by newborn screen. These infants include premature, low-birth weight, and sick babies. These babies might not be able to generate an adequate TSH response in the first weeks of life. As a result, in TSH-based neonatal screening programs, their results may be false negative.[40,41] Maturation and recovery of the HPT axis with an increase in TSH occurs between 2 and 6 weeks of life. Screening programs have revised recommendations for this group of infants. In preterm newborns, the TSH surge, T_4, and T_3 are lower than those in term neonates. The immaturity of the HPT axis in extremely premature infants is characterized by markedly blunted TSH surge, T_4 decrease, and lower and shorter T_3 increase within the first 24 hours of life.

Transient hypothyroxinemia of the preterm neonate is a frequent finding aggravated by general illness; it is due to an immature HPT axis function. LT_4 therapy of preterm hypothyroxinemia remains controversial. Down syndrome is associated with a 14 to 21 times higher than expected incidence of CH and highly prevalent mild TSH elevation, especially in the first months to years of life.[42,43] In patients with Down syndrome, it is recommended to measure TSH at the end of the neonatal period.[38]

Endocrine testing is recommended in all neonates with a familial history of CH or signs/symptoms of CH, such as micropenis with undescended testes, hypoglycemia, prolonged jaundice, or unexplained failure to thrive.[38]

All neonates with CH should be examined carefully for dysmorphic features suggestive of syndromic CH and congenital malformations, particularly cardiac.[38] The Bamforth-Lazarus syndrome is characterized by thyroid dysgenesis, cleft palate, spiky hair with or without bilateral choanal atresia, or bifid epiglottis (biallelic mutation in the FOXE1 gene). Brain-lung-thyroid syndrome is characterized by CH, respiratory distress syndrome, and benign hereditary chorea (due to NKX2-1 haploinsufficiency). Alagille syndrome is associated with thyroid in situ, bile duct hypoplasia, and cardiac malformations. William-Beuren and DiGeorge syndromes have a high prevalence of thyroid hypoplasia. Kabuki and Johanson-Blizzard syndrome is associated with subclinical hypothyroidism. Pendred syndrome can be seen with or without goiter and sensorineural hearing loss (SLC26A4 gene).

Thyroid Function Tests

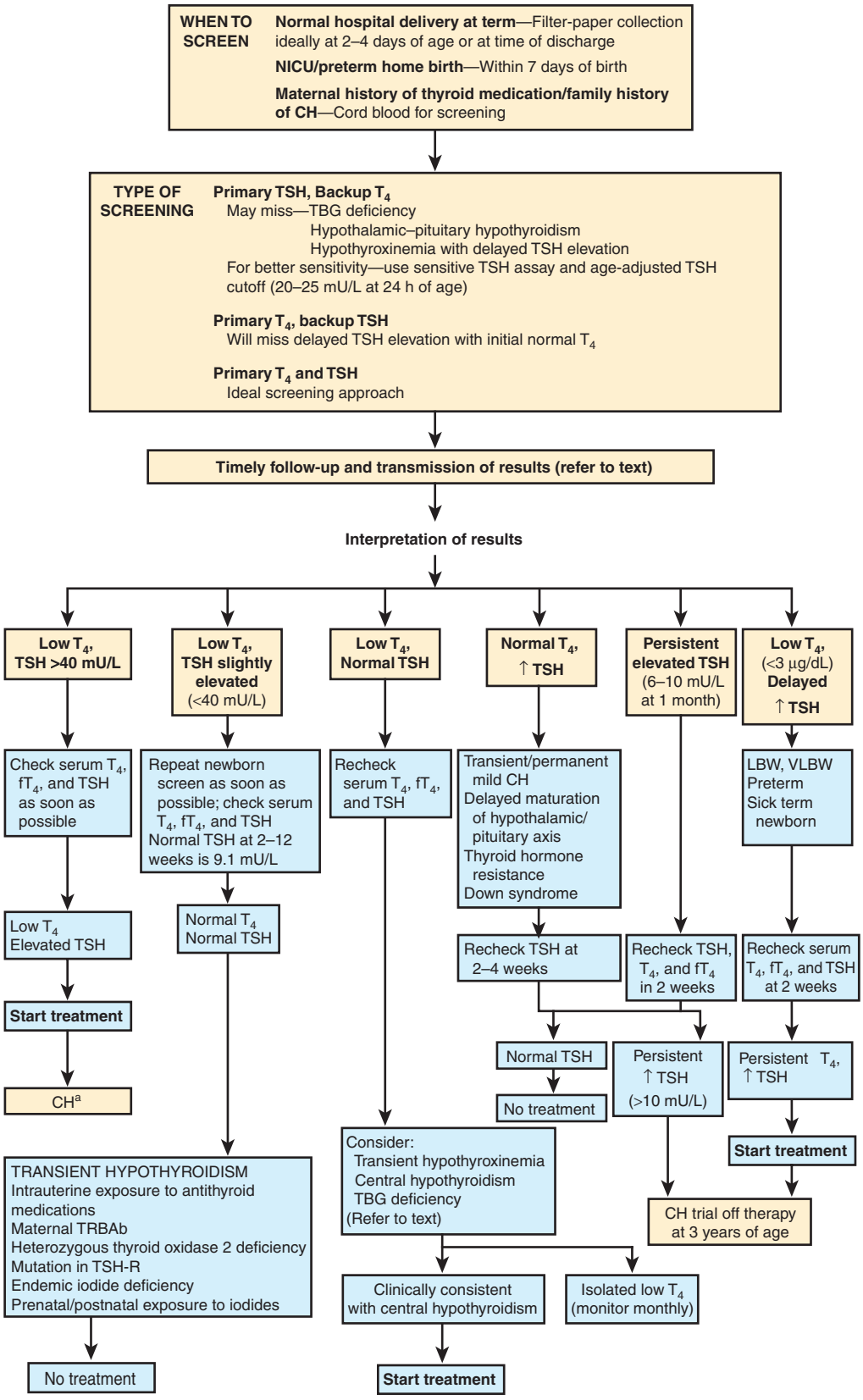
When the diagnosis of CH is suspected, confirmatory thyroid function tests should be performed. It is advisable to assess fT_4 and TSH.[38] Elevated serum TSH value is the most sensitive and specific test to confirm the diagnosis of primary hypothyroidism. Typical laboratory findings for primary hypothyroidism include elevated TSH levels, with T_4 and fT_4 values in the low or low-normal range. A low or undetectable TG concentration[44] with TSH level elevation confirms a dysgenetic or absent thyroid gland,[45] whereas a high TG concentration with TSH level elevation suggests an organification defect.[46] In central hypothyroidism, TSH level is usually normal, T_4 level is low normal, and fT_4 level is in the lowest third of normal.[47]

Interpreting Thyroid Function Tests

When interpreting thyroid function tests, it is critically important to consider the day of life on which the samples were drawn. The recommended period for newborn screening of thyroid function is at 24 hours of life. If screening is done at less than 24 hours of life, it must be redone, regardless of the results. After 24 hours of life, a TSH value greater than 40 μU/L is highly suggestive of hypothyroidism, but confirmatory testing is required. A TSH value above the normal limit for the hours of life and gestational age but below 40 mU/L is indeterminate. Therefore, repeated testing is necessary, and the primary care physician should obtain a second screen (Fig. 86.7). The practice of early hospital discharge (less than 48 hours of age) has led to a higher rate of indeterminate results.

Eight to 10% of infants with CH have TSH values between 20 and 40 mU/L. One in 12 to 1 in 24 hypothyroid infants (1 in 50,000 to 1 in 100,000 newborns) will have a screening TSH level of less than 20 mU/L, with a delayed postnatal increase to hypothyroid levels.[48] Therefore, any infant with suspicious screening or sampling results requires confirmatory testing with measurement of serum fT_4, T_4, and TSH concentrations.[39]

The biochemical profile of hypothalamic–pituitary hypothyroidism comprises low serum T_4 concentration with a normal TSH value. A similar biochemical profile characterizes TBG deficiency (1 in 5000). However, the two states can be differentiated by further assessment of TBG and fT_4 levels. A low serum TBG concentration and a normal fT_4 level identify patients with TBG deficiency. In contrast, a low or low-normal fT_4 level and a normal TBG level identify a patient with hypothalamic-pituitary hypothyroidism. An infant with a low fT_4 concentration needs to be carefully examined for evidence of hypothyroidism, and other tests of pituitary function should be conducted. TRH stimulation testing can be used to differentiate between primary and central hypothyroidism. TRH, however, is not available in the United States. A subnormal TSH response to TRH or a normal but delayed and prolonged TSH response to TRH indicates central hypothyroidism.[47] TSH deficiency may be isolated or associated with other pituitary hormone deficiencies. Frequently it is not possible to



• **Fig. 86.7** Algorithm for evaluating congenital hypothyroidism *(CH)* on the basis of newborn screening results. *fT_4*, Free thyroxine; *LBW*, low birthweight; *NICU*, neonatal intensive care unit; *T_4*, thyroxine; *TBG*, thyroxine-binding globulin; *TRBAb*, thyroid-stimulating hormone receptor–blocking antibody; *TSH*, thyroid-stimulating hormone; *TSH-R*, thyroid-stimulating hormone receptor; *VLBW*, very low birthweight. (Modified from Rose SR, Brown RS, Foley T, et al. Update of newborn screening and therapy for congenital hypothyroidism. *Pediatrics*. 2006;117:2290–2303.)

discriminate between secondary and tertiary hypothyroidism, so the most useful diagnostic term is central hypothyroidism. The presence of midline facial abnormalities, hypoglycemia (growth hormone and/or adrenocorticotropic hormone deficiencies), microphallus (gonadotropin and growth hormone deficiencies), nystagmus or blindness, or polyuria (antidiuretic hormone deficiency) should suggest the possibility of a hypothalamic abnormality. Septo-optic dysplasia, often associated with pituitary hormone deficiencies, can manifest itself as CH. Mutations in HESX1 have been described in septo-optic dysplasia. Alternatively, multiple pituitary hormone deficiencies suggest a genetic defect in the cascade leading to fetal pituitary formation, such as in PROP1, LHX3, LHX4, and POU1F1.

Isolated TRH deficiency may cause low-normal T_4 and low-normal TSH levels. Mutations have been identified in the TRH gene, TRH receptor gene, and the gene encoding the β subunit of TSH. Newborns who are born before term, of low birthweight (<2500 g) or very low birthweight (<1500 g), and ill are found among those with this set of laboratory values. In neonates, inhibition of TSH causing low T_4 concentration can result from dopamine infusions or high-dose glucocorticoids.[39]

A strategy of second newborn screening should be considered for the following conditions: preterm neonates; low birthweight or very low birthweight neonates; ill newborns admitted to the neonatal intensive care unit; specimen collection within the first 24 hours of life; and multiple births.

TABLE 86.3 Genes and Thyroid Development

Gene	Thyroid Phenotype	Other Features
TTF-2/ FOXE-1	Athyreosis	Cleft palate, choanal atresia, kinky hair, bifid epiglottis
TTF-1/ NKX2.1	Athyreosis to normal gland	Respiratory distress syndrome, developmental delays/hypotonia, ataxia/ choreoathetosis
PAX-8	Athyreosis to normal gland	Cysts within thyroid remnants, kidney and urinary tract malformations
GLIS3	Athyreosis to normal gland	Congenital glaucoma, deafness, liver/kidney and pancreatic abnormalities
TSHR	Athyreosis to normal gland	None
NKX2.5	Athyreosis, ectopy	Cardiac defects

FOXE-1, Forkhead box E1; *GLIS3*, GLIS family zinc finger 3; *PAX-8*, paired box 8; *TSHR*, thyroid-stimulating hormone receptor; *TTF-1*, transcription termination factor 1; *TTF-2*, transcription termination factor 2.

Specific Causes of Hypothyroidism

Thyroid Dysgenesis

Eighty-five percent of cases of permanent CH are associated with abnormal development of the thyroid gland, which includes agenesis, ectopy, or hypoplasia of the gland. Thyroid ectopy accounts for approximately two-thirds of cases worldwide.[24] There is female predominance associated with ectopic glands. Ectopic locations of the thyroid gland include lingual, neck, and substernal locations. Most cases are sporadic; however, more recent studies show evidence of genetic factors involved in the pathogenesis.

A unique combination of transcription factors controls the embryonal development of the thyroid gland. Mutations in the genes encoding these transcription factors can lead to varying degrees of abnormalities, from athyreosis to normal glands. Other clinical features can be associated, as noted in Table 86.3. Thyroid ectopy, the most common form of thyroid dysgenesis, remains unexplained.

Mutation in NKX2.5, which encodes a transcription factor involved in heart morphogenesis, has also been reported to be associated with thyroid dysgenesis.[49]

Extrathyroidal abnormalities occur at a higher frequency in children with CH. The most frequent malformations associated with thyroid dysgenesis are cardiac, largely septation defects.[50] Other relatively common malformations include anomalies of the gastrointestinal tract, nervous system, and eyes.[51–53]

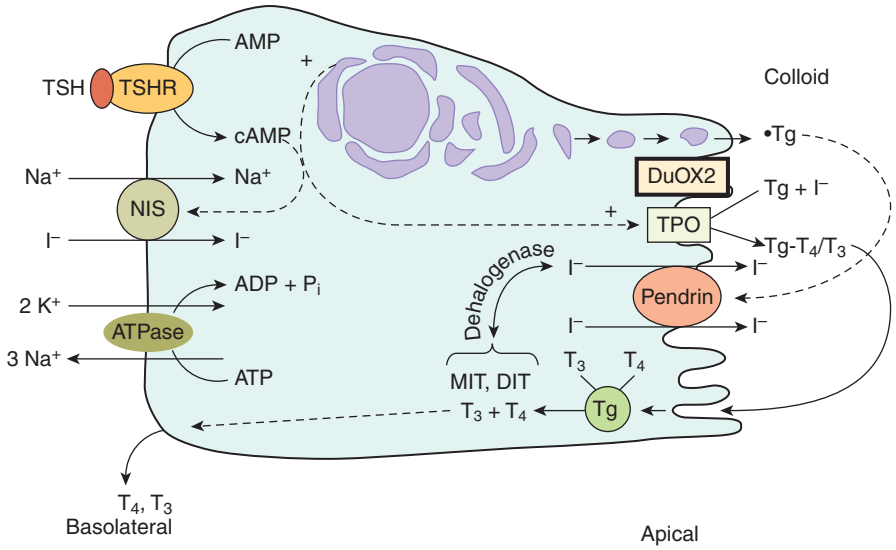
The biochemical picture can differ depending on the amount of thyroid tissue remaining. The presence of residual thyroid tissue is evident by detectable TG levels and normal or near-normal T_3 levels with low T_4 and elevated TSH values. The presence of residual thyroid tissue can be confirmed with a thyroid scan.

Thyroid Dyshormonogenesis

The remaining 10% to 15% of cases of CH involve defects in the synthesis of thyroid hormone. Hereditary defects in virtually all of the steps of thyroid hormone synthesis, secretion, and action have been described (Fig. 86.8). These patients often have normally located and normal-sized thyroid glands. Thyroid enlargement, however, can present at birth or even before, but more commonly, goiter develops later in life. The inheritance pattern is autosomal recessive.[54]

Defects associated with the trapping of iodide or in the oxidation or organification of iodide can result in dyshormonogenesis. These mutations include:

1. Sodium–iodine symporter defect. The gene encoding the sodium–iodine symporter is SLC5A5 on chromosome 19. Hypothyroidism presents most frequently in the neonatal or infantile period. Patients have a goiter on physical examination or ultrasound imaging, contrasting with limited or absent uptake on scintigraphy.
2. Thyroid peroxidase (TPO) defect. TPO is the enzyme responsible for iodide oxidation, organification, and iodothyrosine coupling. Mutations in the thyroid peroxidase gene are the most common cause of thyroid dyshormonogenesis. The enzyme is located at the apical membrane of the follicular cell. The clinical presentation is permanent goitrous, CH, high uptake on thyroid scintigraphy, and a high serum TG level.
3. Defects in H_2O_2 generation. Dual oxidase 1 and dual oxidase 2 are enzymes that generate H_2O_2 at the apical membrane.
4. TG defect. TG is an iodinated glycoprotein. The TG gene is located on chromosome 8. TG serves as the matrix for the synthesis of T_4 and T_3 and the storage of thyroid hormone and iodine. It is the essential substrate for organification and the major protein component of the colloid in the follicular lumen. Goiter and hypothyroidism manifest themselves at birth or before, and patients have low or undetectable TG levels.
5. Pendred syndrome is an autosomal recessive disorder associated with a partial defect in iodine organification, goiter, and sensorineural deafness. It is caused by mutations in the SLC26A4 gene on chromosome 7, which encodes the chloride-iodide



• **Fig. 86.8** A thyroid follicular cell, indicating areas of possible defects in thyroid hormone synthesis (e.g., thyroid dyshormonogenesis). *ADP*, Adenosine diphosphate; *AMP*, adenosine monophosphate; *cAMP*, cyclic AMP; *ATPase*, adenosine triphosphatase; *DIT*, diiodothyronine; *DuOX2*, dual oxidase 2; *K*$^+$, potassium ion; *MIT*, monoiodothyronine; *Na*$^+$, sodium ion; *NIS*, sodium–iodide symporter; P_i, inorganic phosphate; T_3, triiodothyronine; T_4, thyroxine; *Tg*, thyroglobulin; *TPO*, thyroid peroxidase; *TSH*, thyroid-stimulating hormone; *TSHR*, thyroid-stimulating hormone receptor. (Modified from Van Vliet G, Deladoey J. Disorders of the thyroid in the newborn and infant. In: Sperling MA, ed. *Pediatric Endocrinology*. 4th ed. Philadelphia; Elsevier; 2014.)

transporter pendrin. The gene is expressed in the cochlea, thyroid, and kidney. Pendrin functions to maintain the endocochlear potential and is involved in the apical efflux of iodide in the thyroid follicular cells.[55] The thyroid phenotype is usually mild and is seldom identified by neonatal TSH screening. Goiter development and hypothyroidism are variable and depend on nutritional iodine intake.

6. Dehalogenase defects. MIT and DIT, the main iodinated byproducts of thyroid hormonogenesis, are deiodinated in the thyroid cell by dehalogenase. In this way, iodine is conserved within the thyroid gland for another cycle of synthesis of thyroid hormone.[54] Patients with iodothyrosine dehalogenation defects have goiter and CH. The diagnostic hallmark is the presence of MIT and DIT in large amounts in the urine.

When available, genetic testing should be performed using new techniques, such as comparative genomic hybridization (CGH) array, next-generation sequencing (NGS) of gene panels (targeted NGS), or whole exome sequencing (WES).

Antenatal diagnosis, evaluation of fetal thyroid function, and management of fetal hypothyroidism is recommended in cases of goiter discovered during systematic ultrasound examination of the fetus. For the evaluation of fetal thyroid volume, an ultrasound scan is recommended at 20 to 22 gestational weeks to detect fetal thyroid hypertrophy and potential thyroid dysfunction in the fetus. Goiter or an absent thyroid tissue can be documented by this technique. If a large fetal goiter is detected, prenatal care should be provided in a specialized center. Cordocentesis, rather than amniocentesis, is recommended to obtain blood for fetal thyroid function. Norms have been established as a function of gestational age. The test should be carried out only if prenatal intervention is considered. In most cases, fetal thyroid function can be inferred from context and ultrasound criteria, and fetal blood sampling is rarely required. Fetal treatment is recommended by intra-amniotic T_4 injections in a euthyroid pregnant woman with a large fetal goiter associated with hydramnios and/or tracheal occlusion. In a hypothyroid pregnant woman, treating the mother, rather than the fetus, with T_4 is recommended.[38]

Central Hypothyroidism

Congenital central hypothyroidism is uncommon, and isolated TRH/TRH receptor/TSH β subunit deficiency is exceedingly rare. In the United States, the combined incidence of secondary and tertiary hypothyroidism is about 1 in 80,000 to 1 in 100,000 births. Studies in the Netherlands suggest a higher incidence of 1 in 21,000.[56,57] Congenital central hypothyroidism is often associated with mutations in the gene that encode transcription factors involved with pituitary development, including Pit-1, LHX3, LHX4, POU1F1, and HESX1, and can be associated with other hormone deficiencies.

Midline facial, cranial, or intracranial defects should suggest the possibility of hypopituitarism, including altered functioning of the HPT axis. Septo-optic dysplasia, often associated with pituitary hormone deficiencies, can be manifested as secondary or (more commonly) tertiary hypothyroidism. Clinical symptoms of hypopituitarism, such as neonatal hypoglycemia (from growth hormone and/or adrenocorticotropic hormone deficiencies), polyuria (from antidiuretic hormone deficiency), or a small phallus or undescended testicles (from gonadotropin deficiency), whether or not accompanied by the presence of blindness, congenital nystagmus, or midline defects of the brain, should alert the physician to suspect septo-optic dysplasia. Prolonged jaundice with unconjugated hyperbilirubinemia in CH and conjugated hyperbilirubinemia in adrenocorticotropic hormone or cortisol deficiency, can also be seen.

The consequences of central hypothyroidism are as neurologically devastating as those of primary hypothyroidism and therefore also require prompt treatment.[56] However, many newborn

screening programs that use elevated TSH values to recognize thyroid dysfunction are inadequate in identifying newborns with central hypothyroidism.

Central hypothyroidism may be acquired during difficult delivery from traumatic brain injury; however, the fT_4 level may not become abnormal until 2 weeks of age.

The biochemical picture comprises normal or low TSH levels with low or low-normal T_4 levels and low fT_4 levels. Other laboratory evidence includes an abnormal TSH surge of less than 50% and a subnormal TSH response to a TRH stimulation test. Attention to other clinical features such as hypoglycemia, microphallus, and midline facial defects can lead to earlier identification of central hypothyroidism. Other causes of this FT_4–TSH combination are nonthyroidal illness, premature birth (with a correlation between severity and GA/birthweight), and certain forms of reduced sensitivity to thyroid hormone.[58]

In neonates with central CH, it is recommended to start LT_4 treatment only after evidence of intact adrenal function.[38]

Down Syndrome

The association of thyroid abnormalities in individuals with Down syndrome has been well known for several decades, with early case reports of hypothyroidism in trisomy 21 dating back to the 1960s.[59,60] Examination of fetal thyroid glands showed small thyroid follicles and that TSH concentration was consistently in the highest percentiles. This may suggest that the cause of thyroid problems in Down syndrome may be thyroidal in nature.[61]

CH is seen commonly in Down syndrome, with prevalence estimates ranging from 28 to 35 times higher than in the general population.[62] Hypoplasia of the thyroid gland appears to be the most typical occurrence, whereas thyroid agenesis, dysgenesis, and ectopic thyroid are rare causes of CH.[63] When the diagnosis of CH is clear with hyperthyrotropinemia and low T_4 level, treatment should be initiated and guidelines followed as for neonates without Down syndrome. What is more difficult to determine is the natural history and benefit of treatment in those with subclinical hypothyroidism. In one study, Claret et al.[64] found that subclinical hypothyroidism resolved in 73.6% of young children with Down syndrome. The remission rate was higher in those without positive thyroid autoantibodies and without an enlarged thyroid gland. Evidence to support treatment of subclinical hypothyroidism includes improved growth[65,66] and better cognitive and developmental outcomes.[67] Also, there is little in the way of adverse effects of LT_4 treatment when thyroid function is being normalized. However, proponents of not treating subclinical hypothyroidism cite studies such as that of Claret et al. in which a large majority of those with subclinical hypothyroidism progress back to normal thyroid function. Hyperthyroidism is exceedingly rare in the newborn period, with onset most common in the adolescent years. Prevalence estimates for hyperthyroidism in the Down syndrome population range from 0% to 3%.[68]

Consumptive Hypothyroidism

In infants with hepatic hemangiomas and in adults with hemangioendothelioma and malignant fibrous tumors, consumptive hypothyroidism may occur.[69–71] During infancy, hemangiomas occur at a high frequency. Rapid growth during first year of life can be observed followed by involution and gradual regression by adolescence. The first year of life is a critical time for development.[69] There is a concern for neurological damage in infants with hemangiomas when associated consumptive hypothyroidism occurs and goes untreated.[69] Other presentations of consumptive hypothyroidism have been reported as cases of cutaneous hemangiomas[72] and gastric gastrointestinal stroma tumors.[73]

Hypothyroidism in hemangiomas involves increased expression of type III iodothyronine monodeiodinase (MDI III), which accelerates the rate of inactivation of thyroid hormone.[69,74] Type III MDI catalyzes the conversion of T_4 to rT_3 and T_3 to T2. Reverse T_3 and diiodothyronine are inactive metabolites. Type III MDI is particularly expressed in the placenta, uterine endometrium, and neurons of the central nervous system.[75] Due to the high rate of thyroid hormone degradation, hypothyroidism can be refractory to thyroid hormone treatment.[69] Thus, infants with consumptive hypothyroidism may require very high doses of levothyroxine and liothyronine to reduce serum thyrotropin concentrations to normal.[69] The presenting thyroid function tests in consumptive hypothyroidism show low T_3 and T_4, elevated TSH, and elevated serum rT_3. With regression of the hemangioma, the thyroid hormone levels normalize.[69] Some cases have been treated with propranolol therapy alone,[76] liothyronine alone,[77] and a combination of propranolol, levothyroxine, and liothyronine.[78]

Transient Primary Hypothyroidism

Transient CH is associated with excess maternal iodine ingestion or deficiency (pregnant women should ingest 250 μg iodine per day), maternal ingestion of antithyroid drugs, maternal TRBAbs, heterozygous mutations of DUOXA2, and large congenital hepatic hemangiomas (increased type 3 DI activity).[35]

Hypothyroidism in the newborn period can be transient in 10% of cases.[39] Studies in the Netherlands reported an incidence of 1 in 12,000.[57] The newborn screen is initially abnormal, with a low T_4 level and a slightly elevated TSH level. However, repeated laboratory evaluation may demonstrate normalization of thyroid function test results, indicating a transient state of thyroid dysfunction. Because normalization may not occur for several months, thyroid hormone therapy should be initiated to protect the infant's brain development if the TSH level remains elevated at 2 weeks of life. Therapy should be continued until age 3 years when the child should be reevaluated. If the child with no permanent CH and a gland in situ requires LT_4 dose less than 3 μg/kg/day at the age of 6 months, then reevaluation can be done at that time. Transient hypothyroidism is more common in preterm infants.

Transplacental passage of maternal TRBAbs (also known as TSH-binding inhibitory immunoglobulins) is estimated to cause transient thyroid dysfunction in 2% of newborns with CH.[79] This transient thyroid dysfunction is often difficult to differentiate from permanent forms of hypothyroidism in the newborn period. These babies should be treated but will not require lifelong treatment.

Although many women with autoimmune disease are receiving thyroid replacement therapy, some may be clinically euthyroid and thus are not on medications. Some of these women have hyperthyroidism or a history of Graves disease. Therefore, diagnosis of thyroid dysfunction may be delayed until the postpartum period.[79]

Maternal autoimmune thyroid disease may be associated with the production of TRBAbs, a type of immunoglobulin G. TRBAbs, like other immunoglobulins G, do not cross the placenta until after 16 weeks of gestation. TRBAbs can cross the placenta and block TSH receptors in the neonatal thyroid. Therefore, the TRBAbs do not affect thyroid embryogenesis, and infants do not develop permanent abnormalities in thyroid function.[80] The severity and duration of the hypothyroid state in these newborns correlate with the initial titer of the blocking antibody and the duration of its presence in the infant's blood.[79] Subsequent

offspring remain at risk because the antibodies can persist for up to 7 years in maternal sera.[79]

The biochemical profile in transient CH shows low T_4 levels with elevated TSH levels that subsequently normalize. A thyroid scan is not helpful because the TRBAbs are sufficiently potent to block TSH-induced uptake, which can be misleading and suggestive of thyroid agenesis. The distinguishing feature is the presence of TRBAbs in newborn and maternal sera. While routine screening for TRBAbs is not currently indicated, this diagnosis should be suspected in any infant with CH born to a woman who has a history of autoimmune disease or if her previous offspring had thyroid disease.[79]

The Wolff–Chaikoff effect, which is an autoregulatory phenomenon whereby a large amount of ingested iodine acutely inhibits thyroid hormone synthesis within the follicular cells irrespective of the serum level of thyroid-stimulating hormone (TSH), matures at the end of the third trimester. Therefore premature neonates cannot protect themselves from iodine overdose. Thus, the use of iodine-containing disinfectants is contraindicated in preterm babies, as it can cause transient neonatal hypo- or hyperthyroidism.[81]

Euthyroid Sick Syndrome

In acutely ill patients, thyroid function changes take place. It is hypothesized that alterations in thyroid hormone occur as an adaptive response to decreased basal metabolic rates in severely ill patients. The syndrome has been observed in sick infants and children.[82] These patients may have acute or chronic nonthyroidal illnesses. There is a decrease in T_3 activation in the periphery. The consistent finding is an abnormally low serum T_3 level, an increase in the rT_3 level, and a reduction in TSH secretion. Normal TSH secretion can also be observed during mild or moderate illnesses. However, the TSH is inappropriately low in the context of low serum T_3 levels.[75] T_4 may be low or normal and FT_4 may be normal, depending on the metabolic clearance rate of T_4.

In preterm infants, T_4, FT_4, and T_3 levels are naturally lower than those in term infants, and rT_3 is high.[83] Therefore, thyroid tests in a preterm infant may be hard to interpret, especially when infants are sick from nonthyroidal diseases. The most common neonates with euthyroid sick syndrome are preterm infants with respiratory distress syndrome.[83,84] In the pediatric population, nonthyroidal illnesses associated with this syndrome include severe gastroenteritis, acute leukemia, anorexia nervosa, renal disease, burns, and surgical stress.

Euthyroid sick syndrome is found in patients with metabolic stress (e.g., diabetic ketoacidosis), in pediatric patients who underwent cardiac surgery, and in pediatric patients with cancer.[85] There is a sharp rise in rT_3 and a less dramatic fall in T_3 by 2 hours after cardiac surgery. Reverse T_3 returns to normal before T_3. The changes in thyroid function tests are a continuum.[75] There is an inverse relationship between the severity of illness and the T_3 level. In euthyroid sick syndrome, abnormal thyroid function gradually reverts to normal function as the patient's primary illness improves.[37] During recovery, TSH may be transiently elevated (up to 15 mU/L). Treatment with thyroid hormone is not indicated in these patients.

Transient Hypothyroxinemia of Prematurity

In preterm infants, postnatal thyroid hormone concentrations may differ from those in term infants. The thyroid hormone concentrations vary based on the degree of prematurity. There are multiple etiologies such as loss of maternal transfer of T_4[86]; immaturity of hypothalamic-pituitary-thyroid axis[87] and peripheral metabolism of iodothyronines,[88] iodine deficiency,[89] nonthyroidal illness;[83] and decreased TBG concentrations secondary to undernutrition or hepatic dysfunction.[90] Thyroid metabolism can be affected by other factors: exogenous sources of iodine, dopamine infusions, blood transfusion, and glucocorticoid treatment.

In preterm infants, hypothalamic-pituitary-thyroid immaturity is characterized by a limited neonatal TSH surge, decline in T_4 in the first week of life, limited TSH response to hypothyroxinemia, and a prolonged TSH response to TRH. Impairment of T_4 to T_3 and increases in D3 activity may also influence thyroid function tests.[31] Only in the third trimester does hypothalamic TRH begin to increase markedly.[31,91]

In transient hypothyroxinemia in premature infants (before 30 to 32 weeks' gestation), thyroid function tests show low T_4 and FT_4 levels with normal or low TSH levels. Thyroid hormone replacement has not been consistently effective in improving neurologic outcomes or reducing morbidity.[92,93] Research efforts continue to investigate this question. At the current time, therapy is only recommended when low T_4 is accompanied by TSH elevation.[83,92] Abnormalities in thyroid levels in preterm infants can be difficult to interpret when the infants are ill from nonthyroidal diseases. Preterm infants at risk should be monitored by serial determinations of FT_4 and TSH. T_4 treatment should be initiated if the illness state is expected to be persistent and TSH remains elevated for a month or longer. A dosage of 4 to 5 μg/kg of oral levothyroxine was suggested in earlier dosage studies.[94,95]

Low Triiodothyronine Syndrome in Premature Infants

Changes in thyroid function tests during neonatal adaptation are qualitatively similar to those in term infants but occur at lower concentrations in premature infants. The neonatal surge and T_4 and T_3 peak responses diminish with decreasing gestational age.[31] Premature infants have increased susceptibility to neonatal morbidity, including birth trauma, acidosis, hypoxia, hypoglycemia, hypocalcemia, and infection, all superimposed on feeding disorders and relative malnutrition. All of these factors tend to inhibit peripheral T_4 to T_3 conversion. This action leads to the characteristically low T_3 state seen in premature infants. Serum T_3 values may remain low in these infants for 1 to 2 months. Other features of the low T_3 syndrome in premature infants are variable but usually include elevated serum rT_3 levels and normal or low total serum T_4 concentrations. Free T_4 levels usually are in the range of healthy premature infants or matched gestation age and weight.[31] TSH values are low in these infants. Treatment with thyroid hormone is not warranted.[92,93]

Iodine Deficiency

Severe iodine deficiency is associated with cretinism. Iodine deficiency is a rare cause of transient hypothyroidism in North America but may occur in dieting women who are not eating bread or using salt. Many countries have initiated salt iodination.

Iodine is a critical component of thyroid hormone synthesis; therefore, even mild to moderate forms of iodine deficiency can result in an adverse outcome for the fetus. Although the mother with iodine deficiency may be clinically euthyroid with normal T_3 levels, maternal T_4 concentration is low in iodine deficiency. An increase in type 2 DI activity is detected in the fetus in response to iodine deficiency. Because normal development of the fetal neocortex is dependent on maternal T_4, which is the primary source of cerebral T_3, low levels of maternal T_4 place the infants at risk of neurologic cretinism. The fetus can experience several neurologic

manifestations, such as deafness, motor deficits (spasticity, trunk rigidity, flexion dystonia, muscle wasting), and mental retardation. Therefore, it is imperative for the pregnant mother to receive an adequate supply of iodine early in pregnancy to avoid brain damage in the fetus.[96,97] Vitamin supplements that contain at least 250 µg iodine should be used daily.[20]

Preterm infants are at increased risk of iodine deficiency. The premature separation from the maternal supply of iodine and thyroid hormone prevents the preterm infant from accumulating adequate amounts of intrathyroidal hormone. Therefore, the infant is unable to keep up with postnatal thyroid hormone demands. The biochemical profile of iodine deficiency in the newborn comprises low T_4 and elevated TSH levels. Iodine deficiency can lead to a transient state of thyroid dysfunction. An infant is sensitive to maternal iodine nutrition during fetal development.

The thyroid is also protected against iodide excess that might otherwise lead to hyperthyroidism. The sources of excess iodide are pharmaceutical: amiodarone, povidone–iodine, and radiographic dyes. The quantity of iodine organified to TG displays a biphasic response to increasing doses of iodide, at first increasing and then decreasing. The decreasing yield is termed the *Wolff-Chaikoff effect*. The inhibition of iodothyronine formation is reduced over time, the escape phenomenon. This does not occur in the third-trimester fetus, so long-term high iodine intake must be avoided because it will cause fetal hypothyroidism.

Disorders of Thyroid Hormone Carrier Protein

Thyroid hormone in the circulation travels bound to transport proteins (TBG, TBPA, and albumin). As discussed previously, these carrier proteins are produced by the liver. The gene for TBG, the primary transport protein, is located on the long arm of the X chromosome; thus, TBG defects are inherited in an X-linked manner. Given that TBG is the major transport protein, TBG deficiency causes significant thyroid changes in total thyroid hormone concentration. But given that free concentrations remain normal, these individuals are euthyroid. Treatment is not required. Primary T_4 newborn screening methods may be misleading, and confirmation with fT_4 levels is necessary before initiation of treatment.

Thyroxine-Binding Globulin Deficiency

The prevalence of thyroxine-binding globulin (TBG) deficiency varies from 1 in 2500 to 1 in 12,000 newborns.[75,98,99] The frequency in males is much higher, 1 in 2400 to 1 in 2800 males, because it is an X-linked trait.[99] The transmission of this trait is usually from affected males to female offspring.[75] TBG deficiency has no clinical importance but leads to abnormal laboratory tests.

Congenital central hypothyroidism can be detected on newborn screen programs that utilize T_4-reflex thyroid-stimulating hormone (TSH) test method. Congenital central hypothyroidism must be distinguished from TBG deficiency. The diagnosis of congenital central hypothyroidism is made with low serum free T_4 and can be supported by the presence of other pituitary hormone deficiencies. Serum TBG levels play an essential role in the diagnosis of TBG deficiency but do not play a role in the diagnosis of congenital central hypothyroidism.[100]

Serum TBG levels are very low in affected males and are approximately half of normal in carrier females. In about half of the families with this trait, the TBG level shown by radioimmunoassay is very low. In the other half, the defect is partial; serum T_4 levels vary similarly. Affected persons are euthyroid, with normal serum TSH responses to exogenous TRH. Treatment is not indicated.[98]

As many as 26 different mutations have been reported in the TBG gene. These mutations have included a single amino acid substitution or deletion, leading to abnormal post-translational processing.[75] Two novel mutations in the TBG gene identified most recently include a T insertion at the beginning of intron 1 between nucleotides 2 and 3 and a T deletion in exon 1 leading to a truncated protein. Both mutations fail to produce a functional TBG molecule.[101] In partial TBG deficiency, the defects are associated with altered TBG binding of T_4.[75]

Females tend to have partial TBG deficiency, whereas males generally have complete TBG deficiency. A partial deficiency comprises reduced TBG levels and normal TSH and FT_4 with T_4 values at the lower limit of normal. A complete deficiency comprises undetectable TBG levels with normal TSH, normal FT_4, and low levels of T_4. Another useful laboratory tool is T3RU, which is elevated with TBG deficiency.[102] However, this is an indirect measure of TBG levels.

Treatment of Hypothyroidism

Treatment with levothyroxine should begin if the FT_4 is low and the TSH is elevated or if the TSH is greater than 20 mU/L, even if FT_4 is normal. Treatment with levothyroxine should also begin if the TSH greater than 6 mU/L beyond 21 days of age. Treatment of hypothyroidism relies on replacement with exogenous thyroid hormone. LT_4 is the drug of choice because of its uniform potency and reliable absorption.[38] Appropriate doses of synthetic T_4 produce normal serum levels of T_3 via peripheral conversion. The best guide to the adequacy of therapy is the periodic measurement of circulating levels of T_4, fT_4, and TSH. Treatment with LT_4 should be started as soon as possible and no later than the first 2 weeks of life. History and physical examination are important in the follow-up evaluation, but mild hypothyroidism or hyperthyroidism cannot always be excluded on clinical grounds.

The usual starting dosage of thyroid hormone for hypothyroid infants is 10 to 15 µg/kg/day, which approximates to 100 µg/m^2/day. Infants with severe disease, as defined by a very low T_4/fT_4 concentration, should be treated with the highest initial dose. Those with mild to moderate hypothyroidism should be treated with a lower dose. If intravenous treatment is necessary, the dose should be no more than 80% of the oral dose. The aim of treatment is to keep the T_4 level in the upper half of the normal range, approximately 10 to 16 µg/dL, or the fT_4 level in the 1.4 to 2.3 ng/dL range with the TSH level on the lower half of the normal range (0.5 to 2.0 mU/L) during the first 3 years of life. Recent studies have reported neurodevelopmental benefits of quick normalization of T_4 and fT_4 levels within three days of initiation of thyroid hormone therapy with high-dose treatment. Thyroid hormone requirements, however, quickly drop after 2 weeks, and therefore close monitoring of growth and development along with thyroid function tests is necessary.[103,104] The treatment of each patient must be individualized. The adequate dosage of thyroid hormone in the first year usually ranges between 25 and 50 µg daily.

Current recommendations favor the administration of levothyroxine in tablet form. The tablet is crushed and given orally in a small amount of liquid. Liquid formulations should be used only if they are pharmaceutically produced and licensed. The U.S. Food and Drug Administration approved a liquid levothyroxine formulation (Tirosint-SOL) in 2017. There is limited published data on the use of liquid levothyroxine for congenital hypothyroidism. Some theoretical advantages of liquid formulations include the ability to deliver more precisely individualized

doses for infants, unaltered absorption when administered with milk, and better patient satisfaction over tablets. However, two European studies found overtreatment based on TSH suppression in infants with congenital hypothyroidism treated with liquid levothyroxine compared to tablet form, despite equivalent weight-based dosing.[105] LT_4 suspensions prepared by individual pharmacists may lead to an unreliable dosage.[39] Care should be taken to avoid concomitant administration of soy, fiber, calcium, or iron. The thyroid hormone–pituitary feedback setpoint is altered in rare infants with CH, and in such infants, serum TSH concentration remains elevated in the face of a normal or even elevated serum T_4 level.[106]

Infants with presumably transient hypothyroidism resulting from maternal goitrogenic drugs need not be treated unless the low serum T_4 and elevated TSH levels persist beyond 2 weeks of age. Infants with TRBAb-induced hypothyroidism may require treatment for as long as 6 months.

When infants with severe myxedema associated with fluid retention are being treated, potential complications should be kept in mind. Cardiac insufficiency caused by overtaxing of the myxedematous heart, through too rapid a mobilization of the myxedema fluid into the circulation, is well known in the adult. This complication in older children and adults is prevented by administering a small dose of thyroid hormone at first, followed by a gradual increase of the dose. However, infants generally tolerate a rapid restoration to the euthyroid state better than adults, and a prompt restoration of T_4 concentration to a normal value is important for the recovery of brain development and maturation. Nevertheless, excessive thyroid hormone therapy must be avoided, and the dose must be adjusted judiciously if there is evidence of severe myxedema, particularly of the heart.

After initiation of therapy with LT_4, the growth rate should accelerate. Any growth deficit is commonly restored within a few months. Bone age is a sensitive index of thyroid deficiency; however, radiographs are not routinely obtained in the newborn period. Overtreatment can induce tachycardia, excessive nervousness, disturbed sleep patterns, and other problems suggesting thyrotoxicosis. Excessive thyroid hormone administered over a long period can produce premature synostosis of cranial sutures and advancement of bone age.

During the first few years of life, patients should be monitored frequently: the first follow-up examination should occur 2 weeks after the start of LT_4 treatment. Subsequent evaluations should occur at least every 1 to 2 months during the first 6 months of life, every 3 months between 6 months and 3 years, and then twice a year until growth is completed (Box 86.1).[35] Evaluations should occur at more frequent intervals when adherence is questioned, abnormal values are obtained, or the dose of medication has been changed. Clinical observation should be supplemented with monitoring of the growth curve and T_4, fT_4, and TSH levels. Because poor adherence and nonadherence have major sequelae, the initial and ongoing counseling of parents is of great importance.

Patients with permanent CH (e.g., dysgenesis, dyshormonogenesis) require lifetime substitution therapy. After age 3 years, if there is uncertainty about whether the disease is permanent or transient or if the dose of LT_4 has not required an increase, discontinuation of LT_4 therapy for 4 to 6 weeks with close monitoring of the TSH level should distinguish transient from permanent CH. Recent reports indicate that treatment of CH is discontinued within 3 years in more than one-third of children with CH. This is inconsistent with current guidelines; however, it remains unknown how many of the children in whom treatment was discontinued prematurely experience adverse effects or require continued treatment.

• BOX 86.1 Management of Congenital Hypothyroidism

Initial Work-Up

Detailed history and physical examination
- Referral to pediatric endocrinologist
- Recheck serum TSH and FT_4 levels
- Thyroid ultrasonography and/or thyroid scan (see text for recommendations)

Medications

LT_4: 10–15 μg/kg by mouth once daily

Monitoring

Recheck serum FT_4 (or total T_4) and TSH
- Two to 4 weeks after initial treatment has begun
- Every 1–2 months in the first 6 months
- Every 3 months between 6 months and 3 years of age
- Every 6–12 months from 3 years of age to end of growth
- Four weeks after a change in LT_4 dosage

Goal of Therapy

Normalize TSH and maintain T_4 and FT_4 in the upper half of the reference range

FT_4, Free thyroxine; *LT_4*, levothyroxine; *T_4*, thyroxine; *TSH*, thyroid-stimulating hormone.

Neonatal Hyperthyroidism

Epidemiology and Pathophysiology

The overall incidence of neonatal hyperthyroidism is low. It occurs more commonly in pregnancies complicated by Graves disease. Of the offspring born to women with Graves disease, 1% are affected with hyperthyroidism.[107] The thyroid dysfunction in the fetus and newborn is associated with the transplacental passage of TSH receptor-stimulating antibodies/thyroid-stimulating immunoglobulins (TSA/TSI). The mother may have active or inactive Graves disease. The hyperthyroidism is not related to the transfer of maternal thyroid hormone. The antibodies stimulate the fetal and neonatal thyroid gland. It is generally a transient state that clinically resolves by 4 months of age with the clearance of maternal antibodies from the infant's circulation.[108] Rarely, neonatal hyperthyroidism is caused by mutations in the TSH receptor or its mediators. These causes include activating mutations of the stimulatory G protein, as in McCune-Albright syndrome, or an activating mutation of the TSH receptor.[109,110]

In pregnancies complicated by maternal Graves disease, the fetal levels of TSA approximate those of the mother at 30 weeks' gestation. Therefore, fetal thyrotoxicosis generally manifests in the third trimester with fetal tachycardia, fetal goiter, and intrauterine growth retardation. Fetuses of mothers with TSA levels greater than 250% of the upper limit are at increased risk for thyrotoxicosis. Therefore, these fetuses need to be monitored more closely.[111,112]

Clinical Presentation

The clinical manifestations of Graves disease in the newborn include irritability, flushing, diarrhea, vomiting, tachycardia,

hypertension, poor weight gain, thyroid enlargement, and exophthalmos. Thrombocytopenia, hepatosplenomegaly, jaundice, and hyperviscosity syndrome also have been reported. If thyrotoxicity is severe and treatment is inadequate, arrhythmias, congestive heart failure, and death may occur. In some infants, the onset of symptoms and signs may be delayed as long as 8 to 14 days. Late onset of neonatal disease can occur for at least two reasons: (1) postnatal depletion of transplacentally acquired blocking doses of maternal antithyroid drugs and the abrupt increase in conversion of T4 to active T3 shortly after birth in the newborn, and (2) presence of maternal TRBAb, which can block the effect of TSA for several weeks.[111]

Evaluation

The diagnosis of neonatal hyperthyroidism is confirmed by high levels of T4, free T4, and T3 in postnatal blood. Cord blood values may be normal or near normal, whereas levels at 2 to 5 days may be markedly increased; the serum TSH is suppressed below normal levels. Neonatal Graves disease resolves spontaneously as maternal TSA in the newborn is degraded. The usual clinical course of neonatal Graves disease is 3 to 12 weeks.

Management

Fetal thyrotoxicosis is treated by administering antithyroid agents to the mother. Propylthiouracil (PTU) is recommended during pregnancy because it is associated with a lower rate of fetal malformations than methimazole. PTU crosses the placenta to inhibit the fetal production of excess thyroid hormone.[113] Since the concentration of drugs in breast milk is very low, hyperthyroid mothers on antithyroid drugs may breastfeed their infants. Medical treatment of hyperthyroidism in the newborn period depends on the severity of the illness. These medications include iodide or antithyroid agents. Antithyroid agents inhibit thyroid hormone synthesis. Iodide rapidly inhibits hormone release.

Lugol's solution (5% iodine and 10% potassium iodide, containing 126 mg/mL of iodine) is given in a dose of 1 drop (8 mg of iodine) three times daily. Antithyroid agents such as methimazole are administered in doses of 0.25 to 1 mg/kg/day, divided, at 8-hour intervals. PTU is no longer recommended for use in children because of increasing rates of PTU-induced liver failure.[114] Propranolol can be given in a dose of 2 mg/kg/day to decrease β-adrenergic symptoms and inhibit deiodination of T4 to T3. A therapeutic response should be observed within 24 to 36 hours. If a satisfactory response is not observed, the dose of antithyroid drug and iodide can be increased by 50%.

More severe cases may require corticosteroids. Steroids suppress the deiodination of T4 to T3. Digoxin treatment can be used in the case that cardiac failure is present. If hyperthyroidism persists (as in cases with a strong family history of Graves disease, an activating mutation of the stimulatory G protein, or an activating mutation of the TSH receptor), ablative therapy such as thyroidectomy must be performed.[109]

The treatment goal is to achieve a euthyroid state while avoiding hypothyroidism in the infant. Frequent monitoring to adjust methimazole doses is indicated. Another method to treat hyperthyroidism using antithyroid medication is to completely block the synthesis of thyroid hormone while replacing thyroid hormone.

Specific Causes of Hyperthyroidism

Familial Dysalbuminemic Hyperthyroxinemia

Familial dysalbuminemic hyperthyroxinemia (FDH) is characterized by almost a 60-fold increase in the affinity of albumin for T_4 but not for T_3. Mutations in ALB (albumin) gene are inherited in an autosomal dominant manner.[75] The biochemical profile demonstrates increased serum T_4 concentrations but normal FT_4, total serum T_3, and TSH levels. Although the binding of T_4 to albumin is increased, T_3 is less avidly bound, accounting for the preferential increase in serum T_4 concentration. Patients with this disorder are euthyroid with normal thyroid hormone production rates.[115]

The diagnosis of FDH is confirmed by protein electrophoresis of serum containing labeled T_4. The fraction of t4 label associated with TBG, thyroid hormone binding by human serum prealbumin (TBPA), or albumin is measured. The albumin-bound T_4 can be calculated and related to normal values. Measurement of TBG and TBPA concentrations is useful. Euthyroid individuals with FDH have often falsely elevated serum free thyroxine (fT_4) concentrations determined by different automated immunoassays. Refetoff et al. measured serum fT_4 in families with FDH through three methods: (1) direct dialysis coupled with tandem mass spectrometry, (2) direct immunometric assay, and (3) free thyroxine index. The free thyroxine index method greatly reduced the discordance of fT_4 results relative to thyrotropin in FDH.[116] Antithyroid therapy is not necessary for FDH.[117]

Thyroxine-Binding Globulin Excess

The prevalence of TBG excess is estimated to be in 1 in 15,000 to 1 in 25,000 individuals. TBG excess is inherited as an X-linked trait.[98] Persons with increased levels of TBG have increased total serum T_4 concentrations with normal TSH and FT_4 levels. Serum T_3 is modestly increased. These individuals are euthyroid. In these persons, TBG production rates and serum levels are correlated, suggesting that the mechanism for high TBG is increased production, presumably by the liver. TBG levels are increased four- to fivefold in affected persons. Carrier females have intermediate serum TBG values.[75]

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Neonatal Hypoglycemia and Hyperglycemia

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KEY POINTS

- Neonatal hypoglycemia requires diagnostic consideration and urgent management to prevent recurrent hypoglycemia and avoid neurologic injury.
- Neonatal metabolism in the first days of life reflects a transition from the passive glucose consumption of the fetus to the active regulation of glucose of the neonate.
- Diagnosing the cause of hypoglycemia requires an evaluation of the hormonal and metabolic response to hypoglycemia.
- Patients with hyperinsulinemic hypoglycemia should be assessed for diazoxide-responsiveness, and non-responsive patients should have an evaluation to determine if the process is due to focal or diffuse disease.
- Diabetes diagnosed before 6 months of age is very likely to have a genetic cause.
- Patients with neonatal diabetes due to pathogenic variants in the K_{ATP} channel can be treated with oral sulfonylurea in place of insulin therapy.

Introduction

Glucose is the primary metabolic fuel for the neonatal brain. Maintenance of normal glucose levels in the serum and across the blood-brain barrier is essential for normal neurologic function and development. Hypoglycemia in the neonate, therefore, requires thoughtful diagnostic evaluation and urgent treatment to prevent injury to the central nervous system (CNS). The mechanisms underlying neonatal hypoglycemia are best understood as inadequate hormonal and metabolic responses to hypoglycemia, occurring in the context of the necessary shift from fetal to neonatal glucose metabolism in the first days of life. These pathways and pathologies are the focus of the initial portion of this chapter.

In the latter portion of this chapter, we focus on hyperglycemia in neonates. Hyperglycemia most commonly occurs in the context of a physiologic stressor such as sepsis with cortisol and catecholamine release. Intravenous glucose infusion and exogenous glucocorticoid administration can also cause hyperglycemia. Rarely genetic causes of hyperglycemia result in transient neonatal diabetes mellitus (TNDM) or permanent neonatal diabetes mellitus (PNDM).

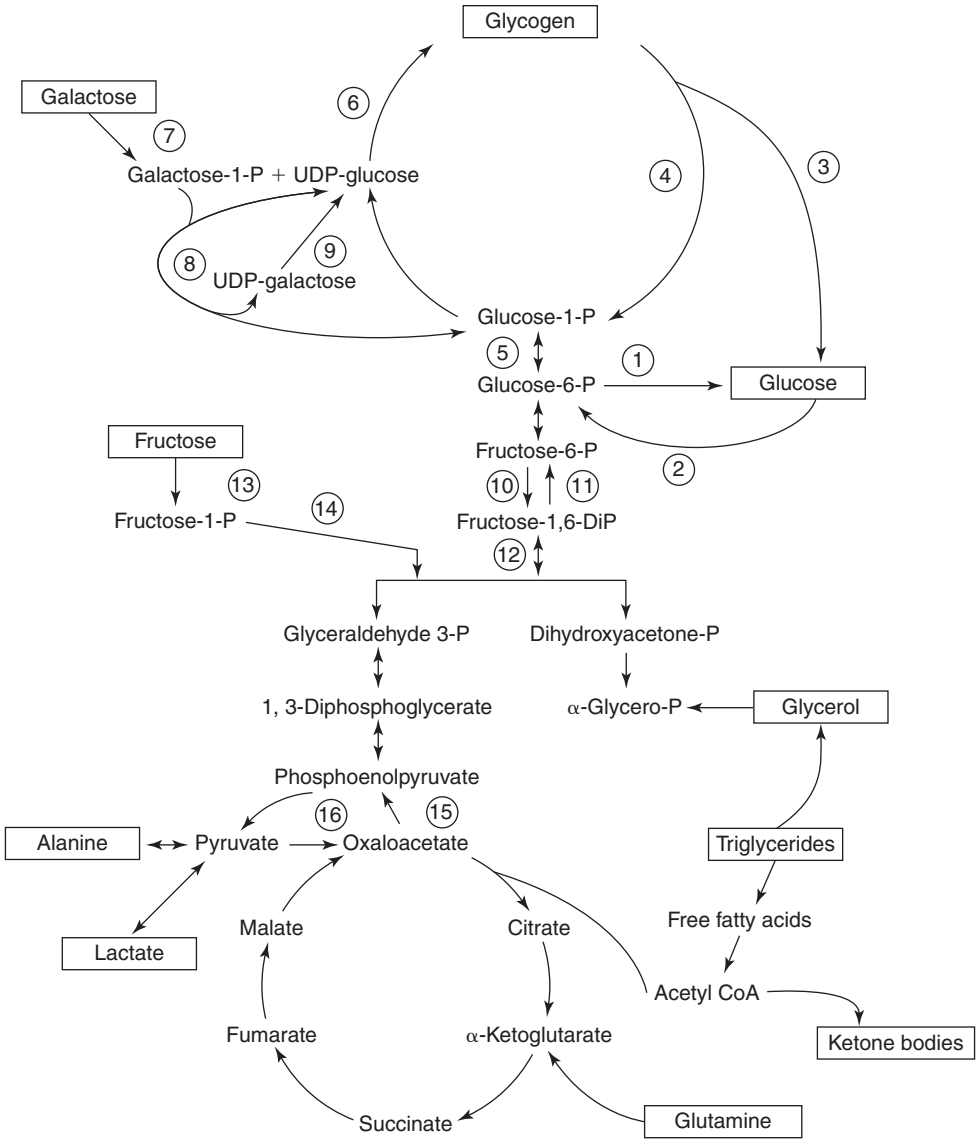
Neonatal Hypoglycemia

Fetal to Neonatal Transition and Energy Metabolism

Fetal glucose supply is dependent on maternal plasma levels and its diffusion across the placenta. There is no evidence for the existence of fetal gluconeogenesis or a robust ability to adjust rapidly to maternal hypoglycemia.[1–3] Once the placental link is interrupted, and glucose is no longer delivered continuously via the umbilical vein, the neonate must maintain normoglycemia and adequate cerebral glucose delivery despite minimal and sporadic enteral carbohydrate intake during the first 24 to 72 hours of life. Glucose homeostasis is accomplished in a manner generally similar to older children who are fasted: via secretion of the counter-regulatory hormones—namely cortisol, glucagon, growth hormone, and catecholamines—and their actions at target tissues, in combination with the suppression of insulin secretion. In concert, these hormonal changes regulate four different metabolic systems: glycogenolysis, gluconeogenesis, lipolysis, and ketogenesis (Fig. 87.1). The result is to facilitate normoglycemia until carbohydrate intake and absorption occur on a more regular basis.

In response to decreased delivery of glucose in the first few hours of life, glucagon and catecholamine levels rapidly increase, and insulin falls. This combination shifts metabolic activity from anabolism to catabolism and induces enzymes necessary for glycogenolysis (glycogen phosphorylase) and gluconeogenesis (pyruvate carboxylase and phosphoenolpyruvate carboxykinase [PEPCK]).[3–5] Glycogenolysis plays the largest role in meeting glucose needs during the first 24 hours (approximately 50%) and causes a depletion of glycogen stores from 50 mg/g of the liver at birth to <10 mg/g of the liver by 24 hours of life.[2] Gluconeogenesis develops somewhat more slowly and is not fully active until 8 to 12 hours of life, providing only 20% to 30% of glucose needs in the first 24 hours.[4,6]

Gluconeogenesis and lipolysis contribute to plasma glucose levels after 8 to 12 hours of life, with their role increasing as glycogen stores are depleted. Lipolysis produces glycerol, which can enter the gluconeogenic pathways, and free fatty acids can be oxidized directly by some organs, including the heart, kidney, and



• **Fig. 87.1** Key Metabolic Pathways of Intermediary Metabolism. *1*, Glucose 6-phosphatase; *2*, glucokinase; *3*, amylo-1,6-glucosidase; *4*, phosphorylase; *5*, phosphoglucomutase; *6*, glycogen synthetase; *7*, galactokinase; *8*, galactose-1-phosphate uridyl transferase; *9*, uridine diphosphogalactose-4-epimerase; *10*, phosphofructokinase; *11*, fructose-1,6-diphosphatase; *12*, fructose-1,6-diphosphate aldolase; *13*, fructokinase; *14*, fructose-1-phosphate aldolase; *15*, phosphoenolpyruvate carboxykinase; *16*, pyruvate carboxylase; *UDP*, uridine diphosphate. (Modified from Sperling MA, Menon RK. Differential diagnosis and management of neonatal hypoglycemia. *Pediatr Clin North Am*. 2004;51:703–723.)

skeletal muscle, but long-chain fatty acids cannot cross the blood-brain barrier.[7] Importantly, partial oxidation of fatty acids in the liver via ketogenesis produces ketones such as beta-hydroxybutyrate (BOHB) and acetoacetate, which the brain can metabolize. However, ketogenesis is impaired in the first 8 to 12 hours of life, coincident with the known transitional hypoglycemia of infancy discussed later.[8,9] The importance of gluconeogenesis, lipolysis, and fatty acid metabolism is highlighted in breastfed infants as the macronutrient profile of colostrum favors protein and fatty acids, compared to the relative carbohydrate predominance of mature human milk.[10]

Glucagon continues to rise gradually over the first few days of life, concurrent with the known, gradual increase and stabilization of glucose levels that normally occurs in infants by 48 to 72 hours of life.[11,12]

The brain is the most metabolically active organ in the neonate, and its demand for glucose is proportional to brain weight.[13,14] Glucose uptake and oxidation in the brain occurs via insulin-independent facilitated diffusion through glucose transporter (GLUT) channels and is dependent on arterial blood glucose concentration. An in vivo study using rats (which are believed to have GLUT channels with similar kinetics to humans) showed that the consumption of glucose in the brain outpaces its replacement via diffusion at an arterial concentration of 36 mg/dL.[15] At this point, cerebral blood flow increases markedly to prevent severe CNS glucose depletion and neurologic sequelae. The relatively large size of the neonatal brain and its high metabolic demand are associated with a two- to threefold higher (per weight) hepatic glucose production compared to adults. Conditions that interfere with hepatic glucose production, therefore, place the infant at risk for

hypoglycemia, some of which are discussed briefly in this chapter, and others are discussed in Chapter 29.

In total, the combined counter-regulatory response and insulin suppression are similar to the starvation response that occurs in older children with two exceptions: (1) the additional complication that maternal factors and immaturity of the counter-regulatory response can interfere with glucose homeostasis in the neonatal period; and (2) the decrease in insulin production and release of glycogen stores is less robust than in older children. These latter factors may explain the "transitional hypoglycemia" seen in normal infants during the first 24 hours of life.

Transitional Neonatal Hypoglycemia

Plasma glucose values in the first hours of life are frequently lower than accepted thresholds for normoglycemia in older children, a phenomenon known as "transitional neonatal hypoglycemia." Serial measurements of glucose in the first days of life in healthy, term appropriate for gestational age (AGA) infants demonstrate average values in the 50s to low 60s (mg/dL).[12,16] If normal is defined as within two standard deviations from the mean, the lower limit of normal may be as low as the high 30s to low 40s in the first few hours of life.[16,17] In support of this, Lubchenco and Bard showed that if feeding is delayed 3 to 6 hours from birth, approximately 10% of healthy, term AGA infants will have glucose <30 mg/dL.[12]

A recent review of the data available on transitional hypoglycemia shows that it is characterized by relative hyperinsulinism as indicated by hypoketosis and preserved glycogen release in response to glucagon.[8,9] An additional factor may be the time required for the enzymatic machinery of gluconeogenesis and glycogenolysis to become active in response to the rise in glucagon and catecholamine secretion after birth.

It is unknown whether the decline in glucose values or the relative hyperinsulinism seen with transitional hypoglycemia serves an adaptive function. The important diagnostic distinction is that this phase of hypoglycemia is transient. Just 2 of the 374 infants (0.5%) in the Lubchenco and Bard cohort had glucose <50 mg/dL prior to feeding on day 3 or 4 of life.

Signs and Symptoms of Hypoglycemia

Neonates with hypoglycemia may have no detectable symptoms and may only be identified incidentally upon measurement of blood glucose levels or in the monitoring of a high-risk infant. When symptoms occur, they may be seen in a progression due to initial counterregulatory hormone responses (such as adrenergic hormones as well as cortisol and growth hormone), which result in symptoms due primarily to the autonomic system (autonomic, or "neurogenic" symptoms and signs). When deficient glucose supply to the brain occurs, neurological dysfunction is detectable and may be considered the symptoms of "neuroglycopenia" (Box 87.1). However, these may be subtle and difficult to recognize clinically and may also be accompanied by other nonspecific symptoms such as apnea, cyanosis, temperature instability (especially hypothermia), and bradycardia.

It is difficult to define a consistent threshold in the neonate below which hypoglycemia produces the above symptoms, especially neuroglycopenia. One often-quoted study of 17 children showed changes in auditory evoked potentials below a whole blood glucose concentration of 47 mg/dL (2.6 mmol/L) but only included four neonates with hypoglycemia (aged 1 to 3 days).[18] In those four neonates, three had no clinical signs at the time of the abnormal recorded evoked potentials, and one was reported to be drowsy. The challenge of defining a true threshold is underscored in these four infants, given that one of these was asymptomatic with normally evoked potentials at a whole blood glucose level of 1.9 mmol/L (34 mg/dL) on day 1 of life, while another infant was symptomatic at 2.5 mmol/L (45 mg/dL).

Blood Glucose Monitoring

While symptomatic, prolonged hypoglycemia in neonates is a risk factor for cerebral injury and poorer neurodevelopmental outcomes, the lower limit of normoglycemia in asymptomatic infants has been difficult to elucidate.[19,20] This is complicated by the pattern of transitional hypoglycemia mentioned above, during which plasma glucose values may drop to levels considered very low for older children. Maternal and neonatal conditions with a high risk for hypoglycemia are well-known, and it is a commonplace for newborn nurseries to have screening protocols for these infants (Box 87.2). Such protocols commonly result in the treatment of asymptomatic infants based on point-of-care (POC) glucose values, with the limited evidence available to define the optimal threshold that minimizes overtreatment while still preventing neuroglycopenia and neurologic damage.

Multiple definitions of the ideal asymptomatic treatment threshold with regard to long-term neurologic outcomes have been proposed: 47 mg/dL,[21] 45 mg/dL,[22] 40 mg/dL,[23] and 30 mg/dL.[24] It is unlikely that a single threshold exists, as the point at

• BOX 87.1 Signs of Neonatal Hypoglycemia

Autonomic	Neuroglycopenic
Sweating	Hypotonia
Pallor	Lethargy
Tachycardia	Coma
Tachypnea	Seizure
Tremor	Weak suck
"Jittery"	Abnormal cry (weak, high pitched)

• BOX 87.2 Maternal and Neonatal Conditions That Increase the Risk of Neonatal Hypoglycemia

Maternal Conditions

Diabetes (gestational or pre-gestational)
Administration of drugs (β sympathomimetics [e.g., terbutaline, oral hypoglycemic agents])
Intrapartum dextrose infusion
Hypertension/preeclampsia

Neonatal Conditions

Prematurity
Intrauterine growth restriction
Hypoxia-ischemia
Large/small for gestational age
Sepsis
Hypothermia
Polycythemia
Presence of syndromic features (microphallus, midline defects, Beckwith-Wiedemann syndrome)

which neurologic injury occurs is likely patient and situation-dependent and related to the availability of ketones and other substrates to the brain.

Recently, a prospective investigation into an appropriate glucose treatment threshold for infants was performed with a cohort of 404 infants of gestational age at least 35 weeks who were at risk for hypoglycemia (infant of mother with diabetes, birth <37 weeks gestational age, and birth weight <10th or >90th percentile).[25] Infants also wore blinded continuous glucose monitoring (CGM) systems to allow the investigators to evaluate for outcomes related to subclinical hypoglycemia missed on the intermittent POC checks. Infants were treated to maintain a blood glucose concentration of at least 47 mg/dL for at least the first 48 hours of life. Neurodevelopmental outcomes were then assessed at 2 years of age using the Bayley Scales of Infant Development III and tests of executive and visual function. The authors found no association between hypoglycemic episodes and neurosensory impairment and concluded that neonatal hypoglycemia was not associated with the adverse neurologic outcomes when treatment was aimed at maintaining a blood concentration of 47 mg/dL in these high-risk infants. A follow-up study with the same cohort of patients aimed to assess higher cognitive function at age 4.5 years. While once again, no neurosensory impairment was seen on follow-up testing, an increased risk of poor executive and visual motor performance was seen. Additionally, the risk was highest in those with severe or recurrent hypoglycemia.[26]

Recent Pediatric Endocrine Society (PES) guidelines make glucose threshold recommendations for infants that are at risk for hypoglycemia and without a known risk for permanent hypoglycemic disorders such as hyperinsulinism, hypopituitarism, or an inborn error of metabolism. According to the PES, such infants should have a treatment threshold of 50 mg/dL in the first 48 hours. Based on evidence that suggests average glucose values in normal infants older than 48 hours of age are no different than those of older children, the authors suggest that these infants should demonstrate an ability to maintain glucose >60 mg/dL during a 6 to 8 hours fast prior to discharge.[27] The PES guidelines are designed to have high sensitivity to detect infants with a pathologic cause of persistent hypoglycemia and to detect these infants before they are discharged from the hospital. The American Academy of Pediatrics (AAP) issued guidelines in 2011 with a focus on the first 24 hours of life, acknowledging that transitional glucose levels in the first few hours of life can be as low as 30 mg/dL.[28] The AAP guidelines recommend a target prefeed glucose of 40 mg/dL in the first 4 hours of life and 45 mg/dL from 4 to 24 hours of life. The differences between the two guidelines reflect the uncertainty regarding the significance of asymptomatic glucose levels <50 mg/dL in the first 48 hours of life, as well as the different weighting of the risks and benefits of continued screening and longer hospital stays in the context of the clinical reality that most term infants in the United States are discharged home between 24 and 48 hours of life.

For diagnostic workup of hypoglycemia after 48 hours of age, we recommend a glucose threshold of 50 mg/dL for collection of a "critical sample" to assess for the counterregulatory hormone response, insulin level, acidosis, and the presence of important metabolic substrates such as BOHB, lactate, serum amino acids, and free fatty acids (Box 87.3). Most importantly, a plasma glucose level should be obtained simultaneously to allow for accurate interpretation of the critical sample, as the POC test result may be artificially low. It's important that the plasma glucose sample reach the lab quickly because glucose in the plasma sample is lost through glycolysis at a rate of 5% to 7%/h.[29] Higher rates of loss can occur with increased ambient temperature and in blood samples with high white blood cell counts.

• BOX 87.3 Components of Critical Sample and Diagnostic Criteria for Hyperinsulinism Based on Critical Sample at Time of Hypoglycemia

Critical Sample

- Serum glucose
- Basic metabolic panel and venous blood gas
- Insulin
- C-peptide
- Growth hormone
- Cortisol
- Free fatty acid
- Beta-hydroxybutyrate
- Lactate
- IGFBP-1
- Ammonia*
- Acyl carnitine profile*
- Serum amino acids*
- Pyruvate*
- Urine organic acid*

*Not necessary to collect at time of hypoglycemia.

Diagnostic Criteria for Hyperinsulinism

- Insulin >2 uIU/mL)
- β-hydroxybutyrate of <1.8 mmol/L
- FFA <1.7 mmol/L
- Glucagon stimulation test rise of ≥30 mg/dL
- IGF-BP1 ≤110 ng/mL

Normoinsulinemic Hypoglycemia

Insulin's role in maintaining normoglycemia is paramount. Insulin simultaneously lowers serum glucose concentration via glucose uptake through insulin-sensitive glucose transporters and represses the effects of counterregulatory hormones, whose primary function during hypoglycemia is to increase serum glucose values. It is therefore relevant to divide a discussion of the causes of hypoglycemia into those that are associated with appropriate suppression of insulin during hypoglycemia (normoinsulinemic hypoglycemia) and those that have inappropriate, elevated insulin levels at the time of hypoglycemia (hyperinsulinemic hypoglycemia) (Box 87.4).

Hypoglycemia in Premature and Small for Gestational Age Infants

Infants born premature or small for gestational age (SGA) are at high risk for transient hypoglycemia due to immaturity of the metabolic pathways described above, exacerbated by inadequate stores of glycogen and triglycerides. The doubling of average fetal weight from 1700 g at 32 weeks to 3400 g at birth is largely due to the accrual of hepatic glycogen and adipose tissue fat stores, which then serve as an important reserve of substrates for energy metabolism in the first days of life. Hypoglycemia can also be caused by delayed maturation of enzymes necessary for gluconeogenesis. Premature infants can have markedly reduced glucose-6-phosphatase activity relative to term infants that may persist for months after birth.[30] There is also a lack of glucose rise after administration of gluconeogenic precursors, which suggests the impaired activity

• BOX 87.4 Causes of Neonatal Hypoglycemia

Hyperinsulinemic

Transient:
- Infants of diabetic mothers
- Intrapartum dextrose infusion to mother
- Stress in peripartum/postnatal period: trauma, asphyxia, hypothermia
- Small for gestational age infants

Permanent:
- K_{ATP} channel defects
- Glutamate dehydrogenase (GLUD1)-activating mutation
- Short-chain 3-hydroxyacyl-coenzyme A dehydrogenase (HADH or SCHAD) mutation
- Glucokinase (GCK) activating mutation
- HNF1A and HNF4A pathogenic variants
- Uncoupling protein-2 (UCP2) pathogenic variants
- Hexokinase-1 (HK1) pathogenic variants
- Beckwith-Wiedemann syndrome (BWS)
- Postfundoplication (dumping syndrome)
- Hyperinsulinism in congenital disorders of glycosylation
- β-cell adenoma—MEN1

Normoinsulinemic

Transient:
- Developmental immaturity in adaptation to fasting: prematurity, SGA
- Increased metabolic expenditure: sepsis, erythroblastosis fetalis, polycythemia
- Maternal conditions: toxemia, administration of tocolytics (β sympathomimetics)

Permanent:
- Hypopituitarism
- Primary adrenal insufficiency
- Inborn errors of metabolism
  - Glycogen storage disease
  - Disorders of gluconeogenesis
  - Defects in fatty acid catabolism and ketogenesis
  - Organic acidurias
  - Galactosemia
  - Hereditary fructose intolerance

of the enzymes of gluconeogenesis.[31] For these reasons, SGA and premature infants should be screened for asymptomatic hypoglycemia and supported with IV dextrose or nasogastric feeding until their hypoglycemia resolves. It is important to note that hypoglycemia in these infants may be multifactorial, as hyperinsulinism may be a contributing factor as well (discussed later).

Counterregulatory Hormone Deficiency

Hypopituitarism

Deficiencies of cortisol, growth hormone (GH), or their combined deficiency in the neonatal period can cause hypoglycemia. Often these two deficiencies occur together in the context of hypopituitarism with adrenocorticotropic hormone (ACTH) hormone and GH deficiency. Infants with congenital hypopituitarism often have other signs of midline malformations such as a midline cleft palate, nystagmus (observed in optic-nerve hypoplasia; of note, however, is that nystagmus is usually not apparent until age 6 weeks, so it is not a distinguishing sign in the neonatal period), seizures (holoprosencephaly), direct hyperbilirubinemia (thyroid hormone deficiency), or micropenis and undescended testes in a male (gonadotropin deficiency). Brain MRI may reveal the underlying cause of hypopituitarism, which may range from severe midline malformations such as alobar holoprosencephaly to more subtle abnormalities such as an isolated ectopic posterior pituitary bright spot or a hypoplastic pituitary gland.

Biochemical evaluation of hypopituitarism requires careful consideration in the first months to a year of life. Conventional stimulation tests used to diagnose GH deficiency in older children have been used in infants, but normal responses are not well established. The stimulation test believed to be safest for use in infants is the glucagon stimulation test, which causes a rapid rise, then rapid fall in glucose, placing the infant at risk for hypoglycemia. Therefore, this testing should only be done under close monitoring, preferably in an ICU setting.[32] As an alternative, taking into account normal physiological processes in the perinatal period, there is evidence that a single random GH measurement in the first week of life can adequately diagnose GH deficiency.[33] Using a post-hoc defined threshold of 7 mcg/L, Binder et al. demonstrated a sensitivity and specificity of 100% and 98%, respectively, for the diagnosis of GH deficiency in the first week of life using a single random GH measurement.[33]

Similarly, optimal testing to diagnose adrenal insufficiency in the neonatal period is a matter of debate. Glucagon stimulation testing can be used to simultaneously assess for GH and ACTH deficiency but carries the risk of hypoglycemia mentioned above. The conventional ACTH stimulation test using Cortrosyn has been used in infants. However, the appropriate dose of 125 mcg, 1 mcg, or 15 mcg/kg has not been well established, and endocrinologist preference varies. Importantly, infants with ACTH deficiency will not develop classical salt-wasting with hyponatremia and hypokalemia due to intact aldosterone production and secretion, which is regulated not by the pituitary but by the renin-angiotensin system. Hyponatremia may occur but is less severe relative to primary adrenal insufficiency and is likely due to reduced free water clearance, for which both cortisol and thyroid hormone play a role.

An important consideration in patients with hypopituitarism is that thyroid hormone should not be replaced until adrenal insufficiency has been either treated or ruled out, as thyroid hormone replacement can precipitate an adrenal crisis if done in the context of untreated adrenal insufficiency.

Isolated Adrenocorticotropic Hormone Deficiency

Isolated ACTH deficiency is very rare and is associated with pathogenic variants in the genes responsible for the production and/or modification of the proopiomelanocortin (POMC) precursor polypeptide. The *TBX19* gene regulates transcription of the *POMC/ACTH* gene in corticotrophs, and deficiency has been associated with adrenal insufficiency in infants.[32] A low estriol level on the prenatal triple-marker screen may be a predictor of TBX19 pathogenic variant and ACTH deficiency in general.[33] Allelic pathogenic variants of the *POMC* gene itself affect all POMC peptides, including α-melanocyte-stimulating hormone (α-MSH), resulting in red hair and fair skin, in addition to ACTH deficiency, with severe obesity developing within the first few months of life.[34] Pathogenic variants in prohormone convertase 1/3 (PCSK1) result in impaired cleavage of ACTH from POMC, as well as several gut hormones. Deficiency results in severe congenital diarrhea and failure to thrive, and a high risk of ACTH deficiency as well as panhypopituitarism, including central diabetes insipidus.[35]

Primary Adrenal Insufficiency

Congenital Adrenal Hyperplasia

Cortisol deficiency in infants is most commonly due to pathology of the adrenal gland (primary adrenal insufficiency) caused by congenital adrenal hyperplasia (CAH). The most common form of CAH, 21-hydroxylase deficiency (incidence 1/10,000 to 1/15,000 annually), results in ambiguous, virilized genitalia in an XX infant driven by overproduction of adrenal androgens synthesized from precursors upstream of the enzyme block. However, in an XY infant, the presentation of CAH due to 21-hydroxylase is more subtle and may present only with hyperpigmentation of the skin, particularly of the scrotum. The classic presentation of hyponatremia, hyperkalemia and severe dehydration due to the cortisol and aldosterone deficiency of primary adrenal insufficiency often do not develop until 7 to 10 days of life, so their absence in a newborn with hypoglycemia does not rule out CAH. Newborn screening for the 21-hydroxylase deficiency form of CAH is now widespread in the U.S., and thus a diagnosis is often suspected early due to elevated 17-hydroxyprogesterone (17-OHP) levels on newborn bloodspot samples. Where hypoglycemia occurs in an infant, especially with any of the features mentioned above of salt wasting or ambiguous genitalia, confirmation of a normal 17OHP should be sought. Treatment is with hydrocortisone and fludrocortisone for glucocorticoid and mineralocorticoid replacement, respectively, along with sodium chloride supplementation due to the low salt content of breastmilk and most formulas.

More rare forms of CAH include 11-beta hydroxylase deficiency (1/100,000 or 1/5000 in Jews of Moroccan ancestry),[34] and the very rare 3-beta hydroxysteroid dehydrogenase (HSD) deficiency. Similar to 21-hydroxylase pathogenic variants, 46 XX infants with 11-beta hydroxylase deficiency resulting in hypoglycemia will have ambiguous genitalia. They may also have hypertension due to excessive production of deoxycorticosterone, a potent mineralocorticoid located upstream of the 11-beta hydroxylase enzyme block. 3-beta HSD deficiency results in undervirilized male genitalia due to deficient testosterone synthesis but may be associated with virilized female genitalia due to elevated DHEA levels. Lipoid CAH is associated with pathogenic variants in the *STAR* gene, which result in loss of function in the steroidogenic acute regulatory protein (StAR), which impairs the transfer of cholesterol into the mitochondria.[35] This disrupts the necessary first step of steroid synthesis, the conversion of cholesterol to pregnenolone. These patients have cortisol deficiency, almost always have aldosterone deficiency, and XY infants can have phenotypically female external genitalia due to impaired androgen synthesis.[36,37]

X-linked Adrenal Hypoplasia Congenita

DAX-1 (dosage-sensitive sex reversal adrenal hypoplasia congenital region of the X-chromosome; also known as NROB1) is a transcription factor located on the short arm of the X-chromosome (Xp21). It is expressed in adrenal, hypothalamic, pituitary, and hypothalamic tissues, but its gene targets are largely unknown.[38] Pathogenic variants in DAX-1 result in underdevelopment or agenesis of the adrenal gland with the potential to cause severe adrenal insufficiency in infancy, otherwise known as adrenal hypoplasia congenita (AHC). Males are classically present in the first weeks of life with vomiting, severe dehydration leading to vascular collapse, and often with hyperpigmentation. Some patients are more mildly affected and present later in childhood with milder adrenal insufficiency. Patients later develop hypogonadotropic hypogonadism, manifesting as delayed pubertal development without elevation of gonadotropins. However, DAX-1 appears to have a direct role in gonadal development, as there is also a component of primary hypogonadism.[39,40] Further implicating its role in gonadal development is the finding that *DAX1* is the likely critical gene for a duplication within Xp21 that causes sex-reversal with female external genitalia and variable presence of Mullerian structures in 46 XY individuals.[41–43] AHC is also seen as a part of Xp21 contiguous gene deletion syndrome, which occurs due to the deletion of DAX1 and nearby genes encoding glycerol kinase (GK) and dystrophin (DMD). Affected patients have severe developmental delay and develop Duchenne's muscular dystrophy in addition to the primary adrenal insufficiency secondary to AHC.

IMAGe Syndrome

IMAGe syndrome (intrauterine growth restriction, metaphyseal dysplasia, congenital adrenal hypoplasia, and genital anomalies) was first described in 1999 in 3 boys who presented with severe adrenal insufficiency shortly after birth.[44] The affected infants also had skeletal abnormalities, micropenis, and hypogonadotropic hypogonadism. Linkage analysis and sequencing in a 5-generation family identified a likely causative variant in the gene encoding cyclin-dependent kinase inhibitor 1C (CDKN1C) located on chromosome 11p15 within an imprinted cluster of genes.[45] Affected patients in the pedigree had maternally transmitted pathogenic variants, suggesting that imprinting silences the paternal allele of CDKN1C. CDKN1C is believed to have a role in inhibiting cell cycle progression, and gain-of-function variants in CDKN1C are established as causative of IMAGe syndrome. Interestingly, pathogenic variants in CDKN1C have also been associated with Beckwith-Wiedemann Syndrome (BWS) as well as Russel-Silver syndrome, demonstrating the importance of this gene in regulating growth.[46]

Adrenocorticotropic Hormone Resistance

Resistance to ACTH at the level of the adrenal gland causes a rare form of primary adrenal insufficiency with retained aldosterone secretion. ACTH is elevated, and cortisol is low. Hyperpigmentation due to excessive production of α-MSH and stimulation of the melanocortin 1 receptor can be present at birth or develop over time. Almost 50% of cases are due to autosomal recessively inherited pathogenic variants in the melanocortin 2 receptor (MC2R, or ACTH receptor) and melanocortin 2 receptor accessory protein (MRAP) genes.[37] Autosomal recessive pathogenic variants in nicotinamide nucleotide transhydrogenase (NNT) caused ACTH resistance in three consanguineous families, presumably due to increased oxidative stress in the adrenal zona fasiculata.[37]

Adrenal Hemorrhage

Adrenal hemorrhage is a rare occurrence in neonates and rarely results in adrenal insufficiency even when it is confirmed radiographically, likely because adrenal hemorrhage occurs bilaterally in only 5% to 10% of cases.[47] Maternal diabetes, high birth weight, fetal acidemia, and birth asphyxia are associated with higher rates of adrenal hemorrhage. Additionally, neonatal adrenal glands are larger than adult adrenal glands, increasing the risk of hemorrhage.[48]

Inborn Errors of Metabolism

The neonatal inborn errors of metabolism are covered extensively in Chapter 29, and the key pathways of metabolism are outlined

in Fig. 87.1. Hypoglycemia resulting from these conditions often requires fasting for at least 4 to 6 hours, and therefore they are less likely to present with hypoglycemia in infancy. However, a few conditions can impact early fasting adaptation and, therefore, can present with hypoglycemia in the neonatal period.

Glycogen Storage Diseases

Many glycogen storage diseases (GSDs) become clinically apparent later in childhood. However, GSD type 1 can present in neonates as it is associated with hypoglycemia after fasting for just 2 to 2.5 hours. GSD 1 results from impaired function of glucose-6-phosphatase, the enzyme responsible for the final step of glycogenolysis and gluconeogenesis in which glucose is produced from glucose-6-phosphate (see Fig. 87.1). Patients have hepatomegaly due to accumulation of fat, and untreated infants typically present with elevated serum lactate, lipids, triglycerides, and uric acid. Administering glucagon 2 to 4 hours after a carbohydrate meal is helpful diagnostically as it causes a rise in lactate but no rise in glucose. Treatment is with frequent feeding as well as more slowly absorbed carbohydrates, such as uncooked cornstarch, to help prolong fasting tolerance.

Defects of Fatty Acid Catabolism and Ketogenesis

Medium-chain acyl-CoA dehydrogenase (MCAD) deficiency is the most common disorder of fatty acid oxidation (1/17,000). Newborn screening for MCAD deficiency is widespread, and infants are often detected based on screening. Defects arise from autosomal recessively inherited pathogenic variants in the *ACADM* gene, and MCAD is responsible for the initial dehydrogenation of acyl-CoAs with a chain length between 4 and 12 carbon atoms. Patients with MCAD present with metabolic decompensation after periods of fasting or with illness, and acylcarnitine profile can show elevations in chain lengths of C6 to C10, with elevations of C8 (octanoylcarnitine) being the most prominent. Hypoglycemia is often a late finding, but lethargy, coma, and severe neurologic injury can occur due to the additional lack of ketone bodies available to the brain. Children typically present between the ages of 3 months and 3 years (average 18 months), but the presentation can occur in the first few weeks of life.[49] Uric acid, ammonia, and liver function tests are often abnormally elevated as well in the context of hepatic steatosis.

Galactosemia

Galactosemia occurs due to pathogenic variants in one of three genes necessary to metabolize galactose to glucose: galactose 1-phosphate uridyl transferase (GALT), galactokinase (GALK), and uridine diphosphate (UDP) galactose 4-epimerase (GALE). Hypoglycemia can be a clinical presentation of galactosemia, often associated with other classic features such as hepatomegaly, jaundice, failure to thrive, cataracts, and *Escherichia coli* sepsis.[50,51]

Hereditary Fructose Intolerance

Hereditary fructose intolerance (HFI) is due to pathogenic variants in the gene for aldolase B (*ALDOB*), responsible for metabolizing fructose-1-phosphate into substrates for gluconeogenesis. Vomiting, abdominal pain, acidosis, and hypoglycemia result after fructose or sucrose ingestion begins in infancy.[52] The accumulation of fructose-1-phosphate leads to liver and kidney dysfunction, the extent of which is proportional to the degree of fructose consumption. Hypoglycemia at least partially results from impaired gluconeogenesis and glycogenolysis in the liver, resulting in impaired response to glucagon.[53] Acute treatment is with IV dextrose, and further organ injury can be prevented by avoidance of dietary fructose and high-fructose corn syrup.

Hyperinsulinemic Hypoglycemia

The next section will focus on infants with hypoglycemia due to excessive insulin production. Hyperinsulinism may be caused by transient or permanent disorders of insulin secretion, and single-gene defects predominate in the permanent forms of hyperinsulinism (Table 87.1). A key management distinction among infants with hyperinsulinism is whether they can be managed with diazoxide and, if not, whether they have a focal or diffuse abnormality of pancreatic insulin regulation. Prior to discussing the causes and management of hyperinsulinism, it is important to consider the physiology of insulin secretion from the pancreatic β-cell.

Mechanism of Insulin Secretion

Depolarization of the pancreatic β-cell leads to insulin secretion, and the most important regulator of membrane polarization in the pancreatic beta cell is the ATP-sensitive potassium channel (K_{ATP} channel) (Fig. 87.2). The K_{ATP} channel is an octameric complex of two different subunits: (1) four inward-rectifying potassium channel subunits (Kir6.2 encoded by the *KCNJ11* gene), and (2) four regulatory sulfonylurea receptor subunits that surround the channel and help regulate the open or closed conformation of the channel (SUR1 encoded by the *ABCC8* gene). The channel is sensitive to the metabolic and energy state of the cell, as manifested by the ATP/ADP ratio in the cellular cytoplasm. Glucose enters the beta cell via an insulin-independent glucose channel (GLUT2 encoded by the *SLCA2A* gene). If glucose concentration is sufficiently high, it is phosphorylated by galactokinase to glucose-6-phosphate, the first step of glycolysis and, therefore, the entry point for glucose in the metabolic pathways that produce ATP (see Fig. 87.1). The increased ATP/ADP ratio causes closure of the K_{ATP} channel, resulting in membrane depolarization. In response to depolarization, voltage-gated calcium channels open, and the increase in intracellular calcium causes the fusion of the insulin-containing vesicles with the cellular membrane and subsequent release of insulin extracellularly.

The essential role that the K_{ATP} channel plays in regulating insulin secretion is evidenced by the large number of pathogenic variants in either KCNJ11 and ABCC8 that cause either congenital hyperinsulinism (decreasing channel presence in the membrane or favoring the closed conformation) or neonatal diabetes (favoring the open channel conformation), both of which are discussed later in this chapter.

Diagnosis of Hyperinsulinism

Estimates of the incidence of congenital hyperinsulinism vary widely depending on the study population but may be as high as 1 in 2500 in regions with high rates of consanguinity.[54] While hyperinsulinism is a common cause of neonatal hypoglycemia; the clinical features may be difficult to recognize and even missed until detection in later life or adulthood. However, infants with hyperinsulinism may have recognizable features of fetal hyperinsulinemia, such as overgrowth and hypertrophic cardiomyopathy. The hypoglycemia is usually present early but can be initially difficult to distinguish from the transient neonatal hypoglycemia seen in the first few hours of life. Alternatively, hypoglycemia may be immediately apparent as more severe and recalcitrant to early

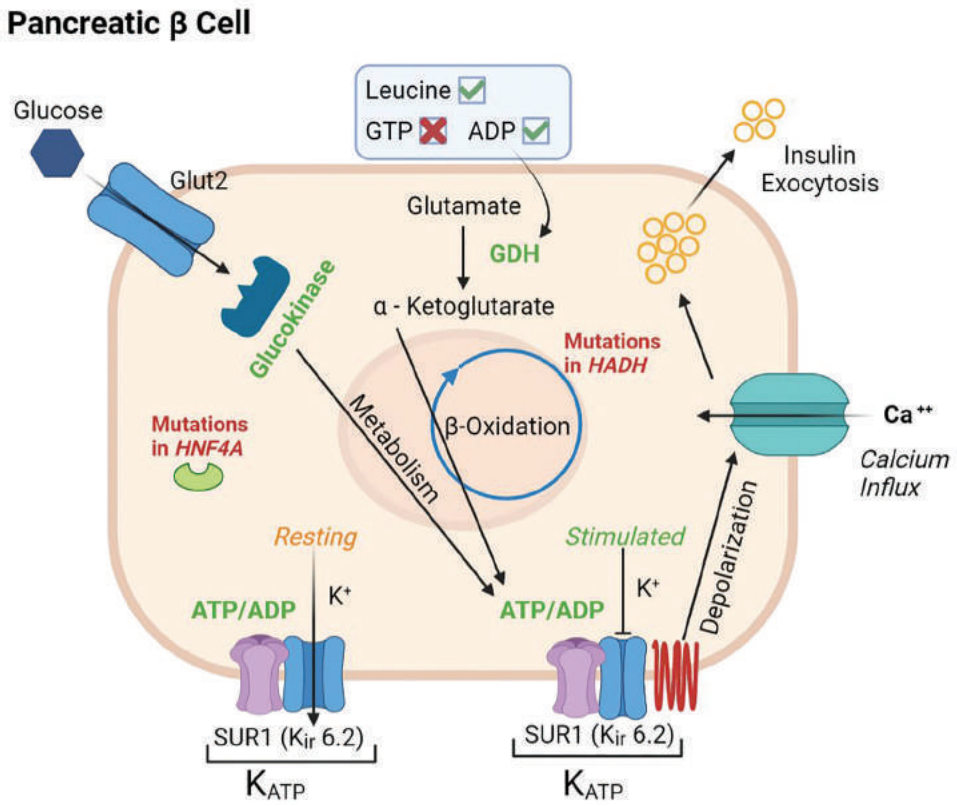
TABLE 87.1 Genetic Causes of Hyperinsulinemic Hypoglycemia

Inheritance	Molecular Defect	Chromosome	Histology	Clinical Features	Treatment
Sporadic	Paternally inherited ABCC8/KCNJ11 mutation with somatic loss of heterozygosity	11p15	Focal	Macrosomia, moderate to severe hypoglycemia in first few days to weeks of life	Poor response to diazoxide, local resection for focal and near-total pancreatectomy for diffuse form
AR	ABCC8/KCNJ11 (inactivating)	11p15	Diffuse	Macrosomia, onset in first few days to weeks of life, family history or consanguinity may be present	Near-total pancreatectomy
AD	ABCC8/KCNJ11 (dominant-negative, inactivating)	11p15	Diffuse	Milder symptoms, may manifest in late infancy	Usually responsive to diazoxide (Pinney, 2008)
AD	GLUD1 (activating)	10q23	Diffuse	Modest hyperinsulinemia and hyperammonemia, onset usually >6 months	Diazoxide, restriction of leucine in diet
AD	GCK (activating)	7p13	Diffuse	Modest hyperinsulinemia, onset usually >6 months	May respond to diazoxide
AR	HADH	4q25	Diffuse	Variable clinical presentation, abnormal acylcarnitine profile	Diazoxide
AD	HNF4α or HNF1α	20q13 and 12q24	Diffuse	Possible Fanconi tubulopathy; may have family history of MODY	Diazoxide
AD	UCP-2	11q13	Diffuse		Diazoxide
AD	HK1	10q22	Diffuse	Variable severity and penetrance	Diazoxide
AR	PGM1	1q31		Congenital disorder of glycosylation; bifid uvula, hepatopathy, hypogonadotropic hypogonadism, poor growth, myopathy, dilated cardiomyopathy[81]	Galactose supplementation may benefit

feeding. Confirming the diagnosis requires an index of suspicion on the part of the clinician, and a search for historical and physical examination findings such as macrosomia and, potentially, signs suggestive of an underlying syndrome known to be associated with hyperinsulinism such as Beckwith-Wiedemann syndrome, Kabuki syndrome, or Turner syndrome. In a state of excess glucose consumption, the normal physiologic rates of glucose supply are insufficient to maintain euglycemia, and thus a supraphysiologic glucose infusion rate (especially if >10 mg/kg/min) in the hypoglycemic infant strongly suggests hyperinsulinism. Where hypoglycemia is recognized and confirmed, the confirmatory diagnosis of hyperinsulinism hinges on the critical detection of inappropriate hyperinsulinemia, including measurable insulin, and the metabolic markers of persistent insulin effect, including suppressed free fatty acid (FFA) mobilization, hypoketosis (low beta-hydroxybutyrate, or BOHB) and an exaggerated glycemic response to glucagon (see Box 87.4).[54,55]

A 2016 retrospective review of infants with hypoglycemia showed that some children with hyperinsulinism do not have detectable insulin at the time of hypoglycemia; of 28 subjects with congenital hyperinsulinism, only 23 had detectable insulin (median value 6.7 uIU/mL). However, in those where ketosis was measured, none had β-hydroxybutyrate levels >1.8 mmol/L.[56] Importantly, bedside β-hydroxybutyrate levels show a good correlation with laboratory values, suggesting clinical utility in making a rapid diagnosis of hyperinsulinism. Similarly, infants without hyperinsulinism have FFA levels on average three times higher than in hypoglycemia due to hyperinsulinism. While previous data suggested an FFA cutoff of 0.5 mmol/L, recent data suggest that this cutoff misses many with true hyperinsulinism and that an FFA cutoff of <1.7 mmol/L provides sensitivity to >85%. Other biochemical data that are consistent with hyperinsulinism but may not yield results in as timely a manner as is ideal for rapid clinical confirmation, include C-peptide levels ≥0.5 ng/mL and IGF-BP1 (an insulin-like growth factor binding protein that is suppressed by insulin) levels ≤110 ng/mL.

Biochemical findings may be challenging to coordinate and collect in the setting of acute hypoglycemia. Therefore, a diagnostic challenge using a provocative fast performed under controlled conditions may be required.[57] This should include the frequent sampling of plasma glucose, insulin, β-hydroxybutyrate, and FFAs. At a point where the glucose falls below 2.8 mmol/L (50 mg/dL), the test can conclude with a glucagon challenge to see the glycemic response to an IV or IM dose of glucagon (1 mg), followed by a collection of glucose levels every 10 minutes for 40 minutes. A rise of ≥30 mg/dL in plasma glucose is considered consistent with hyperinsulinism. This test has high specificity, and while some patients with subsequently confirmed hyperinsulinism in the abovementioned study did not achieve a rise in glucose ≥30 mg/dL, its sensitivity is greater than relying solely on the detection of serum insulin levels at the time of hypoglycemia. While the subsequent detection of urine ketones may provide adjunctive data, ketonuria does not exclude hyperinsulinism, and accurate diagnostic results are more likely to be achieved in a



• **Fig. 87.2** Mechanisms of Insulin Secretion by the β-Cell of Pancreas. Glucose transported into the β cell by the insulin-dependent glucose transporter, GLUT 2, undergoes phosphorylation by glucokinase and subsequent metabolism, resulting in an increase in the intracellular ATP:ADP ratio. The increase in the ATP:ADP ratio closes the K_{ATP} channel and initiates the cascade of events characterized by increase in intracellular potassium concentration, membrane depolarization, calcium influx, and release of insulin from storage granules. Leucine stimulates insulin secretion by allosterically activating glutamate dehydrogenase (GDH) and by increasing the oxidation of glutamate, thereby increasing the ATP:ADP ratio and closure of the KATP channel. Pathogenic variants in the *HADH* gene, which codes for the mitochondrial enzyme L-3-hydroxyacyl-coenzyme A dehydrogenase that catalyzes the penultimate step in the fatty acid β-oxidation pathway, are also associated with CHI. Pathogenic variants in HNF4α cause multiple defects in glucose-stimulated insulin secretion. ×, inhibition; √, and stimulation. (Modified from Sperling MA, Menon RK. Differential diagnosis and management of neonatal hypoglycemia, *Pediatr Clin North Am*. 2004;51:703–723.)

timely fashion at the bedside by blood sampling.[58] These authors thus do not recommend routine reliance on urinary ketosis as a replacement for the above protocol.

Hypoglycemia in Infants of Diabetic Mothers

Transient hyperinsulinemic hypoglycemia is often associated with pregnancies affected by diabetes, including both gestational diabetes as well as permanent maternal diabetes. Infants are typically macrosomic; despite high energy stores in the form of glycogen and fat reserves secondary to fetal hyperinsulinemia, hypoglycemia may persist for several days after disconnection from the high maternal glucose supply. Hypoglycemia in infants of diabetic mothers typically resolves within the first days to the week of life. Studies show that the severity of neonatal hypoglycemia in this setting is impacted by late pregnancy maternal glycemia, with avoidance of maternal hyperglycemia reducing the severity of fetal hyperinsulinemic hypoglycemia.[59,60] Neonates may require high rates of dextrose administration and/or frequent feedings, similar to patients with permanent congenital hyperinsulinism, but may successfully be weaned off high delivered substrate support judiciously over the first week or two of life.

Perinatal Stress and Transient Hyperinsulinism

While the well-described transitional hypoglycemia in normal newborns typically resolves within the first hours to the first day of life, in stressed infants, a persistent form of hypoglycemia may be seen.[61] The mechanism, similar to transitional hypoglycemia in healthy newborns, appears to be related to persistent hyperinsulinism, as ketogenesis and lipolysis remain suppressed. While somewhat unclear, it has been postulated that a lower glucose threshold for insulin secretion persists, given that insulin is a key fetal growth factor, and thus, its persistent secretion may confer a protective effect on the small or stressed neonate.[54] This may be seen in the context of perinatal asphyxia, pre-eclampsia, or SGA and may require interventions with early frequent feeding, medical therapy (including diazoxide directed at the hyperinsulinemia), or continuous feeds to avoid the complications of hypoglycemia.[27,62,63] For SGA infants with hyperinsulinemic hypoglycemia, diazoxide

treatment is often required for as long as 6 months and has been reported to last as long as 22 months.[64]

Genetic Causes of Hyperinsulinemic Hypoglycemia

Pathogenic Variants in the K_{ATP} Channel Genes KCNJ11 and ABCC8

Pathogenic variants in the KCNJ11 and ABCC8 genes located on chromosome 11p15.1 are the most common cause of permanent hyperinsulinism, accounting for about 40% to 50% of cases in neonates, the majority of which affect ABCC8.[65,66] Most commonly, this is due to pathogenic variants that prevent channel formation, trafficking to the cell membrane, or insensitivity to the cellular ATP/ADP ratio. In the absence of a functional K_{ATP} channel the β-cell is permanently depolarized, leading to insulin secretion. Because diazoxide activity requires a functional channel present in the cell membrane, patients with KCNJ11 or ABCC8 pathogenic variants typically do not respond to diazoxide therapy. However, some autosomal dominant mutations in KCNJ11 and ABCC8 are responsive to diazoxide.[67]

The mechanism of inheritance of the pathogenic variant affecting K_{ATP} channel function correlates strongly with the extent of pancreatic involvement. Patients with autosomal recessively inherited (biallelic) pathogenic variants will have diffuse involvement of the pancreas, whereas patients with one paternally inherited pathogenic variant will have a focal disease. The focal lesion arises from a somatic loss of the maternal allele in a sub-population of pancreatic cells, leading to focal unopposed expression of the mutated paternal allele, as well as unbalanced expression of imprinted genes that contribute to cellular hyperplasia.[66,68] Rarely, dominant-negative single allele pathogenic variants can result in diffuse disease. As described later in this section, the extent of pancreatic involvement directly impacts treatment. Patients with the focal histological subtype can be cured by targeted surgical resection, whereas those with the diffuse disease may require near-total pancreatectomy for definitive therapy.

Activating Variant of the Glutamate Dehydrogenase 1 Gene: Hyperinsulinemia-Hyperammonemia Syndrome

After pathogenic variants in KCNJ11 and ABCC8, pathogenic variants in glutamate dehydrogenase 1 (*GLUD1*) are the second most common cause of genetic hyperinsulinism. Glutamate dehydrogenase is an enzyme thought to localize to the mitochondrial matrix protein that converts GDP to GTP, which in turn regulates amino acid and ammonia metabolism. Autosomal dominant gain-of-function pathogenic variants increases the GTP/GDP ratio in the beta cell, leading to the closure of the K_{ATP} channel and subsequent depolarization and insulin secretion. Presentation is typically between 4 and 12 months of age, but infants can present in the first few days of life with hypoglycemia.[69] Leucine is an allosteric activator of GDH, causing hypoglycemia to worsen 30 to 90 minutes after a protein-rich meal. In addition to hyperinsulinemic hypoglycemia, patients also have elevated serum ammonia levels (hyperinsulinemia-hyperammonemia syndrome), although this is believed to have no clinical consequence and does not require therapy.[2] The hypoglycemia is diazoxide-responsive, and carbohydrate-loading is encouraged prior to protein-rich meals to prevent hypoglycemia. Patients may also develop generalized seizures, including absence epilepsy.[70]

L-3-Hydroxyacyl-Coenzyme A Dehydrogenase Gene Pathogenic Variants

L-3-hydroxyacyl-coenzyme A dehydrogenase (SCHAD; encoded by the *HADH* gene) is a mitochondrial enzyme responsible for the β-oxidation of fatty acids. Patients with autosomal recessive pathogenic variants can have a presentation similar to those with hyperinsulinemia-hyperammonemia syndrome.[71] This is due to HADH's additional role as a direct inhibitor of GDH activity via protein-protein interaction.[72] These patients share the same hypoglycemic sensitivity to protein-loading as patients with hyperinsulinemia-hyperammonemia; however, they do not have hyperammonemia and do not have an increased risk for a seizure disorder. Affected patients can be identified by the presence of elevated serum 3-hydroxybutyryl-carnitine and elevated urine 3-hydroxyglutaric acid.[73]

Glucokinase Pathogenic Variants

Glucokinase (GCK) catalyzes the reaction of glucose to glucose-6-phosphate and serves as the "glucose sensor" of the pancreatic beta cell. Dominant, activating pathogenic variants lower the glucose threshold for insulin secretion, causing hypoglycemia. Activating *GCK* pathogenic variants are usually de novo and not inherited from either parent; therefore family history is typically negative. Affected infants can be large for gestational age and have severe hypoglycemia in the neonatal period, but there is a wide spectrum of severity of presentation and hypoglycemia. Diazoxide therapy is effective in some cases, but pancreatectomy is required for some patients.[74]

Hepatocyte Nuclear Factor-4-Alpha and Hepatocyte Nuclear Factor-1-Alpha Pathogenic Variants

HNF4A (hepatocyte nuclear factor-4-alpha) and HNF1A (hepatocyte nuclear factor-1-alpha) are transcription factors expressed in hepatocytes, beta cells, intestinal epithelial cells, and renal tubular cells and are traditionally associated with mature onset diabetes of youth (MODY) type 1 and 3, respectively. Transcriptional regulation targets of HNF4A and HNF1A include each other, glucose transporter 2 (GLUT2), and possibly Kir6.2, but the exact cause of hyperinsulinism in infants is unclear.[75] Interestingly, some patients with neonatal hyperinsulinism due to HNF4A and HNF1A develop diabetes years later in adolescence or young adulthood, consistent with MODY 1 and 3.[76] Diazoxide is an effective therapy for patients with hypoglycemia secondary to HNF4A and HNF1A pathogenic variants. HNF4A hyperinsulinism has also been described in combination with renal Fanconi tubulopathy and hepatomegaly due to increased glycogen stores.[75]

Beckwith-Wiedemann Syndrome

Patients with BWS have characteristic features, including macrosomia, macroglossia, hemihypertrophy, ear pits, umbilical hernia, and a high risk for embryonal tumors. BWS results from epigenetic or genomic abnormalities in the imprinted 11p15.5 region or heterozygous maternally inherited pathogenic variants at CDKN1C. The majority (85%) of patients with BWS do not have a family history of the disorder. Children conceived by assisted reproductive technologies may be at increased risk for BWS and other imprinted disorders. Up to 50% of patients have hyperinsulinemic hypoglycemia with a wide spectrum of severity, most of which resolves in the first few days of life.[77,78] The cause of hyperinsulinism in BWS is variable and may depend on the patient's specific genetic abnormality, which rarely includes

pathogenic variants in either KCNJ11 or ABCC8.[78] Patients with BWS may respond to diazoxide; however, some patients require partial pancreatectomy.

Other Genetic Causes of Hyperinsulinism

Pathogenic variants in uncoupling protein 2 (UCP2) have been shown in a few individuals who presented with diazoxide-sensitive hyperinsulinemic hypoglycemia in infancy.[79] UCP2 has subsequently been shown to regulate mitochondrial, and cellular oxidation and decreased UCP2 activity is thought to increase the oxidation of glucose, thereby increasing the intracellular ATP/ADP ratio and increasing insulin secretion. There are some conflicting data regarding the role of UCP2 in hyperinsulinism, with a large-scale sequencing study not supporting the role of UCP2 in causing hyperinsulinism. However, in another study of a large cohort of diazoxide-responsive patients, 5 out of 211 were found to have one of 4 UCP pathogenic variants. The mechanism of hypoglycemia in patients with UCP2 pathogenic variants appears to be due to an exaggerated insulin response to glucose rather than persistent hyperinsulinism. With the cooperation of a 4-generation pedigree of autosomal dominantly inherited congenital hyperinsulinism, investigators identified a new single gene cause of hyperinsulinism: hexokinase 1 (HK1).[80] Family members were varyingly affected, but all who were treated with diazoxide responded well.

Autosomal recessive pathogenic variants in phosphoglucomutase 1 (PGM1) cause a syndrome of abnormal protein N-glycosylation associated with multifactorial hypoglycemia.[81] Patients demonstrate defects in glycogen breakdown as well as postprandial hyperinsulinemia, both of which have been associated with hypoglycemia in infancy.[54] Other congenital disorders of glycosylation have been associated with hyperinsulinism, including CDG1a (phosphomannomutase 2 deficiency) and CDG1b (mannosephosphate isomerase deficiency).[82]

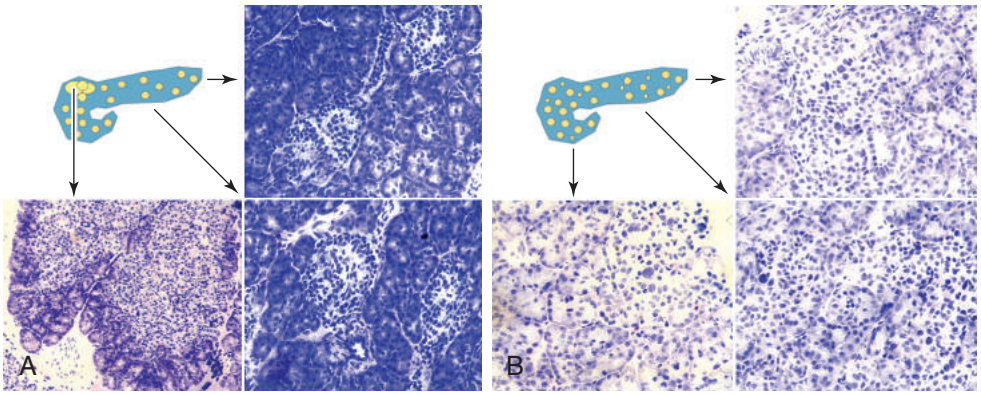
Recently an infant was diagnosed with a combination of congenital hyperinsulinism and hypopituitarism. A variant of uncertain significance (VUS) identified in the *FOXA2* gene was thought to possibly be causative.[83] FOXA2 regulates the expression of ABCC8, KCNJ11, and HADH, as well as several regulators of pituitary gland development, such as SHH, Gli2, and *NKX2-2*. The patient responded to diazoxide, suggesting that at least partial function of Sur1 and Kir6.2 was present.

Postfundoplication Hypoglycemia

Neonates undergoing fundoplication for gastric reflux or other reasons are at risk for postprandial hypoglycemia related to dumping syndrome. Often this hypoglycemia goes undetected—prompting the need for better surveillance, including the use of continuous glucose monitoring.[84] Approximately 25% of neonates develop dumping syndrome after fundoplasty, most of which is identified in the first postoperative week.[85,86] Hypoglycemia occurs 1 to 3 hours after a meal due to an exaggerated insulin response to early postmeal hyperglycemia, which is often detectable as well. Treatment is with gradual gastric feeds, and sometimes continuous feeds are required. Uncooked cornstarch, pectin, octreotide, and acarbose have been attempted with varying degrees of success.[85] More recently, GLP-1 levels were found to be high in children with dumping syndrome, which led to GLP-1 receptor antagonists being studied as a potential treatment for postprandial hypoglycemia from dumping syndrome.[85,87]

Differentiation Between Focal Adenomatous and Diffuse Pancreatic Hyperplasia

Patients with hyperinsulinism who are not responsive to diazoxide therapy should receive specialized imaging to evaluate for a focal pancreatic lesion that can be cured surgically. Positron emission tomography (PET) imaging with 18F-fluoro-L-DOPA exploits the fact that pancreatic β-cells take up the radiolabeled L-DOPA (Fig. 87.3). Simultaneous PET and MRI, therefore, provide visualization of the hyperactive β-cells within the pancreas and allows the differentiation of focal and generalized hyperactivity



• **Fig. 87.3** (A) Frozen sections obtained from three pancreatic sites during surgery for a focal form of hyperinsulinism in the head. Specimens taken from the tail and body show islets of Langerhans at rest with little cytoplasm, leading to crowded nuclei, whereas the focal form aspect from the head is that of multilobular involvement with local signs of β-cell hyperactivity: abundant cytoplasm and large, abnormal nuclei (toluidine blue stain; original magnifications ×200 and ×100). (B) Frozen sections obtained from three pancreatic sites during surgery for diffuse form of hyperinsulinism. On each biopsy, there is one islet showing hyperfunctional signs with abundant cytoplasm and irregular nuclei more than four times the size of the acinar nuclei nearby taken as internal control. The disease involves the whole pancreas and, in consequence, can be called diffuse, which does not mean that all islets are involved in the same manner (toluidine blue stain; original magnification ×200). (From Delonlay P, Simon A, Galmiche-Rolland L, et al. Neonatal hyperinsulinism: clinicopathologic correlation. *Hum Pathol*. 2007;38:387–399.)

(see Fig. 87.3). This distinction is critical as it is far preferable to perform a curative focal resection rather than a "near-total" pancreatectomy which has a more variable outcome ranging from continued hyperinsulinism to diabetes.[88]

In all, approximately half of patients requiring surgery will have a focal lesion.[65] Such imaging is often successful in identifying the appropriate surgical management in those patients with hyperinsulinism refractory to medical management.[89]

Management of Hyperinsulinemic Hypoglycemia

The goal of treatment is to minimize hypoglycemia frequency and duration to prevent impaired neurologic development. First-line treatment is with diazoxide, which opens the beta-cell ATP-dependent potassium channel, limiting depolarization and thereby decreasing insulin secretion. Common side effects of diazoxide include hypertrichosis and fluid retention, sometimes requiring diuretic use.

Pulmonary hypertension has also been described in a small number of patients receiving diazoxide.[90] A retrospective cohort study was able to estimate the risk for pulmonary hypertension to be 7% (13 out of 177 patients), the majority of which were symptomatic.[91] The onset of pulmonary hypertension was most commonly within 2 weeks of starting therapy but could occur at any time while on treatment with diazoxide. Pulmonary hypertension resolved in 10 out of the 13 patients identified but persisted for as long as 12 months after discontinuation of diazoxide. Risk factors for the development of pulmonary hypertension included the presence of congenital heart disease and higher volumes of fluid given prior to the start of diazoxide. The authors proposed a best practice guideline that recommends obtaining an echocardiogram prior to starting diazoxide and 1 to 2 weeks following initiation of therapy.

As mentioned above, not all hyperinsulinism patients will respond to diazoxide (particularly those with K_{ATP} channel pathogenic variants), and second-line therapies include subcutaneous octreotide and glucagon infusion (Table 87.2). Octreotide is known to result in tachyphylaxis, causing diminishing efficacy of the medication. There is also a possible association with necrotizing enterocolitis, which requires special consideration in the neonatal population.[92,93] Lanreotide, a long-acting form of somatostatin administered as a once a month injection, has been successful in treating hyperinsulinism as well.[92,93] Glucagon infusion is effective but not sustainable as a long-term treatment strategy due to the need either for IV infusion or continuous delivery via a subcutaneous catheter. Continuous subcutaneous delivery systems have been described, but they are limited by crystallization of the glucagon and obstruction of the tubing.[94] Sirolimus, a known cause of transplant-related diabetes, has also been used experimentally with some success.[95]

Nutritional factors should also be considered by increasing the carbohydrate content of feeds or by altering the length and frequency of feeding breaks. Continuous dextrose (D20W) delivered enterally is sometimes required to maintain normoglycemia overnight.[96] Families should be taught how to use a glucometer and to be vigilant for signs and symptoms of hypoglycemia. Parental education on the administration of glucagon is necessary prior to discharge.

Patients that require pancreatectomy have a high risk for future development of insulin-dependent diabetes and neurobehavioral problems.[88] Lord et al. recently reported the outcomes for 121 children with congenital hyperinsulinism treated with pancreatectomy, finding that 36% developed diabetes during long-term follow-up, and 20% of these patients developed diabetes in the immediate postoperative period. The risk of diabetes was associated with the percentage of pancreatectomy: 93% of those with diabetes had a 95% pancreatectomy or greater, while those without diabetes had a median 65% pancreatectomy. Also, 48% had neurobehavioral concerns, including 21% of patients with psychiatric or behavioral concerns, 18% with speech delay, and 16% with a learning disability, using parent-reported questionnaires. Quantitative measures of adaptive behavior were abnormal in 27% of patients. The finding that these neurodevelopmental outcomes were similar for the focal and diffuse groups suggests that a shared exposure to recurrent hypoglycemia in the neonatal period may be causative.

Neonatal Hyperglycemia

Insulin is a critical mediator of fetal growth, and therefore infants with hyperglycemia due to persistent insulin deficiency are almost universally born small for gestational age and have a history of intrauterine growth restriction. It is thought that the autoimmune process leading to beta-cell destruction takes at least 6 months to manifest itself, and therefore autoimmune-mediated Type 1 Diabetes does not occur earlier than 6 months of age, with the notable exception of IPEX-related diabetes.[97] Persistent hyperglycemia that meets the criteria for diabetes (>125 mg/dL fasting or >200 mg/dL) in an infant <6 months of age suggests a

TABLE 87.2 Drugs Used in the Management of Neonatal Hyperinsulinism

Drug	Dose/Route	Mechanism of Action	Adverse Effects
Diazoxide	5-15 mg/kg/day in three divided doses orally	Binds to SUR1 subunit, opens K_{ATP} channel	Fluid retention, hypertrichosis, rarely eosinophilia, leukopenia, hypotension
Chlorothiazide (in conjunction with diazoxide to decrease fluid retention)	10-20 mg/kg/day in two divided doses orally	Synergistic response to diazoxide	Hyponatremia, hypokalemia
Octreotide	5–25 μg/kg/day 6–8 hourly SC injection or IV infusion	Inhibits insulin secretion by binding to somatostatin receptors and inducing hyperpolarization of β-cells, direct inhibition of voltage-dependent calcium channels	Anorexia, nausea, abdominal pain, diarrhea, tachyphylaxis Risk of NEC in young infants, thus lower doses are recommended
Glucagon	1–20 μg/kg/h, SC or IV infusion	Increases glycogenolysis and gluconeogenesis	Nausea, vomiting, paradoxical insulin secretion at high dose

monogenic cause strongly, and genetic testing is positive in at least 80% of cases.[98] About 50% of infants with diabetes in the first few months of life will have a resolution by 1 to 2 years of age (transient neonatal diabetes, or TNDM), but a significant portion of these patients will have relapse of diabetes later in childhood or adolescence.[99,100] Family history of early adulthood onset diabetes mellitus, perhaps reported as or assumed initially to be T1DM or T2DM, or MODY, can suggest an inherited abnormality of insulin secretion, but positive family history is not present in 70% of molecularly confirmed cases.[98] Elucidation of the specific genetic etiology has immediate implications for treatment, as those with pathogenic variants in the KCNJ11 or ABCC8 components of the K_{ATP} channel are best treated with oral sulfonylurea rather than insulin.

Transient Stress-Related Hyperglycemia

Most commonly, neonatal hyperglycemia is transient and caused by physiologic stress such as sepsis or other acute illness, particularly in infants receiving continuous intravenous dextrose infusions (Box 87.5).[101] Cortisol and catecholamines secreted during acute illness increase gluconeogenesis, glycogen breakdown, and insulin resistance, all of which increase plasma glucose concentration. The hyperglycemia will improve as the neonate's overall clinical status improves and as glucose infusion rates are minimized to the lowest necessary amount. Insulin infusions may be necessary to control hyperglycemia in the short term.

Neonatal Diabetes Mellitus

Neonatal diabetes mellitus (NDM) is a genetically heterogeneous disease with over 20 known genetic subtypes. Different types of NDM vary widely in their prognosis, from mild self-resolving hyperglycemia to permanent forms associated with severe neurodevelopmental features. Some types of NDM respond to sulfonylureas, while others do not. Thus, early referral for genetic testing should be made as soon as a clinical diagnosis of NDM is made. It is important to recognize that the absence of a family history does not exclude a genetic cause, as most NDM-causing pathogenic variants arise spontaneously.[98]

Transient Neonatal diabetes Mellitus due to Chromosome 6q24 Anomalies

The majority (70%) of patients with transient neonatal diabetes mellitus (TNDM) have an abnormality of the imprinted region of chromosome 6q24. Within this imprinted region are two genes, PLAGL1 and HYMAI, that share a promoter. This promoter is differentially methylated depending on the parent of allelic origin. In the normal situation, only the paternally inherited allele is expressed, while the maternally inherited allele is methylated and not expressed. Situations that lead to a relative increase in the expression of PLAGL1 and HYMAI cause TNDM. These include paternal uniparental disomy, duplication of the 6q24 on the paternal allele, hypomethylation of the maternal allele producing inappropriate PLAGL1 and HYMAI expression from the maternal allele, or multilocus imprinting disturbance (MLID) caused by biallelic pathogenic variants in ZFP57. The mechanism by which overexpression of PLAGL1 and HYMAI leads to TNDM is not fully understood. A second, nonimprinted promoter also controls PLAGL1 expression, which has led to the hypothesis that the postnatal remission of TNDM is due to a switch over to this nonimprinted promoter postnatally. This form of TNDM resolves at a median age of 3 months, but about half of patients will have a return of diabetes during adolescence. Management is with subcutaneous insulin therapy until remission. Associated clinical features include intrauterine growth retardation (IUGR), macroglossia (44%), and umbilical hernia (21%), while other reported findings have included facial dysmorphism (18%), cardiac and renal anomalies (9%), and hand anomalies (8%).[102,103]

Interestingly, reports have shown that a subset of patients with TNDM due to 6q24 abnormalities develops hyperinsulinemic hypoglycemia after the resolution of neonatal diabetes.[104] These patients have reportedly responded to diazoxide therapy and, at follow-up, have still required treatment with diazoxide 1 to 4 years later.

Transient Neonatal Diabetes Mellitus due to K_{ATP} Pathogenic Variants

About 25% of patients with TNDM have normal methylation at 6q24. Most of these 6q24 normal TNDM patients have heterozygous activating pathogenic variants in either KCNJ11 or ABCC8, the two components of the K_{ATP} channel, as described previously. Activating pathogenic variants in these same genes can also cause PNDM; see below. Although some studies have suggested that TNDM-causing pathogenic variants are functionally less severe than PNDM-causing pathogenic variants, some pathogenic variants in KCNJ11 have been reported in both PNDM and TNDM patients, so an absolute genotype-phenotype correlation may not exist.[105,106] Compared with 6q24-associated TNDM, infants with K_{ATP}-associated TNDM have higher birth weight, later diagnosis, later remission, and earlier relapse of hyperglycemia.[107] No significant clinical differences have been noted between KCNJ11 and ABCC8-related TNDM, both of which respond favorably to sulfonylureas.

Additional Genetic Causes of Transient Neonatal Diabetes Mellitus

Several different biallelic pathogenic variants within the promoter of the insulin gene (*INS*) have been reported in TNDM patients.[108] Pathogenic variants within the promoter of the *INS* gene can also cause PNDM. Genotype-phenotype correlations are limited as several of these variants have been reported in both TNDM and PNDM. For example, the INS promoter variant(c.-331C>G) has been reported to cause TNDM in some individuals and PNDM in others, even within the same family. The reason for this variability is unknown.

• BOX 87.5 Differential Diagnoses of Neonatal Hyperglycemia

Common Causes

Excessive intravenous glucose infusion
Impaired glucose homeostasis in preterm/sick/SGA infants
Sepsis
Stress
Corticosteroids

Rare Causes

Transient neonatal diabetes
Permanent neonatal diabetes

Homozygous pathogenic variants in the gene *SLC2A2*, encoding the GLUT2 transporter, which transports glucose into the beta cell, have been reported in 4 unrelated patients with TNDM; parents were first cousins in 3 of these patients.[107,109] Three of the patients presented with apparently isolated diabetes, but eventually, all four demonstrated findings associated with Fanconi-Bickel syndrome (FBS). Biallelic pathogenic variants in *SLC2A2* are known to cause FBS, whose features include renal Fanconi syndrome, poor growth, hepatomegaly, and impaired utilization of glucose and galactose.[110] However, over 95% of patients with biallelic *SLC2A2* pathogenic variants present with symptoms of FBS without evidence of neonatal diabetes, but the reason for this variable expressivity is unknown.

Nonsyndromic Causes of Permanent Neonatal Diabetes Mellitus

Infants with neonatal diabetes without evidence of remission in the first year or two of life are classified as having PNDM. The most common cause of PNDM is heterozygous, activating pathogenic variants in the potassium channel subunit genes KCNJ11 and ABCC8 (more commonly KCNJ11), accounting for almost half of all patients with PNDM.[111,112] These pathogenic variants decrease the potassium channel's sensitivity to the cellular ATP concentration, keeping the channel inappropriately open and inhibiting insulin secretion. 20% of individuals with PNDM due to KCNJ11 pathogenic variants will also have *d*evelopmental delay, *e*pilepsy, and *n*eonatal *d*iabetes (DEND) syndrome.[113] There are clear genotype-phenotype correlations within the *KCNJ11* gene, with some pathogenic variants being associated with DEND syndrome and others with only PNDM. Patients with PNDM due to pathogenic variants in KCNJ11 and ABCC8 typically respond well to sulfonylureas.[114] Interestingly, some neurologic features of DEND have also been reported to respond to sulfonylureas, highlighting the importance of the potassium channel in neuronal cells.[115]

Pathogenic variants within the INS are also a common cause of PNDM, found in approximately 10% of patients with PNDM. *INS* gene pathogenic variants can be homozygous (more common among offspring of consanguineous relationships) or heterozygous, but in both cases, the pathogenic variants lead to inadequate production of insulin protein.[98,108,116]

Rarer genetic causes of nonsyndromic PNDM include biallelic inactivating pathogenic variants in glucokinase (GCK), and the transcription factor PDX1.[117,118] GCK serves as the "glucose sensor" of the beta cell, converting glucose into glucose 6-phosphate. PDX1 is a transcription factor necessary for the formation of the pancreas in utero. Heterozygous pathogenic variants in GCK are a relatively common cause of MODY, accounting for 20% to 50% of MODY patients. Therefore, GCK should be strongly considered in patients with PNDM who have a positive family history of MODY, mild fasting hyperglycemia, or gestational diabetes in a nonobese mother. Some patients with homozygous pathogenic variants of PDX1 have pancreatic agenesis, producing exocrine insufficiency in addition to PNDM, while in others, a pancreatic exocrine function is intact.[118]

Syndromic Causes of Neonatal Diabetes Mellitus

In addition to the genes described above, there are multiple other known genetic causes of NDM, which are typically considered "syndromic" because they are often associated with other non-endocrine features. Although some syndromic forms of NDM present with other features (e.g., congenital heart defects), diabetes is often the initial presentation, making early genetic diagnosis helpful as it can guide management and necessary screening. For example, patients with biallelic pathogenic variants in *EIF2AK3* have Wolcott-Rallison syndrome, which usually presents with neonatal diabetes, while other features (skeletal dysplasia, developmental delays, and liver dysfunction) may not manifest until later. A quarter of NDM patients whose parents are consanguineous have Wolcott-Rallison syndrome, making it the most common cause of PNDM among this group of patients. The remaining syndromic causes of neonatal diabetes are listed in Table 87.3. Because of the considerable number of genetic causes of neonatal diabetes, sequencing multiple genes in parallel is typically the most efficient diagnostic approach.

TABLE 87.3 Syndromic Causes of Neonatal Diabetes

Gene	Syndrome	Reference
EIF2AK3	Wolcott-Rallison syndrome	122,123
FOXP3	IPEX syndrome: severe diarrhea, type 1 DM, dermatitis, X-linked	124,125
GATA4	Neonatal and childhood onset DM, may have pancreatic hypoplasia, cardiac malformations, and neurocognitive defects	126
GATA6	Pancreatic agenesis, ± congenital heart defects	127
GLIS3	NDM with congenital hypothyroidism	128,129
HNF1B	Renal cysts and diabetes (RCAD), neonatal diabetes (NDM)	130
IER3IP1	NDM with microcephaly, lissencephaly, and epileptic encephalopathy	131,132
MNX1	NDM with neurologic features, Currarino syndrome (sacral agenesis, imperforate anus)	133,134
NEUROD1	NDM with cerebellar hypoplasia, sensorineural hearing loss, visual impairment	135
NEUROG3	NDM with congenital malabsorptive diarrhea	136,137
NKX2-2	NDM with developmental delays, hypotonia, short stature and hearing loss	134
PTF1A	NDM with pancreatic and cerebellar agenesis	138
RFX6	NDM with pancreatic hypoplasia, intestinal atresia, gall bladder hypoplasia (Mitchell-Riley syndrome)	139,140
SLC19A2	NDM with deafness and thiamine-responsive megaloblastic anemia (Rogers syndrome)	141
SLC2A2	NDM with renal dysfunction (Fanconi Bickel syndrome)	142
WFS1	Wolfram syndrome, DIDMOAD, low frequency sensorineural hearing loss, optic atrophy	143
PAX6	Neonatal diabetes with brain malformations, microcephaly, and microphthalmia	144
LRBA	Common variable immunodeficiency with autoimmunity	145

Management of Neonatal Hyperglycemia

Management of hyperglycemia in the neonatal period is dictated by the clinical scenario and the results of genetic testing. Transient hyperglycemia is best managed by treating the underlying cause (e.g., sepsis). In addition to close monitoring of glucose, exogenous glucose administration can be decreased to approximately 3 mg/kg/min. If necessary, insulin treatment can commence, starting with a low-dose insulin infusion (e.g., 0.03 units/kg/h).

Treatment of neonatal diabetes requires insulin, at least until a genetic diagnosis is made. For those with a genetic aberration at 6q24, insulin requirements usually drop quite quickly, and treatment is often discontinued by 12 weeks of age.[119] Hyperglycemia may recur with intercurrent illness and then recurs in over half of children, generally at the time of puberty.

Infants with an identified pathogenic variant in *KCNJ11* or *ABCC8* can be treated with sulfonylureas. Generally, it is best to gradually decrease insulin dosing as sulfonylurea treatment is initiated. Sulfonylurea dosing tends to be higher than typically used in adults. While over 90% of those with a *KCNJ11* or *ABCC8* pathogenic variant can successfully transition from insulin to sulfonylurea and maintain near normal glycemic control, the factors associated with sulfonylurea failure are the specific genetic variant and longer duration of diabetes.[120] Treatment with sulfonylureas is not only effective in achieving euglycemia but also has led to improvements in neurological status for patients with DEND syndrome; this appears to be secondary to improved cerebellar perfusion.[121]

Acknowledgments

We would like to thank the authors of the 9th edition of this chapter—Vandana Jain, Ming Chen, and Ram K. Menon—whose work was the starting point for our chapter.

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88 Craniofacial Conditions

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KEY POINTS

- Craniofacial malformations can impact swallowing, breathing, hearing, vision, speech, and development and for some neonates can result in life-threatening airway compromise.
- Early recognition and assessment of craniofacial conditions that include appropriate diagnostic studies, identification of associated health concerns, and family education can have a positive impact on the care and outcome of affected newborns.
- Timely referral to or consultation with a multidisciplinary craniofacial team in a newborn with a craniofacial condition is an important step in the provision of coordinated medical and surgical management. Key members of the craniofacial team are shown in Box 88.1. A list of teams accredited by the American Cleft Palate-Craniofacial Association (ACPA) can be found on the ACPA website: https://acpa-cpf.org/acpa-family-services/find-a-team/.

The neonatal care provider is often the first point of contact for a child born with a craniofacial malformation. Abnormalities of the face and head can be distressing to a new parent, who is immediately wondering, "Is my child going to look, feel, and develop normally?" Having a basic understanding of the relationship between craniofacial abnormalities and feeding, breathing, hearing, vision, speech, and overall development will help care providers to begin to counsel a family. Airway compromise is well described in multiple craniofacial syndromes, and early identification can be lifesaving. Prompt recognition of a constellation of anomalies pointing toward a syndrome or diagnosis will result in better-targeted evaluations and therapies for that patient. Tables 88.1 and 88.2 contain a concise presentation of potential intensive care unit (ICU) issues that may be encountered with craniofacial malformations and syndromes. This chapter highlights the most relevant craniofacial conditions that neonatal care providers will encounter. We describe here the diagnosis, etiology, phenotype, and potential ICU issues as well as basic management and screening recommendations to help guide neonatal practitioners in caring for an infant with craniofacial malformations.

Micrognathia/Robin Sequence

Diagnosis and Etiology

The triad of micrognathia, glossoptosis, and airway obstruction is known as *Robin sequence* (RS) or *Pierre Robin sequence*. Cleft palate is a common feature of RS, although not obligatory to the diagnosis. Approximately one-quarter of infants with cleft palate (CP) were found to have RS in a population-based, case-control study.[1] RS is an etiologically and phenotypically heterogeneous disorder. In a large cohort study of 191 children with RS, 38% had isolated RS, 9% had a chromosome anomaly, 29% had a Mendelian disorder, and 24% had no detectable cause. Twenty-two Mendelian disorders were diagnosed, of which Stickler syndrome was the most frequent.[2] The tremendous heterogeneity and lack of uniformly accepted diagnostic criteria for, or definitions of, RS make it challenging to know the true prevalence. In a review of 42 international studies, the estimated birth prevalence for RS ranged between 1:3900 and 1:122,400 (0.8 to 32.0 per 100,000), with a mean prevalence of 1:24,500.[3]

Phenotype

While there is great variation in severity, RS is characterized by the following phenotypic features: micrognathia, glossoptosis, and resultant base of tongue-level upper airway obstruction (Fig. 88.1A, B).[4] Cleft palate is a common additional feature, occurring in approximately 90%.[5] A wide, U-shaped cleft is classic in RS and should prompt the provider to evaluate for any signs of micrognathia or airway obstruction, while the narrow, V-shaped cleft palate is more typical in infants without RS. Micrognathia, or a small and symmetrically receded mandible, is a subjective diagnosis, although assessing the maxillomandibular discrepancy (distance between the maxillary and mandibular alveolar ridges in the midline) can help with recognition. Glossoptosis is dynamic and defined as displacement of the tongue base into the oropharynx and hypopharynx. Tongue size varies across the spectrum of RS, and the severity of glossoptosis does not always correlate with the degree of micrognathia. Intraoral examination of the infant with glossoptosis may reveal a posteriorly positioned tongue, occasionally pulled up into a palatal cleft. Upper airway obstruction (often presenting with stertor, increased effort, or obstructive apnea) in infants with RS can be associated with feeding difficulties and challenges gaining weight. Clinical judgment can be made about whether the patient represents "isolated RS," "RS plus (RS with other anomalies)" or a syndromic form of RS.

Intensive Care Unit Concerns

Upper airway obstruction in RS is a result of tongue displacement toward the posterior pharyngeal wall or up into the cleft. The tongue can act as a ball valve, leading to inspiratory obstruction.

TABLE 88.1 Craniofacial Syndromes Commonly Associated With Cleft Lip and/or Cleft Palate

Syndrome	Phenotype	ICU Issues	OMIM
Robin sequence[a]	Micrognathia, glossoptosis with upper airway obstruction, cleft palate	Airway obstruction, feeding difficulties	261800
Stickler syndrome[a]	Cleft palate, micrognathia, glossoptosis (Robin sequence), high myopia, risk of retinal detachment and blindness, midface hypoplasia, hearing impairment, arthropathy, pectus, short fourth and fifth metacarpals	Airway obstruction, feeding difficulties	180300, 604841, 184840, 614134, 614284, 609508
22q11.2 deletion syndrome (velocardiofacial syndrome, DiGeorge syndrome)[a]	Cleft palate and submucous cleft palate, small mouth, myopathic facies, retrognathia, prominent nose with squared-off nasal tip, hypoplastic nasal alae, short stature, slender tapering digits	Cardiac anomalies, airway obstruction, feeding difficulties, aspiration	192430, 188400, 611867
Opitz oculogenitolaryngeal syndrome (Opitz BBB/G syndrome)[a]	Hypertelorism, telecanthus, cleft lip and/or palate, dysphagia, esophageal dysmotility, laryngotracheoesophageal cleft (aspiration), hypospadias, bifid scrotum, cryptorchidism, agenesis of the corpus callosum, congenital heart disease, intellectual disability	Laryngotracheoesophageal clefting (stridor, feeding difficulties, choking, aspiration)	145410, 300000
Pallister–Hall syndrome[a]	Cleft palate, flat nasal bridge, short nose, multiple buccal frenula, microglossia, micrognathia, malformed ears, hypothalamic hamartoblastoma, hypopituitarism, postaxial polydactyly with short arms, imperforate anus, genitourinary anomalies, intrauterine growth restriction	Laryngotracheoesophageal clefting (stridor, feeding difficulties, choking, aspiration), panhypopituitarism	146510
IRF6-related disorders (including Van der Woude and popliteal pterygium syndrome)	Cleft lip with or without cleft palate, cleft palate only, lower lip pits or cysts, ankyloglossia; popliteal pterygium syndrome will also have popliteal pterygia, bifid scrotum, cryptorchidism, finger and/or toe syndactyly, abnormalities of the skin around the nails, syngnathia and ankyloblepharon	Not anticipated	119300, 119500
CHARGE syndrome[a]	Coloboma of the eye, heart malformations, choanal atresia, growth retardation, genital anomalies, ear abnormalities and/or deafness, facial palsy, cleft palate, dysphagia	Airway obstruction, bilateral choanal atresia, cardiac anomalies, feeding difficulties, aspiration	214800
Smith–Lemli–Opitz syndrome[a]	Cleft palate, micrognathia, short nose, ptosis, high square forehead, microcephaly, hypospadias, cryptorchidism, ventricular septal defect, tetralogy of Fallot, hypotonia, intellectual disability, postaxial polydactyly, 2–3 toe syndactyly, defect in cholesterol biosynthesis	Cardiac anomalies, airway hypotonia, and airway obstruction	270400
Ectrodactyly, ectodermal dysplasia, and clefting syndrome	Cleft lip and/or palate, split-hand/split-foot, ectodermal dysplasia (sparse hair, dysplastic nails, hypohidrosis, hypodontia), genitourinary anomalies	Not anticipated	129900, 604292, 129400
Ankyloblepharon, ectodermal dysplasia, and clefting syndrome	Cleft lip with or without cleft palate, cleft palate only, intraoral alveolar bands, maxillary hypoplasia, ankyloblepharon (eyelid fusion), ectodermal dysplasia (sparse hair, dysplastic nails, hypohidrosis, anodontia)	Not anticipated	106260
Orofaciodigital syndrome	Median cleft of upper lip, cleft palate, accessory oral frenula, lobulated tongue with hamartomas, broad nasal root, small nostrils, syndactyly, brachydactyly, postaxial polydactyly, polycystic renal disease, agenesis of the corpus callosum	Not anticipated	311200
Kabuki syndrome[a]	Cleft palate, arched eyebrow, long palpebral fissures, eversion of lateral third of lower eyelid, brachydactyly, short fifth metacarpal, cardiac anomalies, postnatal growth deficiency/dwarfism, intellectual disability	Cardiac anomalies	147920, 300867
Fryns syndrome[a]	Cleft lip with or without cleft palate, micrognathia, coarse facies, diaphragmatic hernia, distal limb hypoplasia, malformations of the cardiovascular, gastrointestinal, genitourinary, and central nervous systems	Congenital diaphragmatic hernia, pulmonary hypoplasia; cardiac anomalies	229850
Miller syndrome (postaxial acrofacial dysostosis)[a]	Cleft palate (more than cleft lip), malar and mandibular hypoplasia, downslanting palpebral fissures, lower eyelid coloboma, microtia/atresia, conductive hearing loss, postaxial limb deficiency, absent fifth digit	Airway obstruction	263750

Continued

TABLE 88.1 Craniofacial Syndromes Commonly Associated With Cleft Lip and/or Cleft Palate—cont'd

Syndrome	Phenotype	ICU Issues	OMIM
Treacher Collins syndrome (mandibulofacial dysostosis)[a]	Cleft palate, malar and mandibular hypoplasia, downslanting palpebral fissures, lower eyelid coloboma (missing medial lower eyelid lashes), microtia/atresia, conductive hearing loss	Airway obstruction	154500, 613717, 613715, 248390, 618939
Aarskog syndrome (faciodigitogenital syndrome)	Hypertelorism, widow's peak, ptosis, downslanting palpebral fissures, strabismus, maxillary hypoplasia, broad nasal bridge with anteverted nostrils, occasional cleft lip and/or palate, floppy ears, brachydactyly, clinodactyly, joint laxity, shawl scrotum	Not anticipated	100050, 305400
Wolf–Hirschhorn syndrome (4p deletion syndrome)[a]	Cleft lip and palate, coloboma, hypertelorism, growth deficiency, microcephaly, intellectual disability, cardiac septal defects	Congenital diaphragmatic hernia, cardiac anomalies, seizures, airway hypotonia/obstruction	194190
Amnion rupture sequence[a]	Cleft lip and palate, oblique facial clefts, focal areas of scalp aplasia, constriction bands with terminal limb amputations and syndactylies, occasional anencephaly, encephalocele, and ectopia cordis	Encephalocele, oropharyngeal/ airway deformation	217100

[a]Potential ICU issues.
ICU, Intensive care unit; *OMIM*, online mendelian inheritance in man.

TABLE 88.2 Craniosynostosis Syndromes and Potential Airway Compromise

Syndrome	Key Features	Tracheal Abnormalities	Midface Hypoplasia	OMIM
Apert syndrome[a]	Craniosynostosis (coronal > lambdoid > sagittal), acrobrachycephaly (steep, wide forehead and flat occiput), proptosis, hypertelorism, exotropia, trapezoid-shaped mouth, prognathism, invariable symmetric syndactyly of hands and feet, variable elbow fusion, cognitive impairment, narrow palate with lateral palatal swellings, widely patent sagittal suture connecting anterior and posterior fontanels	Tracheoesophageal fistula, tracheal cartilaginous sleeve less common	Significant maxillary hypoplasia, obstructive sleep apnea syndrome	101200
Crouzon syndrome[a]	Craniosynostosis (coronal > lambdoid > sagittal), brachycephaly, prognathism, exophthalmos, papilledema, hypermetropia, divergent strabismus, atresia of auditory canals, Chiari type 1 malformation and hydrocephalus	Solid cartilaginous trachea or tracheal cartilaginous sleeve	Significant maxillary hypoplasia, obstructive sleep apnea syndrome	123500, 612247
Pfeiffer syndrome types I, II, and III[a]	Craniosynostosis (coronal > sagittal > lambdoid), brachycephaly, hypertelorism, proptosis, broad first digits with radial deviation, variable syndactyly and elbow fusion, cloverleaf skull	Solid cartilaginous trachea or tracheal cartilaginous sleeve	Significant maxillary hypoplasia, obstructive sleep apnea syndrome	101600
Muenke syndrome	Unilateral or bilateral coronal craniosynostosis, brachydactyly, downslanting palpebral fissures, thimble-like middle phalanges, coned epiphysis, carpal and tarsal fusions, sensorineural hearing loss, Klippel-Feil anomaly		Mild maxillary hypoplasia, no airway compromise anticipated	602849
Saethre-Chotzen syndrome[a]	Unilateral or bilateral coronal craniosynostosis, acrocephaly, brachycephaly, low frontal hairline, hypertelorism, facial asymmetry, ptosis, characteristic ear (small pinna with a prominent crus), fifth finger clinodactyly, partial 2–3 syndactyly of the fingers, duplicated halluces		Maxillary hypoplasia	101400
Carpenter syndrome	Craniosynostosis (coronal > lambdoid > sagittal), hypertelorism, proptosis, brachycephaly, brachydactyly, preaxial polysyndactyly, intellectual disability		Maxillary hypoplasia	201000
Jackson–Weiss syndrome	Craniosynostosis (coronal), acrocephaly, hypertelorism, proptosis, midface hypoplasia, radiographic abnormalities of the foot including fusion of the tarsal and metatarsal bones, 2–3 syndactyly, broad short first metatarsals and broad proximal phalanges		Maxillary hypoplasia	123150

[a]Significant risk of airway morbidity.
OMIM, Online mendelian inheritance in man.

• **Fig. 88.1** (A) Infant with Robin sequence and significant micrognathia. (B) U-shaped cleft palate. (C) Infant with Robin sequence and a nasopharyngeal tube in place.

The principal physiologic sequelae of RS are the inability to effectively feed and breathe due to airway obstruction. In the immediate neonatal period, patients with RS may have increased inspiratory work of breathing, cyanosis, and apnea. Rising CO_2 levels may be a signal of worsening airway obstruction and often precedes hypoxemia in the neonate with RS.[6] Obstruction is more common in the supine position and can be exacerbated during feeding and in sleep or in any state where there is loss of pharyngeal tone. Chronic obstruction can lead to failure to thrive, carbon dioxide retention, pulmonary hypertension, and eventually right-sided heart failure (cor pulmonale). Airway exposure is often compromised in the infant with RS, which impacts the ability to safely intubate the neonate with RS.[7]

Airway obstruction is the main cause of feeding and growth issues in infants with RS. Feeding problems can also be related to abnormal coordination, primary swallowing dysfunction, pharyngeal hypotonia, and suction mechanics are complicated by the presence of a cleft palate. Increased energy expenditures because of the increased work of breathing may lead to failure to thrive if the infant is not receiving adequate caloric intake. Gastroesophageal reflux is common in infants with RS, as it is in other infants who have increased work of breathing, and may contribute to episodes of distress and aspiration or apnea.

Management

First and foremost, the airway must be addressed. Placement of a nasopharyngeal (NP) airway or endotracheal tube may be required in an emergency, and it is important to realize that severe, life-threatening airway obstruction can present in the delivery room. RS features are not commonly noted before birth; however, if microgathia or maternal polyhydramnios is a prenatal concern, there should be heightened suspicion for worse airway obstruction. Although uncommon, a prenatal diagnosis of micrognathia allows the involvement of neonatologists and otolaryngologists before and during delivery.

• Box 88.1 Key Members of a Multidisciplinary Craniofacial Team

These are the core members of the craniofacial team that follow a neonate through early adulthood. Each team has slightly different core and ancillary members, and frequently includes other specialists guided by patient-specific needs.

Typical Core Disciplines

- Audiology
- Dentistry
- Feeding and Nutrition Specialist
- Genetics
- Neurosurgery
- Nursing
- Oral Surgery
- Orthodontics
- Otolaryngology
- Pediatrics
- Plastics and Craniofacial Surgery
- Social Services
- Speech and Language Pathology

Other ancillary but important disciplines that are frequently consulted depending on specific patient needs: Child Life, Cardiology, Gastroenterology, Neurodevelopmental Medicine, Ophthalmology, Psychology, Pulmonology, and Sleep Medicine

Key members of the craniofacial team are shown in Box 88.1. Treatment protocols differ across institutions,[8] and an example of the initial evaluation and clinical team discussion for the neonate with tongue-based airway obstruction is provided in Box 88.2. While the threshold for intervention and the management options differ substantially, most neonates with RS can be treated nonsurgically. A number of therapeutic maneuvers can be used to stabilize

• Box 88.2 Evaluation and Decision Making for Neonates With Tongue-Based Airway Obstruction

Initial Evaluation in the Neonatal ICU

- Physical examination (supine vs. prone): attention to craniofacial features, respiratory status, cardiac and limb differences
- Evaluation for presence of glossoptosis, stertor, obstructive apnea, and work of breathing
- Capillary blood gas and total CO_2 level
- Oxygen saturation monitoring
- Growth parameters
- Dysmorphology evaluation
- Craniofacial and otolaryngology consultations
- Consider genetics evaluation if there are multiple anomalies or a concerning family history (micrognathia, cleft palate, childhood hearing loss/myopia/joint problems)
- Consider airway endoscopy (guided by airway severity and response to interventions)
- Consider airway imaging (guided by airway severity and response to interventions)

Multidisciplinary Team Treatment Discussions May Address

- Does the patient need escalation in care to treat airway obstruction?
- Have appropriate subspecialty consults and evaluations been obtained? (Varies by institution, but can include specialists with expertise in neonatal intensive care, craniofacial and pediatric care, airway evaluations, airway surgery, jaw surgery, parent/family support)
- Should the patient undergo CT imaging to assess the craniofacial bony anatomy, level(s) of airway obstruction, and candidacy for MDO (if so, when and how to proceed safely)?
- Has the distal part of the airway been evaluated to look for other levels of airway obstruction?
- Does the patient need a tracheostomy tube, or is he/she a candidate for mandibular distraction?
- What is the family and social context?
- What will the disposition be once airway has been stabilized?

CT, Computed tomography; *ICU*, intensive care unit; *MDO*, mandibular distraction osteogenesis.

the upper airway in RS, ranging from positioning to surgery. Placing the baby in the prone or lateral decubitus position can improve airway patency to some degree, and has the potential to decrease work of breathing.[9] When prone positioning fails to stabilize the airway, alternative approaches include the use of an NP airway, intraoral device such as the Tubingen palatal plate (TPP) or orthodontic airway plate (OAP), noninvasive positive pressure, treatment with tongue–lip adhesion (TLA), and mandibular advancement through distraction osteogenesis. An NP airway provides a temporary way to bypass the infant's airway obstruction (see Fig. 88.1C). An endotracheal tube can be modified so that it can be passed through the nares into the hypopharynx above the epiglottis, bypassing the obstruction at the base of the tongue.[10] The NP airway can be both diagnostic of isolated base of tongue level airway obstruction, and therapeutic, and in some institutions, the infant is discharged home with an NP airway in place.[11] Infants are monitored with oximetry, and parents are taught NP airway suctioning and replacement. The TPP or OAP is a newer therapy in the United States but well established in Europe. This intraoral device can bring the tongue forward to improve airway patency in neonates and infants, allow for full oral feeding, and safe discharge home. Airway compromise and stability are assessed by physical examination, CO_2 levels, oxygenation, overnight sleep studies, and growth, monitored over time.[12,13] While trending oxygen and CO_2 levels is considered the minimum assessments for RS,[14] some centers recommend sleep studies routinely to aid in decision making and to assess the success of interventions.[15] Improved infant normative sleep data, access to quality sleep studies, and understanding long-term outcomes will impact approaches to neonates at risk for early obstructive sleep apnea.

The infant's clinical status, a perceived need for long-term respiratory support, and failure of less invasive interventions will determine whether invasive surgery is recommended.[16] Tracheotomy is considered a gold standard to bypass severe tongue-based airway obstruction, and the preferred option for infants who are not candidates for less invasive treatments, for example, those with multilevel airway obstruction and those who need longer-term mechanical ventilation. However, other surgical interventions may avoid a tracheostomy tube.

Children with isolated airway obstruction at the base of the tongue without other medical comorbidities may be considered for mandibular distraction osteogenesis (MDO).[17,18] The surgery consists of surgical osteotomy and placement of a distraction device to slowly increase mandibular length and bring the base of the tongue forward, thereby increasing the airway space. This procedure will not achieve respiratory stabilization in patients with concomitant airway anomalies, lung disease, central apnea, or the need for positive pressure ventilation. In some institutions, TLA may be a temporizing measure to reduce base of tongue-level obstruction while allowing for mandibular growth.[19]

Airway endoscopy helps to delineate the level of obstruction, and computed tomography (CT) of the facial skeleton provides optimal understanding of jaw anatomy and tooth bud position before MDO. For many infants with RS needing an ICU, the patterns of obstruction are more complex. In addition to glossoptosis, other mechanisms may contribute to airway obstruction in individuals with RS, such as pharyngeal hypotonia and/or compromised airway clearance in the infant with a concomitant neurological disorder. Recognition of other causes of respiratory compromise, for example, poor secretion handling, laryngotracheomalacia, or ventilatory muscle weakness, affects treatment decisions. Children with RS associated with syndromes, skeletal dysplasia, or neurologic conditions may have multiple causes of respiratory compromise such that a tracheostomy may be the best approach to alleviate respiratory compromise. Thus infants with RS who have airway obstruction unresponsive to positional techniques for whom surgical options are being considered should have a comprehensive airway evaluation as well as a diagnostic evaluation for an underlying syndrome or associated malformations that might impact respiratory status and response to therapies. The multidisciplinary approach and considerations of all therapeutic options and potential outcomes should be considered for the neonate with RS requiring airway escalation.

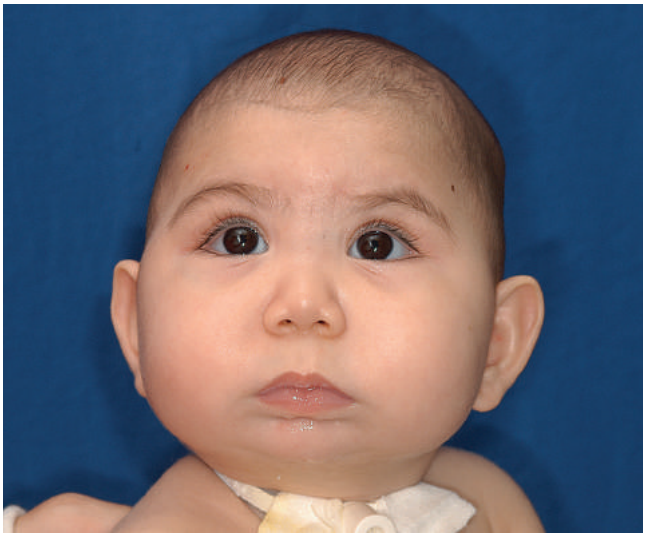
Nutrition can be maintained with a fortified breast milk or formula given by side-lying feeding using a cleft feeder, or via a feeding tube; placement of a surgical gastrostomy tube is more common among infants with a syndromic form of RS.[20] Oral feeding can and should be introduced when the airway is stable, and consultation with a feeding therapist is crucial. As tone and tongue position improve, and growth ensues, swallow coordination and safe feeding can also improve. A formal swallow evaluation may be helpful for the infant with persistent feeding challenges. Close observation for symptoms of gastroesophageal reflux with proactive treatment to prevent reflux and aspiration should also be considered.

Genetics consultation is recommended, as identification of an associated syndrome will have implications for treatment decisions and additional screening.

Screening and Surveillance

Syndrome diagnoses may become more apparent over time, and reassessments investigating a unifying diagnosis should be continued as the child with RS grows.[21] Associated anomalies can impact respiratory function, including skeletal dysplasias. CNS anomalies and hypotonia will impact care needs and prognosis. Congenital heart defects are present in up to 25% of babies with RS who die in early infancy.[22] It has been reported that a portion of individuals with RS experience developmental delay, cognitive impairment, and poorer school achievement.[23] Overall morbidity and mortality are higher in syndromic RS, RS plus, and RS with associated neurological anomalies compared with isolated RS.[24] Diagnostic work-up should include investigation of common associated anomalies and syndromes.[25,26] Specific genetic and syndrome diagnosis will guide surveillance protocols, but for all infants and children with RS, we recommend:

- An eye exam in the first 6 months of life to evaluate for ocular features of Stickler syndrome
- Hearing assessment annually, more frequently if hearing loss is detected
- Close monitoring of development and referral to early intervention services for developmental assessment, monitoring, and support
- Monitoring for obstructive sleep apnea, with a low threshold for a sleep study referral
- Monitoring dental eruption, occlusion and facial growth over time; most children will benefit from orthodontic management, and some will be candidates for mandibular or bimaxillary advancement surgery in adolescence



• **Fig. 88.2** Infant with Stickler syndrome, showing a flat face, depressed nasal bridge, and epicanthal folds. This infant also has Robin sequence and required tracheostomy.

Stickler Syndrome

The most common syndrome associated with RS is Stickler syndrome (SS). Approximately one-third of individuals with RS will have Stickler syndrome.[21] Stickler syndrome is most commonly an autosomal dominant (with variable expressivity) connective tissue disorder with ophthalmic, orofacial, auditory, and articular manifestations.[27] SS may present with a wide range of findings, including RS, cleft palate without RS, hearing loss, or early onset osteoarthritis. Ocular forms of SS can present with congenital high myopia, cataracts, and risk for retinal detachment. Midface hypoplasia in SS can produce a flat and occasionally concave facial profile, and other facial features can include a depressed nasal bridge, short nose, anteverted nares, micrognathia, telecanthus, and epicanthal folds (Fig. 88.2). Hearing loss can be sensorineural with increasing prevalence with age (most common) with or without conductive hearing loss. Skeletal features associated with some forms of SS include early-onset arthritis, joint hypermobility, scoliosis, and kyphosis.[24,28]

The diagnosis of Stickler syndrome should be considered in any neonate with RS or a cleft palate, especially when associated with myopia or hearing loss. Spondyloepiphyseal dysplasia is not usually apparent in the newborn period. Mutations affecting multiple collagen genes have been associated with Stickler syndrome, and clinical molecular testing by sequence analysis is sensitive and available. More than 90% of individuals with Stickler syndrome are found to have a mutation in either *COL2A1* or *COL11A1*.[27] The diagnosis should also be considered in any newborn with a family history of RS or SS features.

In addition to appropriate management of feeding, breathing, and growth (as described for RS), management of Stickler syndrome includes active detection of the ocular features of the syndrome, as the associated risk of retinal detachment and blindness are preventable. An initial ophthalmologic evaluation is recommended for all children with RS aged between 6 and 12 months or at the time of a definitive molecular diagnosis of Stickler syndrome and then routine surveillance thereafter.

Orofacial Clefts

Orofacial clefts of the primary and secondary palate are among the most common congenital anomalies. Classified as either cleft lip with or without cleft palate (CL±P) or cleft palate only (CPO), these two phenotypes are thought to be distinct in origin. On an average day in the United States, 17 infants are born with an orofacial cleft,[29] and prevalence varies by phenotype (Table 88.3).

TABLE 88.3 Orofacial Clefting Prevalence and Relative Risk for Recurrence

Phenotype	Prevalence[29]	Babies Affected per Year in the United States[29]	Relative Risk for Recurrence for Offspring (%)[40]	Relative Risk for Recurrence for a Subsequent Sibling (%)[40]
Cleft lip with cleft palate	1 in every 1563 births	2518	4.1	4.6
Cleft lip without cleft palate	1 in every 2807 births	1402	3.5	2.2
Cleft palate	1 in every 1687 births	2333	4.2	3.3

Diagnosis and Etiology

Cleft lip and palate is the most common type of orofacial clefting, followed by cleft lip, then CPO. Less prevalent are atypical clefts (macrostomia or lateral cleft, Tessier or oblique, and midline clefts). Unilateral CL±P is more common than bilateral involvement.[1] A bifid uvula can be a normal variant, found in 2% to 4% of births, but can also be a sign of an associated submucous cleft palate, which can have the same functional impact as an overt CP.[30]

The causes of most orofacial clefts are unknown and are nonsyndromic (isolated) in 70% to 75% of infants with CL±P and approximately 55% of those with CPO.[31,32] Neonates with orofacial clefting who are born prematurely or have low birth weight may have a higher incidence of associated congenital malformations.[33] Racial and ethnic variation in the prevalence of clefts has been described. In the US, rates are closest to those of the area from which the population originated[34] with the highest prevalence of CL±P found in Native Americans, followed by whites and Hispanics, and the lowest overall prevalence of CL±P demonstrated in African Americans.[35] The cause of nonsyndromic clefts is complex and multifactorial, likely resulting from an interaction between environmental and genetic factors. Known environmental risk factors include maternal tobacco and alcohol use, anticonvulsant treatment, and nutritional status.[34,36] There is some evidence showing a protective association with preconception folate supplementation in preventing nonsyndromic orofacial clefts.[37–39] Although many candidate genes have been described, in the absence of a family history of cleft or lip pits, routine clinical genetic testing for a child with isolated CL±P is not recommended. Recurrence risk information for the parents of a child with CL±P or for the affected individual depends upon either the specific syndrome/genetic diagnosis or the empiric risks for those with nonsyndromic clefts. Recurrence risk for nonsyndromic clefts differs based on the cleft phenotype and the number of affected individuals in a family (see Table 88.3).[40]

Anatomy

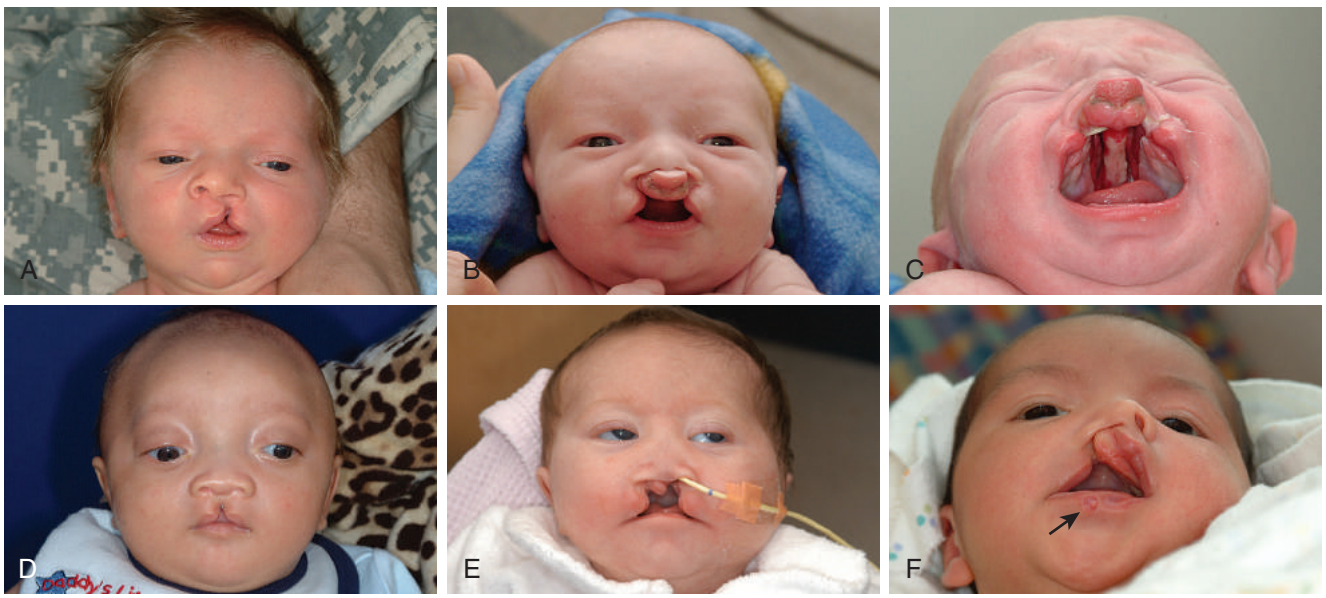
Embryologic development of the primary palate begins early in gestation, and the upper lip and primary palate have usually fused by the seventh week of gestation. A failure of fusion of the medial and lateral nasal processes with the maxillary process produces CL±P. Clefts can affect the primary palate (lip, alveolus, or anterior portion of the hard palate that extends to the incisive foramen) and secondary palate (posterior hard palate and soft palate). Clefts of the primary and secondary palate can be unilateral or bilateral and complete or incomplete. A complete cleft of the primary palate leaves no residual tissue between the alar base and the lip, whereas an incomplete cleft does not extend through the floor of the nose (Fig. 88.3A–C, F). A submucous cleft palate is a defect in the musculature of the palate with intact overlying mucosa.

Phenotype

The cleft of the primary and secondary palate affects facial shape and growth (see Fig. 88.3A–C). Children with cleft palate (CP) are at increased risk of eustachian tube dysfunction, recurrent otitis media, acquired hearing loss, as well as speech issues in childhood. Feeding difficulties, nasal regurgitation of feeds, and difficulty gaining weight may also occur in infants with a CP (submucous and overt clefts of the palate). Associated dental findings include hypodontia and natal teeth.

Lateral facial clefting or macrostomia is pathogenically distinct from isolated CL±P and is often associated with syndromes, including craniofacial microsomia and Treacher Collins syndrome. Amniotic rupture sequence can be associated with oblique facial clefts and may be associated with underlying central nervous system (CNS) malformations and transverse limb anomalies.

A true median cleft of the upper lip is the rarest type of facial cleft (see Fig. 88.3D). Midline clefts can be associated with other congenital defects as can be seen in orofaciodigital syndrome and frontonasal dysplasia, and CNS malformations are common in



• **Fig. 88.3** (A) Infant with a unilateral incomplete cleft lip. (B, C) Infant with bilateral complete cleft lip and palate. (D) Infant with midline cleft and hypertelorism. He also has a frontonasal encephalocele. (E) Infant with premaxillary agenesis and holoprosencephaly. (F) Infant with Van der Woude syndrome with unilateral complete cleft lip and a lip pit (*arrow*).

children with midline clefts. Some midline clefts are not true clefts but represent hypoplasia or agenesis of the primary palate or premaxillary agenesis, which can be associated with holoprosencephaly (HPE) sequence (see Fig. 88.3E). Infants with HPE often have a depressed nasal tip and a short columella and appear hypoteloric (compared with FND or frontonasal encephalocele, where a midline cleft may be present, but the infant has a broad nasal tip, wide columella and hypertelorism).

Orofacial clefts are rarely associated with clefting of airway structures, such as cleft larynx or extension of clefting into the trachea. Opitz G/BBB syndrome is a multiple congenital anomaly syndrome characterized by facial anomalies (100% are hypertelorics and 50% have CL±P), genitourinary abnormalities (90% have hypospadias), and laryngotracheoesophageal (LTE) defects (present in 70%).[41] Autosomal dominant and X-linked recessive forms of Opitz G/BBB syndrome are recognized. Pallister–Hall syndrome (PHS) is characterized by a constellation of findings that include hypothalamic hamartoma (resulting in seizures and pituitary dysfunction), polydactyly, airway clefting, and other anomalies (genitourinary, renal, pulmonary, and imperforate anus). Bifid epiglottis is the most common airway manifestation in PHS, although LTE clefts have been reported. LTE defects may range from LTE dysmotility in mild forms to laryngeal or tracheoesophageal clefts in more severe forms.

Syndromes Associated With Cleft Lip and/or Palate

It is estimated that there are more than 400 syndromes associated with orofacial clefts.[22] Associated malformations occur in about 30% of children with CL±P.[42] In considering a diagnosis of a syndrome, one should categorize the type of cleft (CL±P, U-shaped or V-shaped cleft palate, or more atypical orofacial cleft) and look for any other malformations. Table 88.1 describes the syndromes most commonly associated with clefting, their key features, and potential ICU issues. A referral to a clinical geneticist is recommended when an underlying diagnosis is suspected.

Intensive Care Unit Concerns

Most infants with CL±P do not require ICU care. Thus an infant with an apparently isolated cleft who develops significant respiratory or electrolyte abnormalities requiring ICU care should be considered syndromic until proven otherwise. In these infants, a genetics consultation should be pursued.

The newborn with a midline cleft or premaxillary agenesis is at risk of serious underlying CNS anomalies, including HPE. In the presence of HPE, the detection of associated medical issues is essential. Endocrine abnormalities can arise because the midline malformation affects the development of the hypothalamus and the pituitary gland. Clinical manifestations include growth hormone deficiency, adrenal hypoplasia, hypogonadism, diabetes insipidus, and thyroid deficiency. Neurologic manifestations warrant close attention, including seizures, hypotonia, spasticity, autonomic dysfunction, and developmental delays.

With an LTE cleft, there is communication between the airway and the esophagus, allowing tracheal aspiration of oral contents, including saliva and feeds. Clefting of the larynx may result in stridor, a hoarse cry, respiratory distress, swallowing dysfunction, feeding difficulties, regurgitation, aspiration, hypoxia, recurrent pneumonias, and eventually severe respiratory compromise if unrecognized. An infant boy with hypertelorism, hypospadias, orofacial clefting, and symptoms of airway obstruction or aspiration should be evaluated for Opitz syndrome. Infants with PHS may also have respiratory distress due to airway clefting, as well as other potentially life-threatening clinical manifestations such as seizures and severe panhypopituitarism. Genetic evaluation and consideration of molecular testing for Opitz syndrome and PHS can be coordinated through a geneticist.

Management

The specifics of management of orofacial clefting are center-specific. Because of the potential impact of the orofacial cleft on breathing, eating, hearing, speech, facial growth, and dental health, it is recommended that infants and children with clefts be referred to a multidisciplinary care team for long-term management. Infants cared for with a multidisciplinary cleft or craniofacial team have better long-term functional and aesthetic outcomes.[42] The nearest cleft team may be found through the American Cleft Palate-Craniofacial Association (ACPA) team listings. Overviews of recommended team care for patients with cleft lip/palate can be accessed electronically.[43,44]

On the initial assessment, the provider should assess the cleft and examine the infant for dysmorphic features and other anomalies. Hearing should be evaluated by evoked otoacoustic emissions or by brainstem auditory evoked response if the newborn does not pass the initial hearing screen.[45] Although this finding is often attributed to middle ear effusion because of the high prevalence of middle ear disease in children with CP, the incidences of sensorineural hearing loss, conductive hearing loss, and mixed hearing loss are higher in children with clefts.[46] A neonate with a complete cleft lip should be evaluated by a craniofacial or cleft team in the first 2 weeks of life, and some centers offer taping or presurgical molding (such as nasoalveolar molding) that can be initiated in early infancy. Many mothers will be able to breastfeed an infant born with an isolated cleft lip. Breastfeeding a baby with CP (with or without cleft lip) will prove extremely challenging because the open palate will not generate the negative pressure needed for sucking. Thus the mother of infants with CP with or without cleft lip should be encouraged to provide expressed breast milk with the use of a specialized cleft feeder. Lactation counselor support should be offered to all mothers to discuss feeding at the breast or pumping to provide expressed breast milk to the infant. A variety of cleft nipples/bottles exist to allow oral feeding (http://www.cleftline.org/who-we-are/what-we-do/feeding-your-baby/). There are assisted milk delivery systems such as the Medela special needs feeder (formerly known as the Haberman) and the Mead Johnson squeeze bottle. There are also infant-driven systems, such as the Dr. Brown's specialty feeding system (with valve and varied nipple sizes allowing flow variation) and the Pigeon system. Infants with CP tend to swallow more air during feedings and should feed in an upright position, as gravity will help prevent nasal regurgitation. If the child is still having difficulty feeding safely or efficiently, a feeding therapist should be consulted. If a feeding specialist is not available, a lactation counselor or the nearest ACPA Cleft/Craniofacial team's nurse coordinator can be an additional helpful resource for feeding support.

Adequate weight gain is important for overall health, development, and readiness for the surgical procedures that occur in the first year of life. Newborns with clefts are considered nutritionally high risk, but a child with an isolated orofacial cleft should be expected to follow typical growth charts. Infants with suboptimal weight gain may require additional nutrition support from a

dietitian to help determine caloric needs and to closely monitor growth.

Surgical timelines and approach differ between teams but often span from infancy into early adulthood. In general, surgery to repair the cleft lip and associated nasal deformity occurs within the first 6 months of life. Palatoplasty typically occurs between 9 and 12 months of age with the primary goal to normalize palate muscle function to facilitate normal speech development.

Newborns with orofacial clefting should have a follow-up with their primary care pediatrician and be evaluated by a cleft/craniofacial specialist as soon as possible after discharge from the birth and NICU hospitalization, ideally within 1 week from discharge.

Screening and Surveillance

Routine screening laboratory and imaging studies are not typically recommended in the neonate with an isolated cleft. For children with syndromes, surveillance is guided by syndrome-specific protocols, with some special considerations noted here:

- Although rare, airway or laryngeal clefts can cause respiratory distress, coughing, choking, stridor, recurrent croup, and recurrent aspiration. Recommended evaluations include a clinical swallow evaluation, videofluoroscopy, functional endoscopic evaluation of swallow, and the gold standard for diagnosis is microlaryngoscopy and bronchoscopy. Given the risk of gastrointestinal manifestations such as gastroesophageal reflux, dysmotility, and aspiration, anti-reflux precautions should be initiated in infants with suspected or confirmed LTE defects. Early diagnosis and proper repair of the laryngeal cleft are essential to prevent injury to the lungs. Significant LTE defects will need to be managed surgically,[47] and tracheostomy may be necessary initially to ensure airway stability and safety.
- In the presence of a midline cleft, it is important to evaluate the neonate for underlying CNS malformations such as HPE. In any child with a midline cleft or facial features consistent with premaxillary agenesis/hypoplasia, CNS imaging (CT or MRI) is recommended. Consultation with a geneticist or genetic counselor may provide insight into the genetics, molecular testing options, and recurrence risk of HPE. Treatment of HPE is supportive and based on symptoms. The outcome depends on the severity of HPE and the associated medical and neurologic manifestations.

22q11.2 Deletion Syndrome

Diagnosis and Etiology

22q11.2 deletion syndrome (22q11.2DS) is the most common microdeletion syndrome, with an estimated prevalence of 1 in 4000 births, in which affected individuals are missing a region (typically 3 Mb, encompassing approximately 40 genes) on one copy of chromosome 22.[48,49] 22q11.2DS is associated with more than 180 clinical features, and phenotypic variation is a hallmark of this genetic condition.[49] In some cases, this condition is diagnosed prenatally. Testing may occur as part of the evaluation for fetuses with congenital heart disease or because of a parental history of 22q11.2DS. The clinical indications for genetic testing for this condition in neonates include congenital heart malformations (particularly conotruncal anomalies), hypocalcemia, dysphagia, CP, other palatal dysfunction (e.g., submucous CP, velopharyngeal insufficiency with intact palate), and immunodeficiency identified on newborn screening or by noting thymic hypo-/aplasia, such as during heart surgery.[50] Overt CP is less common than submucous CP and velopharyngeal insufficiency; genetic testing is more definitively indicated for CP when other features associated with 22q11.2DS are also observed.

Phenotype

22q11.2DS commonly presents with multiorgan system involvement, including cardiac and palatal abnormalities, immune differences, endocrine and gastrointestinal problems, developmental delay, and later-onset conditions across the life span, including variable cognitive deficits and psychiatric illness.

Several craniofacial features have been observed in individuals with 22q11.2DS; however, many of these are subtle and may not be apparent in the newborn period. Common features identified include small ears with overfolded helices, a long face, tubular conformation of the nose, nasal alar hypoplasia, and hooded eyelids.[50] In neonates, some of the most indicative findings include dysphagia and/or nasal regurgitation (including in the absence of an overt CP, due to palatal dysfunction), congenital heart disease, and hypocalcemia.

Intensive Care Unit Concerns

About two thirds of patients with 22q11.2DS have congenital heart disease, sometimes severe, which often leads to prolonged neonatal hospital stays. If a seizure occurs in the neonatal period, especially in the setting of known congenital heart disease, 22q11.2DS and hypocalcemia should be strongly suspected. Hypocalcemia is most common in the newborn period and is triggered by physiologic stressors (e.g., peripartum period, surgery, infection). Importantly, hypocalcemia and neonatal seizures caused by it have been linked with worse intellectual outcomes for patients.[51] Feeding challenges can be due to cleft palate, palatal dysfunction, and dysphagia. Rarely, severe immunodeficiency can be present, increasing the risk for serious infections. It can be identified on newborn screening for T-cell receptor excision circles (TRECs).[52] About one-third of patients with 22q11.2DS have structural urinary tract abnormalities. Cervical spine anomalies can occur; routine screening in infancy is not recommended, but neonates should be monitored for symptoms of cord compression and cervical spine instability.[48] In addition, infants with 22q11.2DS often have airway obstruction, most commonly due to tracheomalacia, subglottic stenosis, laryngomalacia, glottic web, and bronchomalacia; this is most commonly observed in patients who also have congenital heart disease.[53]

Management

Chest x-ray, EKG, echocardiogram, and cardiology consultation should be pursued in suspected and confirmed cases of this condition, and 22q11.2 deletion testing should be considered in cases of confirmed congenital heart disease.[54] Calcium and parathyroid levels should be checked, as the neonatal period is the most common time for hypoparathyroidism to present itself.[48,51] A complete blood count, screening for leukopenia and thrombocytopenia, and flow cytometry for T and B cells should be obtained. An immunologist should be consulted if any concern arises for abnormalities in these studies, newborn screening, or clinical suspicion of hypo- or athymia. Renal ultrasonography should be obtained for all suspected and confirmed cases.[48] Newborns should have a palatal examination to evaluate for overt or submucous CP, as

well as a diagnostic hearing test. Infants with evidence of dysphagia, regardless of the presence/absence of CP, benefit from an evaluation by a feeding therapist to determine if a swallow study is needed and/or if other feeding interventions, such as the introduction of a specialized cleft feeder, would be helpful. Families of infants diagnosed with 22q11.2DS should receive genetic counseling. Ophthalmology evaluation is warranted for all confirmed diagnoses, as well. Thyroid function should be assessed with newborn screening.

Screening and Surveillance

Long term, children with 22q11.2DS benefit from a multi-disciplinary team, often including pediatrics, cardiology, immunology, behavioral health, psychiatry, feeding therapy, speech, social work, nursing, audiology, and endocrinology. In addition, other subspecialties may need to be involved, depending on which chronic issues are present, such as constipation, urologic abnormalities, cervical spine instability, and scoliosis. Some children's hospitals and academic health centers include dedicated 22q11.2DS clinics. Many infants and toddlers with 22q11.2DS have persistent dysphagia and swallowing problems, and it is not uncommon for them to depend on supplemental tube feedings for months or years. As they get older, medical issues often stabilize, and the focus shifts to understanding and supporting learning differences, behavioral health, and mental health. Older children and young adults are at an increased risk for psychiatric disorders, including depression, anxiety, ADHD, and schizophrenia.

Craniosynostosis

Diagnosis and Etiology

Craniosynostosis refers to the premature fusion of one or more cranial sutures (metopic, sagittal, right or left coronal, right or left lambdoid) that normally separate the bony plates of the cranium. The birth prevalence of all craniosynostoses has been estimated at 1 in 2500 live births, with shifting epidemiology and a more recent study estimating an increase of prevalence to 1 in 1400 live births.[55,56]

Typically, patent sutures allow the calvaria to expand as the brain grows, producing a normal head shape and size. If one or more sutures fuse prematurely, this typically happens prenatally, and there is restricted growth perpendicular to the fused sutures and compensatory growth in the patent sutures, producing a progressively abnormal head shape.[57] Physical exam by an experienced craniofacial provider can be sufficient for the diagnosis, and a CT scan is typically needed to confirm the extent of synostosis and for surgical planning. Plain skull radiographs in neonates are unreliable and not helpful.[58] Craniosynostosis is a heterogeneous disorder with health consequences that range from an abnormal head shape and increased intracranial pressure (ICP) to secondary visual and intellectual impairments.

The causes of craniosynostosis are heterogeneous, with monogenic, chromosomal, polygenic, and environmental/teratogenic factors all playing key roles. A genetic diagnosis can currently be identified in 25% of individuals with craniosynostosis. Nonsyndromic single suture craniosynostosis accounts for 65% of patients.[59] Syndromic craniosynostosis may involve single or multiple fused sutures, additional anomalies (such as limb, cardiac, CNS, and tracheal malformations), and developmental delay. Multiple suture involvement is usually considered hereditary even when it does not fit a classic pattern of anomalies. Advances in high-throughput DNA sequencing have led to the identification of causative variants and genetic pathways in these relatively common congenital anomalies.[60,61] Our understanding of the genetic causes of craniosynostosis is increasing, and for the growing proportion of syndromic forms, identification of the primary genetic cause is possible with the use of clinically available genetic tests.

Single Suture Synostosis

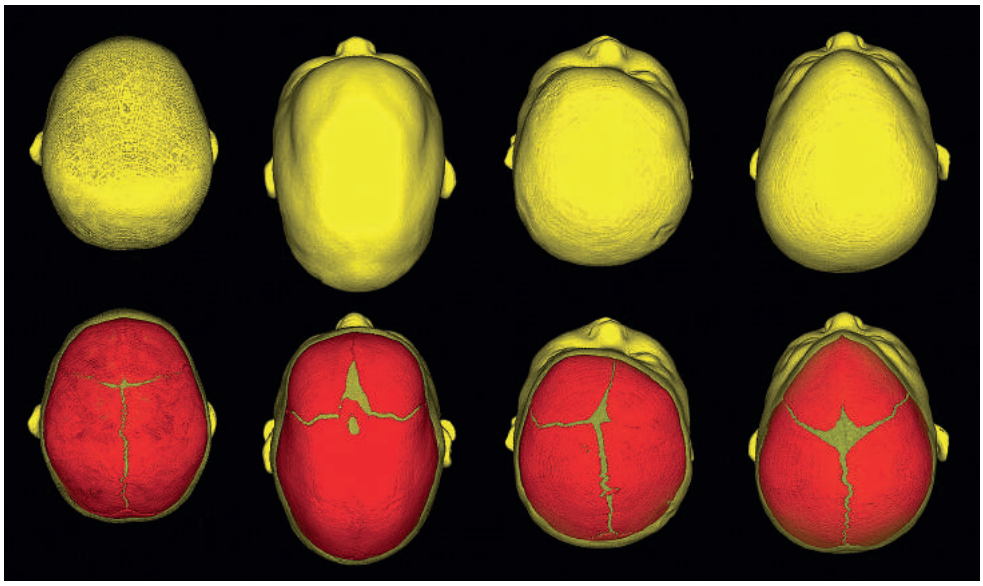
There is a concern that children with untreated single suture synostosis are at risk for elevated ICP, local brain injury, and later developmental delays. For this reason, early recognition and referral are thought to be key to devising optimal treatment plans to protect the developing brain.[62]

Sagittal synostosis is the most common single suture synostosis (approximately 60%).[63] Known risk factors include male sex, intrauterine head constraint, twin gestation, maternal thyroid hormone dysregulation, and maternal smoking. Uncommon but reported associated anomalies include congenital heart defects and genitourinary tract malformations. Syndromes with synostosis involving only the sagittal suture are rare. Premature union of the sagittal suture hinders normal calvarial expansion, leading to scaphocephaly, an elongated, narrow calvarium, decreased biparietal width, frontal bossing, and occipital elongation (Fig. 88.4). Premature fusion of the suture before birth leads to abnormal head shape in the newborn period. A breech-positioned neonate can have dolichocephaly that may mimic sagittal synostosis. However, in sagittal synostosis, frontal bossing and biparietal narrowing progress, whereas the head shape in a breech-positioned infant will begin to normalize in the first months of life.

Metopic synostosis has become increasingly common, representing approximately 20% to 30% of single suture synostosis. Risk factors include male sex, twin gestation, and in-utero exposure to valproate.[64] Syndromes, associated anomalies, and chromosomal abnormalities occur in approximately one-quarter of individuals with metopic synostosis.[65,66] Premature fusion of the metopic suture results in a triangular head shape, or trigonocephaly, and additional features including a midline forehead ridge, frontotemporal narrowing, pterion constriction, hypotelorism, and an increased biparietal diameter (see Fig. 88.4). Isolated metopic ridging is common in infancy, does not distort forehead shape, and is not associated with metopic synostosis.

Coronal synostosis represents about 10% to 20% of single suture synostosis and presents with anterior plagiocephaly. Recognizable skull differences in unicoronal craniosynostosis include a flat supraorbital rim and orbit that appears higher on the affected side, with a frontal bulge on the contralateral side (see Fig. 88.4). In addition to orbital and frontal asymmetry, the nose often twists away from the coronal fusion. Genetic syndromes are more frequently seen in individuals with coronal synostosis, including Saethre-Chotzen syndrome, Muenke syndrome, and craniofrontonasal dysplasia. All families of children with coronal synostosis should be offered genetic consultation and/or genetic testing to include *FGFR2, FGFR3, TWIST1*, *TCF12*, and *EFNB1* on the basis of clinical examination.

Lambdoid synostosis (1% to 3% of single suture craniosynostosis) is the least common form of single suture synostosis. It is characterized by flattening of the ipsilateral occiput, posterior–inferior displacement of the ear, bulge of the mastoid process on the fused side, a skull base tilted downward on the affected side, and may exhibit facial scoliosis or asymmetry. This head shape is



• **Fig. 88.4** Head shapes in single suture synostosis. *From left to right:* normal head shape, sagittal synostosis, right coronal synostosis, and metopic synostosis.

often confused with positional deformational plagiocephaly, but skull base tilt and vertical ear displacement should not be present in positional plagiocephaly.

Multiple Suture Synostosis

Multiple suture (or *multisuture*) *synostosis* describes patients who have two or more fused sutures. Although children with multisuture synostosis are more likely to have a known syndromic form of craniosynostosis such as Apert, Crouzon, Pfeiffer, or Muenke syndromes, some have chromosome aberrations or patterns of craniosynostosis with associated anomalies not previously described. With 20 known hereditary forms of craniosynostosis, genetic consultation and counseling are of critical importance in the management of these conditions.[60,61,67] Discussed in this section are select major syndromes with craniosynostosis that may have medical issues in the newborn period. See Table 88.2 for a description of key phenotypic features and potential airway compromise.

Apert syndrome was initially described as acrocephaly with four-limb syndactyly. The symmetric hand and foot involvement with syndactyly and symphalangism is an important clue to the diagnosis (Fig. 88.5). Inheritance is autosomal dominant and Apert is associated with advanced paternal age. Neurocognitive outcomes vary, but a moderate to severe degree of cognitive impairment is most common. Multiple mutations in *FGFR2* causing Apert syndrome have been identified.[68]

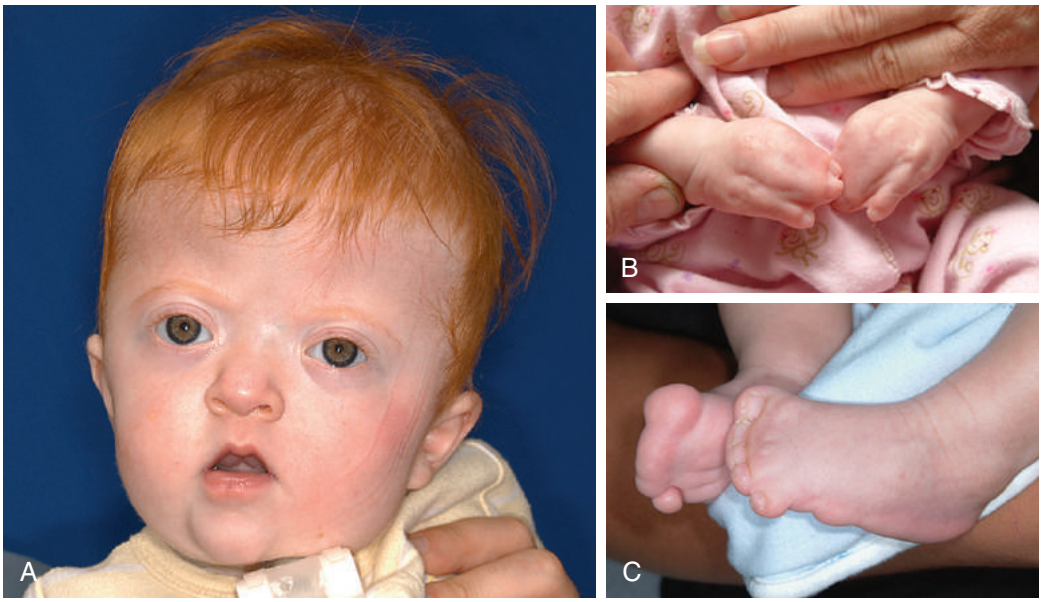
Crouzon syndrome is an autosomal dominant condition that demonstrates wide phenotypic variability. Shallow orbits with proptosis are an important diagnostic finding, although this feature may be subtler in the newborn (Fig. 88.6). Significant abnormalities involving the CNS include the frequent presence of a Chiari type 1 malformation, with progressive hydrocephalus and risk for intracranial hypertension. Compared with Apert syndrome, Crouzon syndrome is associated with more extensive suture involvement, smaller cranial volume, and more severe intracranial constraint; however, cognitive development is usually normal. Like Apert syndrome, Crouzon syndrome is caused by mutations in *FGFR2*. A less common form of Crouzon syndrome with acanthosis nigricans skin findings developing in the first 2 years of life is caused by a transmembrane mutation in *FGFR3*.

Pfeiffer syndrome is a hereditary craniosynostosis that shares significant overlap, both phenotypically and genetically, with Crouzon syndrome. It is an autosomal dominant disorder with craniosynostosis accompanied by proptosis, broad and deviated thumbs, and large first toes (Fig. 88.7). Mutations in *FGFR1* and *FGFR2* cause Pfeiffer syndrome. Type 1 Pfeiffer syndrome involves mild manifestations including brachycephaly, midface hypoplasia, and digital malformations. Type 2 consists of cloverleaf skull, extreme proptosis, digital malformations, elbow ankylosis, developmental delay, and neurologic complications. Type 3 is similar to type 2 but without a cloverleaf skull. CNS and spine anomalies are common in Pfeiffer syndrome.[69]

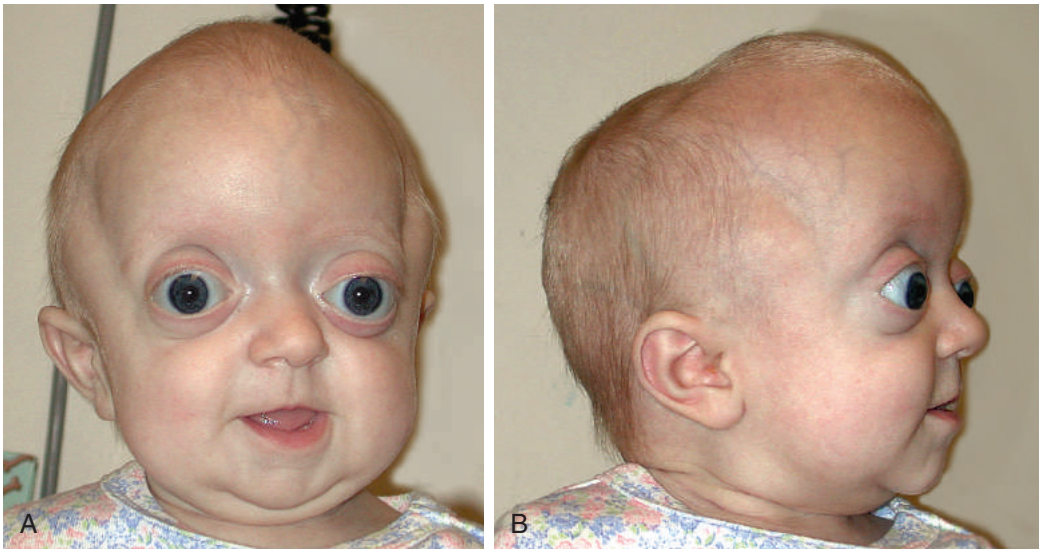
Muenke syndrome is an autosomal dominant syndrome caused by a single P250R mutation in the *FGFR3* gene. Like Apert syndrome, Muenke syndrome is associated with advanced paternal age. Individuals with Muenke syndrome may have coronal craniosynostosis (unilateral or bilateral), macrocephaly, variable degrees of proptosis, a high prevalence of sensorineural hearing loss, and do not typically have significant midface hypoplasia (Fig. 88.8).

Saethre-Chotzen syndrome is caused by a mutation in the *TWIST1* gene on chromosome 7. The inheritance is autosomal dominant, and many children with Saethre-Chotzen syndrome will have an affected parent. In addition to craniosynostosis, affected individuals commonly have a low frontal hairline, ptosis, 2 to 3 syndactyly of the fingers, cervical spine anomalies, and duplicated halluces. Although learning difficulties may be noted, cognitive impairment is not typical of Saethre-Chotzen syndrome caused by intragenic mutations. Children with deletions rather than point mutations often demonstrate significant developmental delays.

ERF-related craniosynostosis is a recently recognized syndromic form of craniosynostosis caused by variants in the *ERF* gene.[70] The multisutural involvement varies, including pansynostosis and a pattern involving the sagittal and lambdoid sutures (Mercedes-Benz pattern), and can be postnatal in onset with insidious and progressive effects on head shape and unsuspected



• **Fig. 88.5** (A) Infant with Apert syndrome, a high and full forehead, proptosis and exotropia, midface hypoplasia, and a trapezoid-shaped mouth. (B, C) Hands and feet in Apert syndrome. Note the syndactyly symmetrically affecting hands and feet. All five digits may be webbed, or a single toe, finger, or thumb may be free.



• **Fig. 88.6** (A) Infant with Crouzon syndrome with acro brachycephaly. (B) Proptosis and midface retrusion are seen in the lateral view.

• **Fig. 88.7** (A, B) Infant with Pfeiffer syndrome, brachycephaly, a high forehead, midface hypoplasia, proptosis, and ocular hypertelorism. (C) An older child with Pfeiffer syndrome and the typical broad thumbs with radial deviation.

• **Fig. 88.8** (A, B) Infant with Muenke syndrome, acrobrachycephaly due to bicoronal synostosis, and absence of proptosis. (C) Sibling of the infant in (A, B) also with Muenke syndrome; note the downslanting palpebral fissures.

intracranial hypertension. Facial features include hypertelorism, mild exorbitism, and malar hypoplasia. Chiari malformation and developmental concerns are common.

Cloverleaf skull can result from any form of multisuture craniosynostosis. The skull forms a trilobular appearance, as the cerebrum bulges through the sagittal and squamosal sutures, because of craniosynostosis affecting the coronal, metopic, and lambdoid sutures. Cloverleaf skull is most commonly associated with a syndrome, and it is estimated that up to 20% of cases represent Pfeiffer syndrome.

Intensive Care Unit Concerns

The most significant concerns for the newborn with craniosynostosis are airway compromise due to upper airway obstruction and intracranial hypertension.

Midface hypoplasia and tracheal anomalies that may be present in syndromic craniosynostosis can lead to significant airway compromise (see Table 88.2). With midface hypoplasia, there is decreased nasal and oropharyngeal space because of a small maxilla, narrowing at the level of the posterior choanae, and posterior displacement of bony and soft tissue structures, leading to breathing problems, obstructive sleep apnea, asphyxia, and even death (Fig. 88.9). Obstructive sleep apnea is common in Apert, Pfeiffer, and Crouzon syndromes.

Cartilaginous tracheal abnormalities can be present in multisuture craniosynostosis syndromes. Vertically fused tracheal cartilage (also referred to as tracheal cartilaginous sleeve, solid cartilaginous trachea, and stovepipe trachea) in Crouzon and Pfeiffer syndromes may produce a rigid trachea resulting in upper airway stenosis, inability to clear secretions, and increased risk of injury because of decreased distensibility. Characteristic tracheal cartilaginous rings are fused to form a continuous sleeve of cartilage, which may extend from below the subglottis to the carina or bronchus; rarely, the cartilaginous sleeve can begin more proximally, at the level of the cricoid cartilage. Infants with congenital tracheal anomalies may have stridor, increased work of breathing, and distress, particularly with respiratory illnesses.

Neurologic abnormalities such as hydrocephalus and increased ICP may arise, especially in multisuture craniosynostosis. Increased ICP due to constraint of the growing brain within a restricted calvarium is usually chronic, causing symptomatic intracranial hypertension when brain growth is rapid during the first 2 years of life. ICP issues in the neonate are not usually life threatening, given the open fontanel and compensatory splaying of normal sutures or erosion of the calvarium, but brain injury and encephalomalacia may result if cranial expansion is not performed.

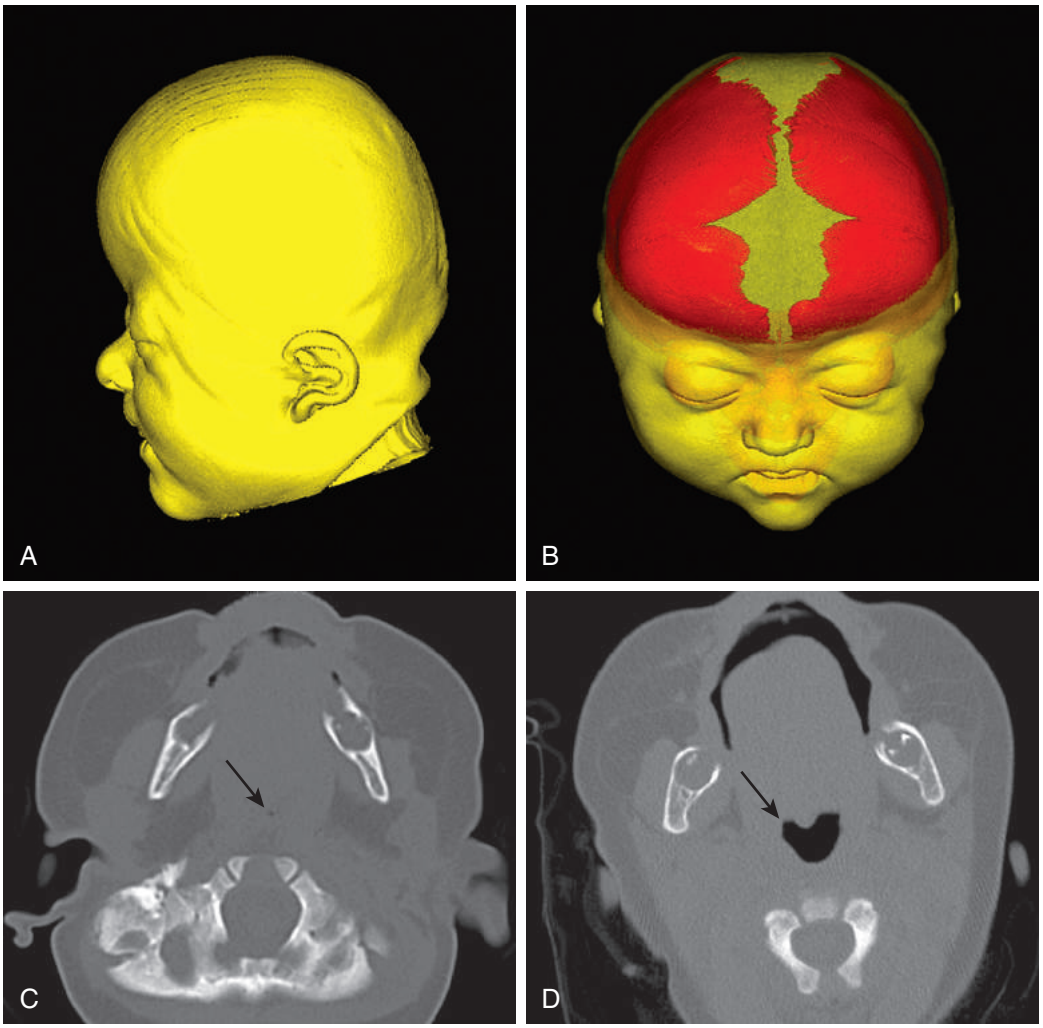
Hydrocephalus, which is more common in Crouzon and Pfeiffer syndromes compared with other multisuture synostosis syndromes, can occur as a result of obstruction of cerebrospinal fluid at the basal cistern, aqueductal stenosis, or impeded venous flow or when there is an associated Chiari malformation. Hydrocephalus is extremely common in cloverleaf skull. Individuals with multisuture craniosynostosis (particularly Apert syndrome) more commonly have nonprogressive distortion ventriculomegaly or compensated hydrocephalus, which does not require shunting.[71] Abnormalities of the corpus callosum and septum pellucidum have been described in Apert syndrome, and neuroimaging and genetic advances will illustrate links between brain architecture, phenotype, and genotype.[72] Seizures presenting in multisuture craniosynostosis syndromes are more commonly due to encephalopathy rather than increased ICP.

Chiari malformation is frequently diagnosed in syndromic craniosynostosis. Cerebellar tonsillar herniation, especially in the setting of cord compression, can affect control of breathing and lead to central sleep apnea, ranging from mild to profound. Treatment of airway obstruction can unmask central apnea and continued monitoring for apnea over time is necessary in syndromic craniosynostosis.

Management

The evaluation of the patient with craniosynostosis includes recognizing and confirming the type of suture fusion, clinical syndrome identification, evaluation for associated anomalies, and preparedness for surgical repair.

A detailed physical examination should be performed as part of the initial evaluation, looking for any other anomalies, with specific attention to cleft palate, limb defects, heart defects, and ear anomalies. The assessment of cranial and face shape, the fontanelles, presence of sutural ridging, skull base symmetry, and ear position is important. Proptosis and exorbitism due to shallow orbits are important to recognize as exposure keratopathy is the major etiology of corneal pathology encountered in multisuture craniosynostosis, and ophthalmology involvement early can be vision sparing. If proptosis is present, as can occur in Apert, Crouzon, and Pfeiffer syndromes, ocular lubricants help to prevent exposure keratopathy. Although rare, severe proptosis can lead to globe luxation and may need surgical intervention such as tarsorrhaphy, in addition to eye surface lubrication,



• **Fig. 88.9** (A, B) Three-dimensional reconstruction of a child with Apert syndrome with significant midface hypoplasia, leading to upper airway obstruction. Also notable is acrobrachycephaly due to bicoronal synostosis and the typical pattern of sagittal suture patency. (C) CT scan axial slice at the level of the skull base in a newborn with Apert syndrome. The *arrow* pointing to the airway illustrates significant airway obstruction. (D) CT scan of a newborn illustrating a normal airway (*arrow*).

to preserve eye health. During the neonatal period and as a child grows, an ophthalmologist with experience in craniosynostosis is recommended.[73]

CT with three-dimensional reconstruction will ultimately confirm the diagnosis of craniosynostosis, delineate the degree of suture involvement, and help with preoperative planning. Although the specific timing of the surgical treatment may differ between teams, it is generally accepted that individuals with synostosis should undergo cranial surgery in the first year of life. Cranioplasty involves the release of fused sutures and repositioning and reconstruction of the calvaria to expand the skull to prevent increased ICP and progressive abnormal craniofacial development. Several techniques, including endoscopic strip craniectomy, calvarial distraction, and traditional cranioplasty, are currently used. Consultation with a craniofacial team should be initiated when craniosynostosis is suspected, as the timing of some surgical interventions are performed in the first few days or weeks of life. A comprehensive guideline has been recently updated by Mathijssen in 2021.[74]

Attention to facial shape, especially the degree of maxillary hypoplasia, is important in determining the risk of airway compromise due to midface hypoplasia. If concerning airway symptoms are present, such as snoring, stridor, or apnea, consultation with a sleep specialist and polysomnography may help to quantify the presence and severity of early obstructive sleep apnea, and identify perhaps more subtle central apnea and the need for positive pressure ventilation. Awareness of potential airway compromise and proactive airway management are crucial in many craniosynostosis syndromes. Temporizing measures to bypass airway obstruction include placement of nasal stents, endotracheal intubation, and tracheotomy. Specific airway management in syndromic craniosynostosis will depend on the level and severity of obstruction. Consultation with an otolaryngologist and airway endoscopy to identify the types and degree of airway narrowing is essential in infants with multisuture craniosynostosis and airway obstruction. Particular attention to the presence of tracheal malformations, such as vertically fused tracheal cartilage, is crucial in craniosynostosis syndrome as early recognition of tracheal malformations

can be lifesaving.[75] With the increased awareness of this condition, the diagnosis of tracheal malformations is increasingly made on direct laryngoscopy/bronchoscopy or with MRI, and ultrasound is an emerging tool.[76] Infants with tracheal anomalies benefit from skilled complex airway management that may include airway surgery or even tracheotomy with custom airways to achieve airway patency and prevent mortality in infancy.[77] Serious caution must be exercised in the placement and care of tracheostomies in patients with tracheal cartilaginous sleeves because of unique airway shape, abnormal tissue healing, and granulation tissue formation. Midface advancement surgery may be necessary for some children who have nasal level airway obstruction, swallowing, feeding, and dental malocclusion. This is usually performed later in childhood.

The family and prenatal history, including documentation of affected family members, teratogen exposure, maternal thyroid disease, and in utero constraint (oligohydramnios, twins, fetal movement), and the birth history should be ascertained, specifically looking for risk factors.

For all individuals with craniosynostosis, we recommend the early involvement of a craniofacial team including members specializing in pediatrics, genetics, neurosurgery, ophthalmology, oral surgery, orthodontics, otolaryngology, nursing, nutrition, plastic surgery, and social work.[78] Prenatal involvement of craniofacial and airway specialists is especially critical for planning the safe delivery and post-partum care when multisuture craniosynostosis is anticipated.

Screening and Surveillance

Many genetic causes of craniosynostosis require screening for additional health characteristics as well as complications. Accurate and prompt diagnosis requires a combination of careful clinical evaluation and correctly targeted diagnostic testing, proceeding to exome/whole genome sequencing if necessary.[59] The role of the geneticist in understanding the causes of single suture craniosynostosis is evolving. The families of children with multisuture synostosis with the presence or absence of associated syndrome should be offered appropriate genetic consultation, molecular testing, genetic counseling, and surveillance monitoring guided by the unique genotype.[79] Below are some important considerations:

- CNS: In all children with craniosynostosis, and particularly in those with multisuture involvement, it is important to monitor for any signs or symptoms of increased ICP. Evaluation for hydrocephalus should be a part of the initial assessment of all children with multisuture craniosynostosis. Ventriculomegaly may be identified by the initial diagnostic head CT, and follow-up imaging should be pursued if any acceleration in OFC or bulging fontanelle is noted. MRI of the brain may be helpful in defining any associated CNS anomalies, and screening for Chiari malformation is recommended for children with multisuture craniosynostosis, or craniosynostosis with Chiari symptoms.
- Spine: In coronal synostosis and syndromes including Apert, Crouzon, Pfeiffer, and Saethre-Chotzen, associated vertebral anomalies, including fusions and instability, may be present, detected on spine radiographs, and more accurately visualized with CT C-spine imaging that can be coordinated with CT head imaging in the young infant.
- Eyes: Early ophthalmology consultation and ongoing surveillance are valuable in the management of proptosis, monitoring for optic neuropathy, vision and eye alignment given the high risk for strabismus.
- Hearing: Conductive and mixed hearing loss, most commonly due to middle ear disease, ossicular abnormalities, and external auditory canal stenosis or atresia, can be present in syndromic craniosynostosis. Early amplification (for example with a bone conduction sound processor on a soft band in the setting of canal atresia) may be indicated to support communication. Sensorineural hearing loss has been described in Saethre–Chotzen syndrome and Muenke syndrome. Timely hearing screening is recommended for all children with craniosynostosis, and continued monitoring for progressive hearing loss is indicated for children with coronal and multisuture craniosynostosis.
- Development: Developmental monitoring and referral to early intervention services is recommended for all infants with single and multi-suture craniosynostosis, especially those with craniosynostosis syndromes. Although school-age children with repaired single suture craniosynostosis have been found to have evidence of mild developmental delays, the pathogenesis and direct relationship to synostosis have not been determined.[80]
- Sleep: Monitoring for sleep apnea in infants and children with multisuture craniosynostosis and syndromes is recommended, with a low threshold for referral for a sleep study.
- Heart: In Apert syndrome, a cardiac and genitourinary evaluation is recommended.
- GI: Low threshold to obtain imaging to rule out malrotation in the infant with emesis and multisuture craniosynostosis, given the association with intestinal abnormalities.[81]
- Limbs: If any limb abnormalities are seen, as in Apert, Jackson–Weiss, Pfeiffer, and Saethre–Chotzen syndromes, radiographs with orthopedic or hand specialist consultation should be obtained.

Disorders of the First and Second Branchial Arches

Craniofacial Microsomia

Craniofacial microsomia (CFM), a congenital malformation in which there is asymmetric deficiency in skeletal and soft tissue on one or both sides of the face, is the most frequently encountered form of facial asymmetry. CFM affects approximately 1 in 3000 to 1 in 5600 births.[82,83]

Diagnosis and Etiology

Individuals with features of CFM have been classified under a variety of different diagnoses (hemifacial microsomia, oculoauriculovertebral spectrum, facioauriculovertebral syndrome, first and second branchial arch syndrome, otomandibular dysostosis, Goldenhar syndrome, lateral facial dysplasia) attesting to the phenotypic variability. There are no accepted diagnostic criteria, but the presence of ipsilateral mandibular and ear defects is most common. Infants with CFM are often born small for their gestational age, and the perinatal history may include polyhydramnios due to fetal swallowing dysfunction. Various causes, both environmental and heritable, have been studied, and for most, the cause is thought to be multifactorial. Most often CFM is a sporadic condition with a recurrence risk of approximately 2% for future pregnancies unless there is a known family history of microtia or CFM.[84]

Phenotype

CFM is primarily a condition of the first or second branchial arches, resulting in the underdevelopment of the ear, temporomandibular joint, mandibular ramus and body, and mastication muscles. Asymmetric bilateral facial involvement is common (Fig. 88.10A). The affected external ear can be underdeveloped and small (microtia) or malformed, may be lower in position compared with the ear on the contralateral side (see Fig. 88.10B), can present with no external ear (anotia), and may be accompanied by preauricular tags. Hearing loss may result from maldevelopment of the ossicular chain and a stenotic or atretic external auditory canal and can affect one or both sides. Second branchial arch defects can involve the facial nerve and muscles of facial expression, which can be difficult to appreciate in a newborn.

A common classification system for CFM is the OMENS system, which characterizes the degree of involvement of facial structures: *o*rbital distortion, *m*andibular hypoplasia, *e*ar anomaly, *n*erve involvement, and *s*oft tissue deficiency.[85,86] Isolated microtia may represent a forme fruste of CFM. Other craniofacial features include external auditory canal stenosis or atresia, unilateral macrostomia (transverse facial cleft leading to lateral displacement of the oral commissure and the most common form of orofacial clefting in CFM), cleft lip and/or palate, temporomandibular joint ankylosis, ankyloglossia, preauricular or facial pits (most common in the distribution of the facial nerve), midface hypoplasia and malocclusion, epibulbar lipodermoids (see Fig. 88.10C), microphthalmia, eyelid and ocular colobomas, facial nerve palsy or paresis, and other cranial nerve palsies. There can be extreme variability of phenotypic expression, and the severity of mandible, ear, and facial involvement varies from mild to more impacted (see Fig. 88.10D). Goldenhar syndrome has historically been described as a subgroup variant of CFM characterized by vertebral anomalies and epibulbar dermoids in addition to the ear and jaw findings. Extracraniofacial anomalies are common in CFM, with one large study describing a prevalence of 47%, and higher among those with bilateral CFM or more severe bony and soft tissue involvement. Extracraniofacial anomalies can include vertebral (28%; scoliosis, block vertebrae, hemivertebrae), cardiac (21%; septal defects, valve anomalies, tetralogy of Fallot), CNS (11%; hydrocephalus, ventriculomegaly, intracranial cyst), urogenital tract (11%; renal aplasia, undescended testicle, hydronephrosis), GI tract (9%; inguinal hernia, imperforate anus, esophageal atresia), and respiratory tract (3%; laryngomalacia).[87]

In CFM, deficient growth of the hypoplastic mandible and the compensatory growth of the contralateral maxilla and zygoma contribute to facial asymmetry that progresses with growth. Conversely, facial and skull asymmetry caused by deformation (intrauterine or postnatally with plagiocephaly and torticollis) will improve with time, repositioning, and treatment of torticollis.

Branchial Arch Malformation Syndromes

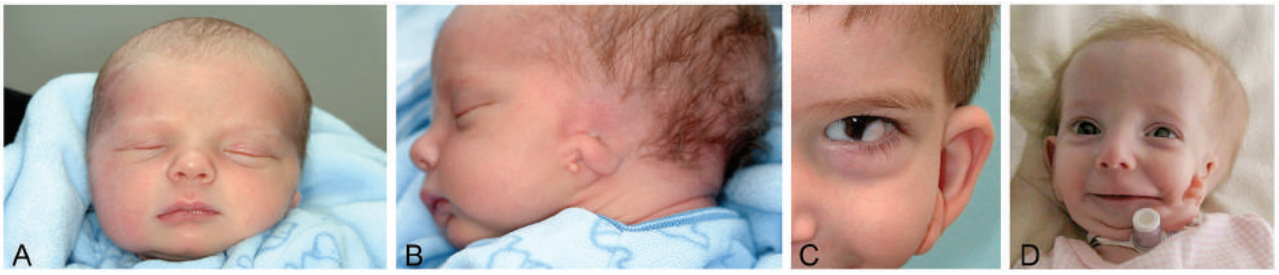
While multiple syndromes can be associated with malformations of the first and second branchial arches, presented in this chapter are two syndromes with particular relevance to the neonatologist.

Moebius Syndrome

Moebius syndrome is a rare congenital condition affecting approximately 2000 people worldwide.[88] The sixth and seventh cranial nerves are universally affected. Sixth nerve palsy leads to an inability to abduct the eyes beyond the midline. This is usually bilateral but may be unilateral or asymmetric. Paralysis of facial muscles results from the seventh nerve palsy. While newborns may have a "masklike facies," the presentation is challenging to recognize in the newborn period.[89] Feeding difficulties may result from problems with swallowing and sucking, aspiration, and palatal weakness related to more widespread cranial nerve involvement. Both abnormalities of cranial nerve nuclei and neural connection issues are hypothesized to cause Moebius syndrome. Many associated features have been described, and hypotonia is common, also impacting swallowing and breathing in infancy.[90] Associations with chest wall abnormalities, including the absence of the pectoralis muscle, suggest a pathogenic relationship with the Poland anomaly. Exposure conjunctivitis and keratopathy can occur in children with facial paralysis and lagophthalmos and should be prevented with ocular lubricants. Limb defects occur in more than half of children with Moebius syndrome, most commonly talipes deformity; however, transverse limb anomalies are also seen. Individuals with hypoglossia-hypodactylia or Hanhart syndrome can have severe limb deformities, ankyloglossia, and temporomandibular joint ankylosis, in addition to Moebius syndrome–like features and micrognathia. As a consequence, they are at risk of significant swallowing dysfunction and airway compromise.[91]

Treacher Collins Syndrome

Treacher Collins syndrome (TCS) is a disorder of craniofacial development that affects approximately 1 in 50,000 live births.[92] As in CFM, the tissues affected in TCS arise from the first and second branchial arches. The major clinical features of TCS include hypoplasia of facial bones (mandible and zygoma), microtia,

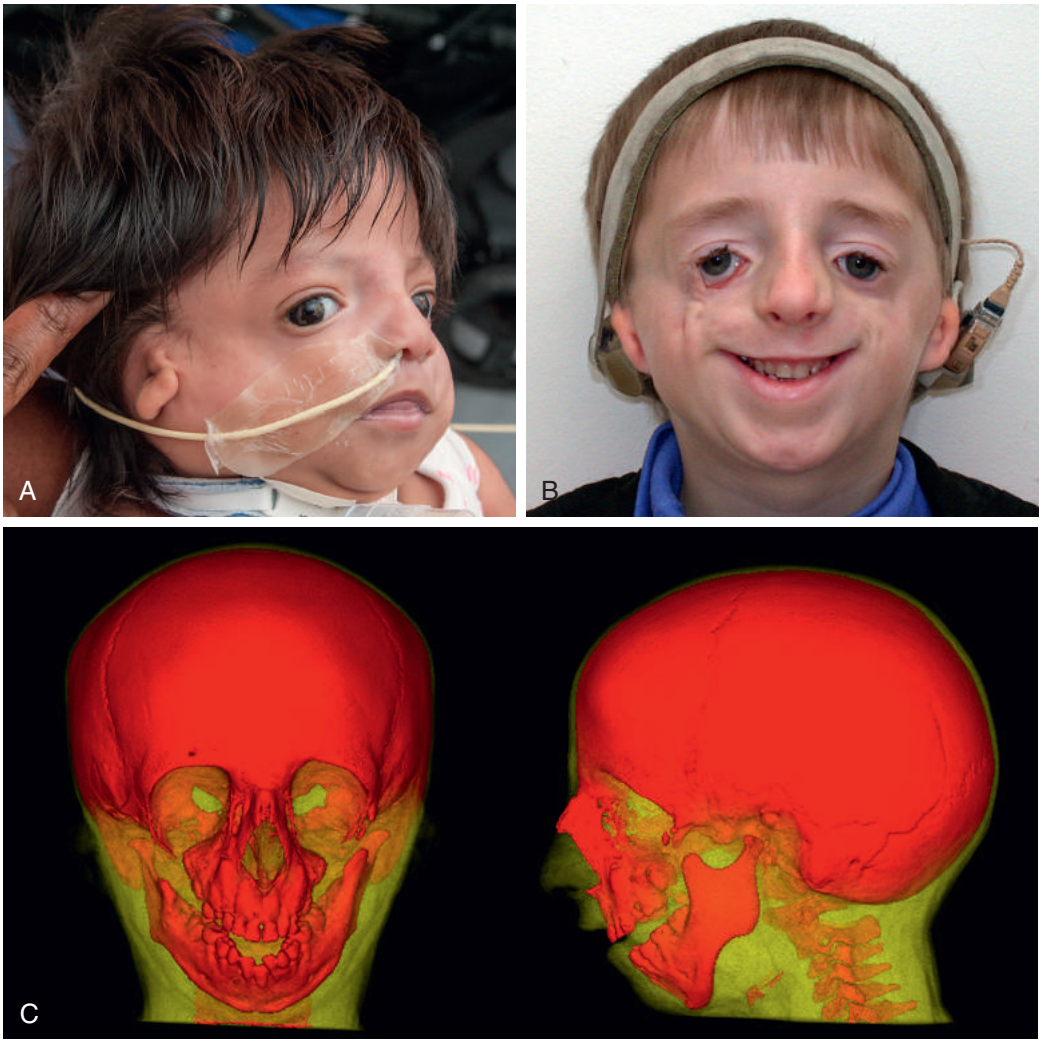


• **Fig. 88.10** (A, B) Infant with craniofacial microsomia, mandibular asymmetry, and left-sided microtia. (C) Child with an epibulbar lipodermoid and craniofacial microsomia. (D) Infant with more severe mandible hypoplasia, airway obstruction, and an associated tracheostomy tube.

external auditory canal atresia, bilateral conductive hearing loss, downward sloping palpebral fissures, and lower eyelid colobomas, as well as risk of exposure keratitis (Fig. 88.11A, B).[93] Cleft palate may occur and hearing loss is present in up to 50% of individuals with TCS.[94] In severe cases, the zygomatic arch may be absent. Extracraniofacial features are rare in TCS, and limb anomalies can distinguish other forms of mandibulofacial dysostoses from TCS: for example, Miller syndrome with craniofacial features similar to TCS plus postaxial limb anomalies affecting the fifth digital ray of all four limbs, and Nagar syndrome with craniofacial features similar to TCS plus preaxial limb anomalies, hypoplastic/absent thumbs and or radii. Mutations in one of four genes (*TCOF1, POLR1B, POLR1C, POLR1D)* are causative of TCS and mutations in the *TCOF1* gene account for 71% to 93% of affected individuals. The diagnosis of TCS is usually made clinically and can be confirmed with genetic testing.[95] In newborns with TCS, airway management may be required to address narrowing of the airway or extreme shortening of the mandible (see Fig. 88.11C). When compared with that in CFM, the mandibular hypoplasia in TCS is usually bilateral and symmetric, leading to an even higher risk of upper airway obstruction. In addition to glossoptosis and mandible involvement similar to RS, choanal stenosis or atresia can be present in neonates with TCS predisposing to multilevel airway involvement not effectively resolved with neonatal mandible advancement.[96,97] Among infants with TCS and significant airway compromise, there is an increased need for tracheostomy, and risk of death in the neonatal period.

Intensive Care Unit Concerns

Mandibular hypoplasia in CFM can lead to upper airway obstruction that may be obvious on physical examination, presenting with stertor or stridor and increased work of breathing, or may be more subtle, with noisy breathing occurring with sleep or feeding. Bilateral severe mandibular and maxillary involvement in TCS leads to airway obstruction at the level of the nasopharynx and base of the tongue and substantial respiratory compromise. As multilevel airway obstruction is common in TCS, airway endoscopy to help target treatment options should be pursued for any neonate with TCS or CFM and signs of airway obstruction.



• **Fig. 88.11** (A) Infant with Treacher Collins syndrome (TCS), microtia, severe mandibular and zygomatic hypoplasia, and airway obstruction requiring tracheostomy. (B) An older child with TCS, downslanting palpebral fissures, eyelid colobomas, and bilateral microtia wearing a hearing augmentation device. (C) Three-dimensional reconstruction of TCS. Note the severe mandibular hypoplasia, which may lead to significant airway compromise. Also notable are zygoma hypoplasia and orbital defects seen in TCS.

Infants with CFM may have feeding difficulties that may be related to macrostomia affecting lip seal; among infants with CFM and TCS, swallow coordination issues and dysphagia are attributed to both palate dysfunction and more commonly hypoglossal dysfunction and muscular and bony underdevelopment. Infants with Moebius syndrome may have cranial nerve palsies that affect swallow and oral coordination and are consequently at high risk of aspiration. Close monitoring and support of feeding, swallowing, and growth is recommended in all of the branchial arch conditions.

Management

In newborns with suspected CFM, an evaluation for associated anomalies should be undertaken. All children with external ear anomalies or any evidence of first or second branchial arch abnormalities should undergo diagnostic hearing testing in the newborn period, with follow-up audiometry in the first year of life. If there is any hearing loss, ongoing monitoring of hearing is routine. It is also important to monitor ear health and eustachian tube function in the patent/hearing ear. CT imaging to assess middle and inner ear anatomy is not recommended in the neonatal period. Consultation to discuss ear reconstruction and atresia repair typically occur by 4 years of age, although consultation for hearing amplification should occur as soon as possible in infants with hearing loss, and infants diagnosed with hearing loss should receive intervention services as soon as possible, but no later than 6 months of age (https://www.cdc.gov/ncbddd/hearingloss/treatment.html). Additionally, aural habilitation support is helpful.

Mild airway obstruction in CFM and TCS may be reduced with prone positioning. However, infants with severe unilateral or bilateral mandibular hypoplasia or multilevel airway obstruction may have significant airway compromise and require tracheostomy placement. In cases with airway compromise or signs of obstructive sleep apnea, early referral to a craniofacial center to determine optimal and safe airway management should be pursued.[98] The timing of surgery to address mandibular underdevelopment is typically in later childhood and depends on the degree of mandibular hypoplasia, mandibular growth, occlusion, and airway involvement.[99] For children with severe hypoplasia of the mandible, bone grafting may be necessary for jaw reconstruction before mandible distraction. Oral feeding should be introduced when the airway is stable. Given the risk of feeding difficulty and aspiration in infants with malformations of the first and second branchial arches, early consultations with a dietitian and a feeding therapist are helpful.

Screening and Surveillance for Craniofacial Microsomia

- Diagnostic hearing test in infancy and regular assessments of hearing guided by initial audiologic assessment and hearing loss risk
- CT to assess middle and inner ear anatomy and guide atresia repair options at 4 years of age.
- Renal ultrasound in infancy to evaluate for structural malformations
- Cardiac examination (echocardiogram) in infancy if any clinical concerns or murmur
- Ophthalmology consultation to manage epibulbar lipodermoids, colobomas (if present), and prevent exposure keratopathy
- Cervical spine screening radiographs to identify vertebral anomalies (defects in segmentation). If the newborn has no symptoms of cervical spine abnormality, screening four-view cervical spine radiographs can be deferred until the child is 2 to 3 years old, when vertebrae are more reliably imaged. Appropriate cervical spine imaging is recommended in children undergoing surgery before 2 years of age and children with head tilt or signs of vertebral anomalies.
- Spine monitoring for progressive scoliosis
- Monitoring for obstructive sleep apnea, with a low threshold for referral for a sleep study
- Dental and occlusal monitoring through childhood

CHARGE Syndrome

Diagnosis and Etiology

The term *CHARGE* (*c*oloboma, *h*eart defect, *a*tresia choanae, growth *r*etardation, *g*enital hypoplasia, *e*ar anomalies/deafness) was first coined by Pagon, given the observation that the associated malformations occurred more frequently together than one would expect on the basis of chance.[100] Over time, the facial features and associated malformations were better characterized as a syndrome, with mutations in at least one major gene described.

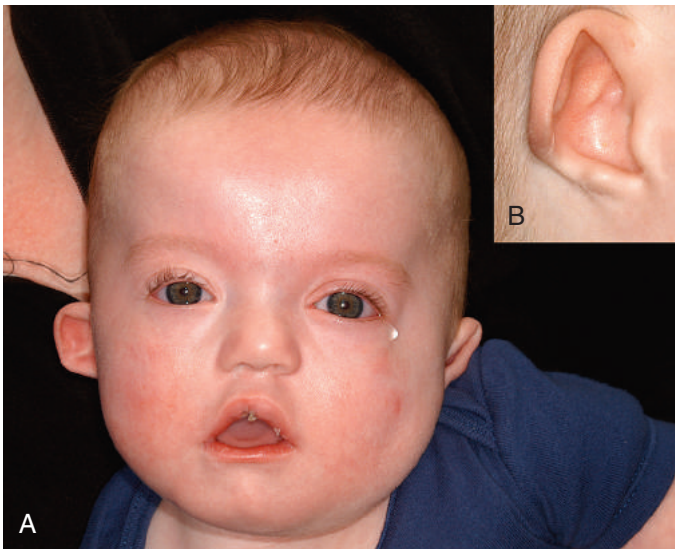
This multiple malformation condition has a prevalence of approximately 1 in 10,000 births.[101] Mutations in the *CHD7* gene account for most cases, but CHARGE syndrome remains a clinical diagnosis, with some individuals meeting the classic criteria without a *CHD7* abnormality.[102] Molecular testing for mutations in the *CHD7* gene is especially useful in atypical cases where the diagnosis is being considered but can also be performed to confirm the diagnosis and assist in counseling for the parents and the patient. For children in whom *CHD7* gene testing results are normal, further genetic testing is warranted.[103]

The clinical diagnosis of CHARGE syndrome is summarized in Table 88.4. Additional findings include renal, spinal, hand, neck, and shoulder anomalies.[101,103] With improving diagnostics, the

TABLE 88.4 Clinical Diagnosis of CHARGE Syndrome

Major Criteria	Minor Criteria
Coloboma (80%–90%)	Cardiovascular malformations (conotruncal and aortic arch most common)
Choanal atresia/stenosis (50%–60%)	Genital hypoplasia
Cranial nerve dysfunction (especially I, VII, VIII, IX, X) (40%–90%)	Cleft lip and/or palate
Characteristic CHARGE ear findings (inner, middle, outer) (90%–100%)	Tracheoesophageal fistula
	Distinctive CHARGE facies
	Growth deficiency
	Developmental delay

CHARGE syndrome strongly suspected if all major criteria or 3 major and 3 minor criteria are present.



• **Fig. 88.12** (A) Child with CHARGE syndrome with (B) classic ear malformation—hypoplastic lobes, cupped and low set.

phenotype is expanding, and gastrointestinal problems, immunodeficiency, and neuromuscular problems are also described.[103] Polyhydramnios is commonly present prenatally, secondary to upper airway obstruction and/or swallowing dysfunction.

Phenotype

Distinctive ear anomalies (hypoplastic lobes, cupped/lop, low-set, and posteriorly rotated) occur in most cases (Fig. 88.12). Facial features include a square face with malar flattening, broad forehead, facial asymmetry, pinched nostrils, full nasal tip, and long philtrum. Ocular colobomas can range from iris involvement to anophthalmia. A minority of cases have cleft lip and/or palate. A heart murmur may indicate congenital heart disease. Limited neck range of motion may indicate cervical spine anomalies. In the neonatal period, breathing and feeding difficulties are often the most prominent features, as the characteristic facial and ear features may not be as pronounced.

Intensive Care Unit Concerns

The most important potential postnatal emergency in CHARGE syndrome is bilateral posterior choanal atresia.[104] Neonates with bilateral choanal atresia will have breathing difficulty and cyanosis within the first hour of life. Crying relieves the cyanosis by allowing the obligate nose breather to take in air through the mouth; feeding exacerbates respiratory distress. Left untreated, asphyxiation and death can occur. Symptoms of bilateral choanal stenosis or unilateral atresia may not present until after the newborn period with chronic rhinorrhea and nasal airway obstruction exacerbated by respiratory infections. Feeding difficulties and sialorrhea are significant causes of morbidity. These issues, and secondary growth problems, are common in early infancy and may be attributed to swallowing dysfunction, pharyngeal incoordination, gastroesophageal reflux, and aspiration. Cranial nerve palsies (specifically of V, IX, and X) may contribute to swallowing dysfunction, and tracheoesophageal fistula (TEF), if present, contributes to aspiration risk.

Swallowing dysfunction and gastroesophageal reflux can cause descending and ascending aspiration and lower respiratory tract disease, leading to chronic respiratory distress. Infants with CHARGE may also have micrognathia and glossoptosis, putting them at risk of airway obstruction at the level of the pharynx/hypopharynx. Infants with CHARGE syndrome may require multiple surgical procedures during the first year of life and are at increased risk of postoperative airway events.[104,105]

Cyanotic heart disease may present in the immediate newborn period because of tetralogy of Fallot, outflow tract anomalies, and interrupted aortic arch. There should be a very low threshold to obtain an echocardiogram and involve cardiology in a neonate with possible CHARGE syndrome.

Although it is well described that infants with CHARGE syndrome who survive the newborn period are more likely to survive childhood, the risk of death in infancy remains. Bilateral choanal atresia, TEF, cyanotic heart disease, atrioventricular septal defects, CNS malformations, and ventriculomegaly have all been associated with reduced life expectancy in individuals with CHARGE syndrome.[104,106] A study of 77 individuals with CHARGE syndrome found mortality to be 13%; the ages at the time of death range from less than 1 week old to 9 years old.[106]

Management

Many children with CHARGE syndrome will require intensive medical management and undergo multiple surgical interventions in infancy and early childhood. Early management targets airway stabilization and circulatory support. Neonates suspected to have CHARGE syndrome require immediate evaluation of their airway and cardiac structure and function. An oral airway should be placed if bilateral choanal atresia is suspected. Once the airway has been secured, a confirmatory CT scan of the nasal passages can be obtained; a CT of the temporal bones should be included and may reveal the characteristic inner ear findings of CHARGE syndrome (Mondini malformation of the cochlea and/or absent or hypoplastic semicircular canals). If the oral airway does not allow adequate air entry, endotracheal intubation may be required. In consultation with a pediatric otolaryngologist, trans-nasal stents may be placed to keep the nasal passages patent in choanal stenosis (and postoperatively after choanal atresia repair). Given the significant risk of cyanotic heart defects, an echocardiogram should be obtained as soon as feasible. If heart surgery is needed, documentation of the presence/absence/removal of the thymus should be reported.

Infants with confirmed or suspected CHARGE syndrome should have audiologic and ophthalmologic evaluations in the neonatal period and should be referred to early intervention services. While there is no consensus on immune screening in CHARGE, given the emerging data and implications, a full blood count with a lymphocyte differential and calcium level (because of the connection between immunodeficiency and abnormalities of the parathyroid glands observed in CHARGE syndrome, not unlike 22q11.2DS) should be considered in the neonatal period in CHARGE syndrome.[107] Consultation with an immunologist should occur for the individual with CHARGE syndrome and recurrent infections.[108] Underdevelopment of the genitals and genitourinary anomalies may be present. If there is a concern for hypogonadism, the pituitary-gonadal axis can be evaluated in infancy and will help determine the option for sex steroid therapy. Screening renal ultrasonography should also be performed in all suspected cases.[109]

Consultations with a feeding specialist and a dietitian are recommended in the newborn period. If the clinical bedside feeding evaluation or video fluoroscopic swallow study are concerning for swallowing dysfunction or aspiration, supplemental tube feeding should be initiated. With prolonged feeding issues, gastrostomy tube feeding is often necessary. Infants with severe gastroesophageal reflux and/or aspiration risk may benefit from post-pyloric feeding with a nasoduodenal or more secure gastrojejunal feeding tube.

Screening and Surveillance

CHARGE syndrome has potential impacts on nearly all body systems, and it is difficult to summarize them here. Trider et al. (2017) provides an exceptional summary of health supervision for these patients. Some important highlights include:

- Gonadotropins screening at 3 months of age
- Screening for lymphopenia and hypocalcemia due to overlap in phenotype with 22q11.2 deletion syndrome
- Referral to deaf-blind resources
- Assessment for potential cochlear implants
- Treatment of GI motility issues
- Holistic neurodevelopmental and early intervention services.

Macroglossia/Beckwith-Wiedemann Syndrome

Diagnosis and Etiology

Beckwith-Wiedemann syndrome (BWS) has been estimated to affect 1 in 10,340 live births.[110] The genetics of BWS is complex and variable. Most cases are sporadic and may result from chromosomal rearrangement, mutations, or epigenetic effects (DNA methylation changes) affecting imprinted genes on chromosome band 11p15.5. Approximately 80% of individuals with features of BWS are found to have an 11p15.5 abnormality by clinically available testing.[111] An international group of experts has developed consensus criteria for classical BWS, which require a score ≥4 in Table 88.5 for clinical diagnosis.[112] As children with BWS are at risk of neoplasms in early childhood, recognition and diagnosis of BWS are consequential. Infants conceived by in vitro fertilization may be at higher risk of BWS.[113] Although genetic testing can provide confirmation of diagnosis in 80% of individuals, clinical suspicion alone should initiate medical management and tumor surveillance studies. At this time, initiation of screening studies and consultation with genetics are recommended.[112]

Phenotype

Most clinical features outlined in Table 88.5 can present in the neonatal period. Macroglossia (Fig. 88.13) is the most frequent and most obvious manifestation of BWS, present 85% to 95% of the time.[112] It is defined as a tongue that protrudes beyond the alveoli at rest.[114] Other craniofacial features include capillary nevus flammeus, large fontanelle, mandibular prognathism, prominent eyes, infraorbital creases, anterior earlobe linear creases, and posterior helical pits. Additional findings in BWS include renal and cardiac defects; cleft palate is also described, albeit less often.[115] The risk of embryonal tumors (Wilms tumor, hepatoblastoma, neuroblastoma, rhabdomyosarcoma) in childhood is estimated to be 7.5%, of which 95% present in the first 8 years of life.[112]

Some features suggestive of BWS may present prenatally, including polyhydramnios (due to swallowing dysfunction), preeclampsia, fetal macrosomia, and a large placenta. Prematurity is also associated with BWS.[112]

Intensive Care Unit Concerns

Hypoglycemia due to hyperinsulinemia occurs in 30% to 60% of neonates with BWS, usually within the first days of life.[116] Polycythemia can occur and is a potential marker of a congenital Wilms tumor.[117]

Upper airway obstructive symptoms typically present in later infancy, although they may present in the newborn period if macroglossia is severe. The enlarged tongue can occlude the upper airway, leading to respiratory distress, apnea, and hypoxia. Macroglossia can also contribute to feeding issues, dysphagia, and aspiration.

Mortality among infants with BWS has been reported to be as high as 21% and is related to complications of prematurity and macroglossia.[111] Congenital heart disease is present in 13% to 20% of neonates with BWS and can include cardiomegaly, cardiomyopathy, patent ductus arteriosus, patent foramen ovale, atrial and ventricular septal defects, long QT syndrome, and more severe defects.[112]

Management

Neonatal hypoglycemia should be managed according to standard protocols. If it persists or is refractory to therapy, additional biochemical testing and consultation with an endocrinologist are helpful to guide treatment.[116,118] In severe cases, subtotal pancreatectomy may be a treatment option.[112,116] If present, polycythemia may need to be treated and could have implications for

TABLE 88.5 Clinical Diagnosis of Beckwith-Wiedemann Syndrome

Cardinal Features (2 Points Each)	Suggestive Features (1 Point Each)
Macroglossia	Birth weight >2 standard deviations above mean
Omphalocele	Facial nevus simplex
Lateralized overgrowth	Polyhydramnios and/or placentomegaly
Multifocal and/or bilateral Wilms tumor or nephroblastomatosis	Ear creases and/or pits
Hyperinsulinism beyond 1 week of age and needing escalated treatment	Neonatal hypoglycemia lasting <1 week
Pathology features: adrenal cortex cytomegaly, placental mesenchymal dysplasia, pancreatic adenomatosis	Neuroblastoma, rhabdomyosarcoma, unilateral Wilms tumor, hepatoblastoma, adrenocortical carcinoma, pheochromocytoma
	Nephro- and/or hepatomegaly
	Umbilical hernia and/or diastasis recti

A score of ≥4 meets criteria for BWS. A score of ≥2 warrants geneticist involvement to evaluate for possible BWS.

• **Fig. 88.13** (A) Premature newborn with Beckwith-Wiedemann syndrome, macroglossia, and rectus diastasis. (B) Same child at 6 months of age. Macroglossia has increased, and he now has a tracheostomy.

anesthesia. Neonates with an omphalocele may require surgery in the first few days of life.

There is no definitive approach to the management of macroglossia, and even confirmation of its presence can be challenging. Otolaryngology involvement and endoscopic airway assessment to assess the degree of airway narrowing due to base of tongue airway obstruction should be considered in symptomatic infants. Polysomnography can quantify the severity of airway obstruction and complement airway endoscopy to guide airway management. If endotracheal intubation is needed, it is important to exercise caution, because macroglossia can affect airway exposure. If macroglossia results in significant airway obstruction or prolonged intubation, tracheostomy may be needed as a temporizing measure. After infancy, progressive tongue growth will slow, and as jaw growth accelerates, airway compromise may decrease. Some children may benefit from a surgical reduction of the tongue,[119] which is usually performed between 2 and 4 years of age but may be offered as early as 3 to 6 months at some centers. Whenever possible, surgical intervention should be pursued at a multidisciplinary center with an experienced surgical team.[112,116]

Referrals to an infant feeding specialist and dietitian are recommended if macroglossia or associated airway obstruction result in feeding difficulties. Oral feeding should not be attempted if upper airway obstruction is significant, and some infants may require surgical placement of a gastrostomy tube for long-term nutritional support.

It is important to perform a thorough cardiac evaluation, including an electrocardiogram and echocardiogram if any cardiac abnormalities are suspected.

Neurodevelopment is typically normal but can be impacted by prematurity, hypoglycemia, and unbalanced chromosomal translocations. Other overgrowth syndromes, such as Sotos, Costello, and Simpson-Golabi-Behmel syndromes, are more commonly linked with developmental delay. Trisomy 21 also can present with macroglossia. A thorough evaluation of neurodevelopment is important for neonates with features of BWS.

Surveillance for tumors begins in the neonate with BWS or at the time of diagnosis. Abdominal ultrasonography to assess for organomegaly, tumors, and renal abnormalities should be considered when BWS is suspected clinically. Genetic consultation must be pursued as soon as the diagnosis is suspected.

Screening and Surveillance

Referral to a craniofacial team may be helpful in the management of airway obstruction in BWS, including evaluation for macroglossia and facial hemihypertrophy and later orthodontic and orthognathic surgical management to address developing compensatory prognathia and malocclusion. Ongoing follow-up with a geneticist and genetic counselor are essential, as the underlying genetic causes of BWS have differing cancer risk profiles and this can guide specific strategies for ongoing screening, treatments, and therapies. Some large children's hospitals or academic centers may have a dedicated multidisciplinary BWS clinic or program.

- Until the specific underlying genotype is identified, an abdominal ultrasound should be performed every 3 months to screen for embryonal tumors. The underlying genetics are complex and influence surveillance, and there are currently differing regional surveillance guidelines.[112,120] The geneticist involved can guide tumor surveillance based on the genotype identified.
- Abdominal ultrasounds can also monitor for the increased risk of hypercalciuria, nephrolithiasis, renal cysts, pelviectasis (which might indicate vesicoureteral reflux), and nephromegaly.
- At a minimum, ultrasound evaluation of the kidneys and bladder should be done at diagnosis and transition to adult care in patients who do not warrant more regular abdominal ultrasounds.[112]

- Ongoing cardiac surveillance will vary depending on the cardiac anomaly identified at the time of diagnosis.
- Monitoring for obstructive sleep apnea in infancy and early childhood, with a low threshold for referral for a sleep study
- Monitoring dental eruption, occlusion, and facial growth. Most children with macroglossia and mandible overgrowth benefit from orthodontic management, and some will be candidates for jaw surgery in adolescence
- Primary care providers should monitor children with BWS for leg-length discrepancy annually and refer them to an orthopedic surgeon if it is confirmed.[112]

Other Notable Craniofacial Conditions

Frontonasal Dysplasia, Hypertelorism, Encephalocele

Diagnosis and Etiology

Frontonasal dysplasia (FND; also known as *frontonasal malformation, median cleft face syndrome*, and *frontal nasal syndrome*) is a malformation resulting from abnormal morphogenesis of the frontonasal process. The development of the facial midline is abnormal, leading to ocular hypertelorism and associated craniofacial features. Most cases of FND are sporadic.

Phenotype

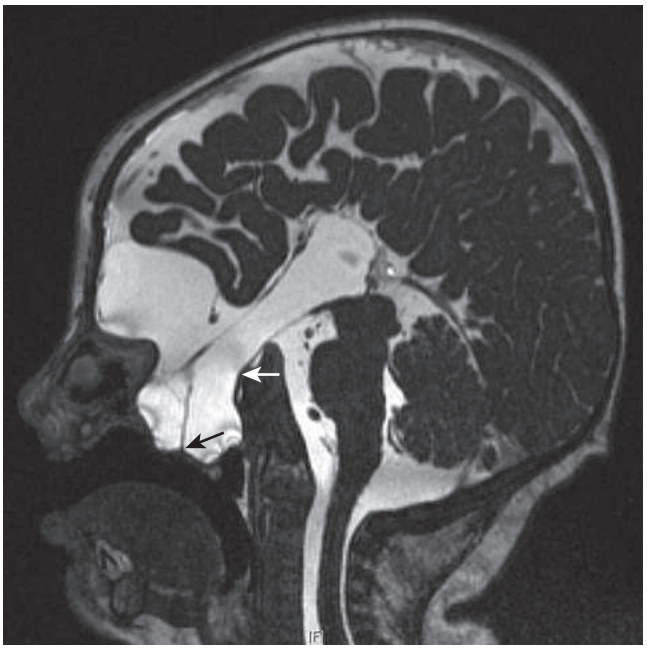
FND has been defined clinically as containing two or more of the following craniofacial features[121]:

- Ocular hypertelorism (interpupillary distance >4.5 cm in a term neonate)
- Broadening of the nasal root
- Midline facial cleft affecting the nose, lip, or palate
- Unilateral or bilateral clefting of the alae nasi
- Hypoplastic nasal tip
- Anterior cranium bifidum
- V-shaped frontal hairline

FND is a diverse and genetically heterogeneous condition found in isolation without other concerns, or can be associated with a pattern of other malformations,[122] or as a spectrum of syndromes with known genetic changes[123] such as craniofrontonasal syndrome.[124]

In addition to hypertelorism, eye anomalies, including epibulbar dermoids, colobomas, ptosis, nystagmus, or cataracts, may be present in FND and are associated with a more severe phenotype and an increased incidence of CNS abnormalities. Associated CNS manifestations include encephalocele or meningocele (most commonly frontonasal location), agenesis of the corpus callosum, and abnormal neuronal migration. When FND is associated with CNS anomalies, there is an increased association with cognitive impairment.[122]

Craniofrontonasal syndrome is an X-linked condition in a subset of patients with frontonasal malformations who also present with coronal craniosynostosis and variable skeletal and ectodermal defects. Similar to FND, facial features include hypertelorism, frontal bossing, broad nasal bridge, and a bifid nasal tip. Children with CFNS often have significant facial asymmetry due to unicoronal synostosis. In this X-linked condition, females are more severely affected (and typically have hypertelorism and grooved nails), and mutations are detected in the *EFNB1* gene. Affected individuals usually have normal intelligence.[124]



• **Fig. 88.14** MRI of an infant with frontonasal dysplasia and a midline cleft lip. The scan reveals a moderate-sized meningocele extending into the posterior nasopharynx. The *white arrow* points to midbrain meningocele coming through the cribriform plate; the *black arrow* points to the intraoral meningocele.

Intensive Care Unit Concerns

Intracranial abnormalities associated with FND may put the infant at risk of CNS manifestations such as hydrocephalus or seizures. If the pituitary gland is involved or deficient, as can be seen with HPE sequence, there can be serious endocrine abnormalities (as discussed in Orofacial Clefts). Also, frontonasal encephalocele may contribute to upper airway compromise at the level of the nasopharynx (Fig. 88.14).

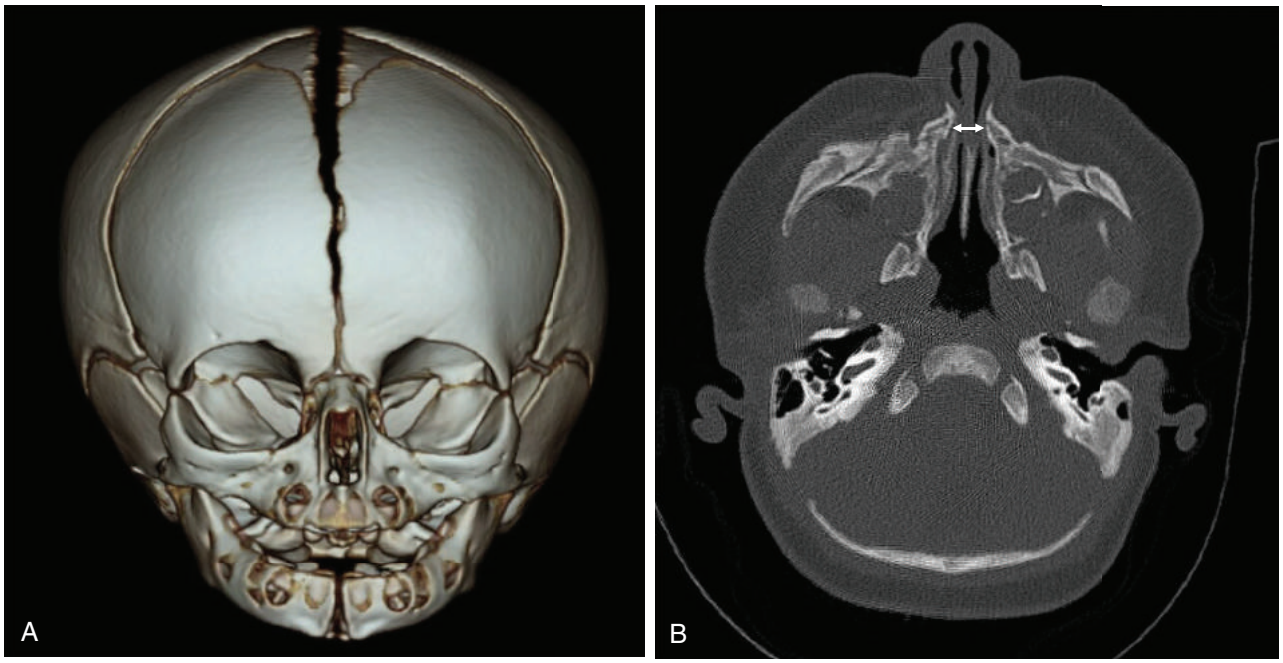
Management

In any infant with hypertelorism or features that raise suspicion for FND, awareness of potential underlying malformations is critical, and cranial imaging by CT scan or MRI should be considered. Instrumentation of the nose and mouth, including placement of a nasogastric tube or suction catheter, should be avoided or used with caution until the CNS anatomy has been delineated. Because infants with FND have a high incidence of frontonasal encephalocele or meningocele, placement of these catheters could lead to brain injury. If an infant with FND needs urgent or emergent endotracheal intubation, intraoral structures should be examined carefully to prevent injury to potential herniating CNS structures. Management of seizures or any electrolyte derangements should be managed as per the neonatal ICU standard protocol. Consultation with a craniofacial team, including ophthalmology, can clarify the work-up and management (including potential surgical interventions) for individuals with FND.

Congenital Nasal Pyriform Aperture Stenosis

Diagnosis and Etiology

Congenital nasal pyriform aperture stenosis (CNPAS) is a rare but notable cause of nasal obstruction in the neonate. The pyriform aperture (PA) is the pear-shaped maxillary nasal inlet and is



• **Fig. 88.15** (A) 3D reconstruction of CT scan with congenital nasal pyriform aperture stenosis (CNPAS). (B) CT scan axial slice with arrow showing CNPAS measuring 4mm.

the narrowest portion of the nasal airway (Fig. 88.15). CNPAS is caused by a bony overgrowth of the maxilla at the pyriform aperture during embryogenesis. Any decrease in the cross-sectional area leads to a significant increase in nasal airway resistance. The true prevalence of CNPAS is unknown due to varying degrees of clinical presentation and stenosis but has been estimated at 1 in 25,000 births.[125] First described in the medical literature in 1989, case reports and publications have more than doubled in recent years.

Age and symptoms at presentation often depend on the degree of narrowing. CNPAS may not be diagnosed immediately due to its rare occurrence and nonspecific presentation as other types of nasal obstruction, most frequently mistaken as neonatal rhinitis or choanal atresia or stenosis. Most infants have symptoms shortly after birth or in the first few weeks of life. Presentation can be mild with intermittent noisy breathing, constant congestion, and stertor, and for some infants, nasal airway obstruction presents with difficulties feeding, cyanosis with feeds, or failure to thrive. More severe obstruction may present with obstructive apneas and occasionally, infants may have life-threatening respiratory distress.

When suspected clinically, the diagnosis of CNPAS is confirmed with nasal endoscopy and imaging. Respiratory distress or cyanosis with feeding, constant congestion that improves with crying, or difficulty/inability to pass a 5 to 6 French catheter or NG through the PA should prompt further investigation into nasal airway obstruction. Anterior rhinoscopy will reveal a narrow anterior nasal valve passage, typically effecting both nares. Otolaryngology may have difficulty passing a flexible fiberoptic scope through the PA to visualize the rest of the nasal cavity.

A definitive diagnosis is made by measuring the width of the PA at the level of the inferior meatus on axial cuts of a maxillofacial computed tomography scan. In a term infant, the PA averages 16.9 mm (depending on the study); with CNPAS being defined as a PA that measures less than 11 mm in a term neonate.[126]

Phenotype

Physical exam findings in the infant with CNPAS can include microcephaly, midface hypoplasia, absent maxillary labial frenulum, and prominent central incisor; with the last two findings being relatively unique to CNPAS.[127] CNPAS can occur as an isolated condition or in combination with other midline defects, most commonly a solitary median maxillary central incisor (60%). It is considered a microform of the holoprosencephaly spectrum and can have CNS anomalies: pituitary anomalies, corpus callosum abnormalities, Arnold-Chiari I malformation, optic nerve hypoplasia, olfactory bulb agenesis, hydrocephalus, and a shallow sella turcica. Up to 40% of patients manifest endocrine dysfunction due to agenesis or hypoplasia of the hypothalamus and anterior or posterior pituitary gland.[128]

CNPAS has been associated with multiple chromosomal abnormalities and syndromes in the literature including Apert and Crouzon syndromes; tuberous sclerosis; craniosynostosis; RHYNS; VACTERL; deletion of Xp22.2, 22q11,[128] 18p, 13q, 5q[129]; and ring 18.[125]

Intensive Care Unit Concerns

Most infants with CNPAS are admitted to the NICU for respiratory distress or cyanosis with feeding. Use of an oral airway, supplemental oxygen, or CPAP are common initial first steps to stabilize the airway in the neonate with CNPAS and respiratory distress.

A complete metabolic panel can assess an infant's glucose, electrolytes, and bilirubin levels. Hypoglycemia and conjugated hyperbilirubinemia are highly predictive of pituitary dysfunction.[130] For any infant suspected or confirmed to have CNPAS, an endocrinology consult and evaluation is recommended to monitor for a disorder of the hypothalamic-pituitary axis that can include diabetes insipidus, adrenal insufficiency, hypothyroidism, growth hormone deficiency, and hypogonadism. Early diagnosis and appropriate hormonal replacement are crucial as morbidity

and mortality are risks among the cohort of neonates that have respiratory distress and may need surgery.[130] Recurrent hypoglycemic seizures, electrolyte imbalance, and subsequent cardiac arrest have been reported in infants with CNPAS.[131]

Nutrition can be supported with supplemental nasogastric or orogastric feeds, and continued oral trials are appropriate as respiratory status allows. Working with a feeding therapist is key to developing a safe feeding plan, including pacing, preventing overexertion, and providing a positive feeding experience for the neonate.

Management and Screening

Once diagnosed, treatment of CNPAS can be conservative or surgical, depending on the degree of obstruction and severity of associated symptoms. Initial medical management consists of saline irrigation, intranasal steroids, and topical decongestants with the timing of medications optimized for feeds. Conservative management should be attempted for up to 2 weeks before considering surgery.[129]

During this time, it is paramount to evaluate the neonate for endocrine dysfunction, electrolyte abnormalities, CNS anomalies, or any other associated issues. Brain MRI is recommended for all infants with CNPAS. An ectopic posterior pituitary, anterior pituitary aplasia/hypoplasia, and a pituitary stalk abnormality (in descending order of specificity) on MRI is highly suggestive of pituitary dysfunction, and a structurally normal pituitary does not rule out pituitary dysfunction. Endocrine follow-up through at least 1 year of life is recommended for all infants with CNPAS, and low or plateaued linear growth at 1 year is a predictor of long-term pituitary dysfunction.[130] Given the rarity of CNPAS and potential concomitant clinical abnormalities and syndromes that would affect management, genetic consultation is warranted.

Failure of medical management is determined by persistence of symptoms: stertor, wheezing, increased work of breathing, inability to wean from respiratory or airway support, sleep apnea, inability to feed, or failure to thrive.[125] In these cases, before performing surgery, an alternative procedure has emerged using dilation of the anterior nasal opening. This technique uses a balloon or dilators to provide slight concentric pressure on the bone/cartilage tissue that has natural plasticity due to the presence of circulating maternal estrogens.[132]

Ultimately, surgical intervention may be needed for infants with persistent symptoms. The mean PA width in neonates needing surgical intervention varied from 4.8 mm to 6.6 mm.[133] The most common technique is a sublabial approach where the excessive bony growth around the PA is removed to widen the bony nasal entry. It is considered sufficient to open each side so that at least a 3.5-mm endotracheal tube can be passed without difficulty. Postoperatively, nasal stents are generally left in place for 2 to 4 weeks; however, there is no standardization of technique or timeline for stent usage. Some otolaryngologists may recommend topical or parenteral steroids after surgery. Newer techniques are emerging using endoscopic repairs or using silicon splints along the nasal septum. As with any nasal surgery, postoperative nasal irrigation with saline solution is important to clean the nasal passages and maintain the patency of a stent or other surgically placed foreign body.[125] Infants who have surgical treatment are found to have good long-term success, with few needing subsequent nasal airway surgery.[133]

Prenatal Screening for Fetal Face Anomalies

The American College of Obstetricians and Gynecologists recommend routine surveillance of pregnancy with an ultrasound at 18 to 22 weeks' gestation and have included essential elements to a standardized examination of fetal anatomy that now includes visualization of the upper lip.[134] Most studies estimating the incidence of prenatal recognition and diagnosis of orofacial clefting focus on this anatomic examination. Adequate evaluation of the facial structures with ultrasonography can be achieved by 16 to 17 weeks' gestation. However, the accuracy of this evaluation is impacted by multiple factors such as fetal size, position, limb positioning, and movement; maternal factors such as maternal abdominal scars and maternal body habitus; and other factors including oligohydramnios, type or technology of ultrasound machine, and experience of the ultrasonographer.[134,135] Ideally, many facial features can be visualized with routine two-dimensional ultrasonography at 18 weeks' gestation with standard 3-plane facial views: orbital size and position, eye size (including microphthalmia and anophthalmia), shape of nose, nasal hypoplasia, length of the philtrum, clefts of the upper lip, frontal bossing, retrognathia, micrognathia, macroglossia, and soft tissue abnormalities.[136]

Cleft lip with or without cleft palate can be detected by prenatal ultrasonography, whereas cleft palate only (CPO) without lip and alveolar involvement may be obscured by the tongue, thus making a prenatal diagnosis of CPO more difficult using traditional 2D ultrasound. A systematic review evaluating the diagnostic accuracy of orofacial clefts detected by second-trimester anatomy scans showed that with 2D ultrasound, detection of CL±P was 9% to 100%, and only 0% to 22% in cases of CPO. Using 3D and 4D ultrasounds, detection was 86% to 90% for CL±P and 0% to 89% of cases of CPO.[137] Advances in ultrasound technology and techniques allow ultrasonographers to provide a more detailed, comprehensive, and systematic evaluation of fetal anatomy and should improve detection rates of all craniofacial conditions.[138]

Among high-risk pregnancies, fetal MRI has higher accuracy in confirming and diagnosing anomalies of the head, face, and neck[135,139] and extracraniofacial anomalies otherwise not detected on ultrasound.[140] Some centers have started using fetal MRI to predict the need for immediate neonate airway intervention due to airway compromise caused by glossoptosis in Robin sequence,[141] micrognathia, or craniofacial masses.[142] When fetal airway compromise is anticipated based on prenatal imaging with polyhydramnios, severe micrognathia, mass induced in utero neck extension, neck vessel compression,[142] tracheal compression/deviation, or a solid neck mass,[143] delivery may be coordinated at a tertiary care center with obstetric, neonatology, pediatric otolaryngology, and pediatric anesthesia cooperation to perform an ex utero intrapartum treatment (EXIT) procedure. EXIT procedure allows controlled treatment of fetal airway obstruction while maternal-fetal circulation is maintained as a bridge to a secure airway, resection of obstructing lesion, or onto ECMO for severe cardiac anomalies or congenital diaphragmatic hernias.[142]

Improvement in fetal imaging modalities has shifted the diagnosis of craniofacial anomalies from detection at birth to prenatal diagnosis, and this facilitates parental counseling and planning of delivery and postnatal treatment.[135] Prenatal counseling with a craniofacial team has been shown to decrease negative parental perceptions of orofacial clefting[144] and reduce rates of postpartum

depression.[145] Families who meet members of an experienced craniofacial team before delivery have the opportunity to build trust with their baby's providers, to know what to expect after their child's birth, and to be armed with knowledge, tools, and partners to help their child receive the best care possible.

Acknowledgment

Thank you to our mentors and authors of the previous version of this important craniofacial chapter: Michael L. Cunningham MD, PhD, and Anne V. Hing, MD.

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Common Neonatal Orthopedic Conditions

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KEY POINTS

- Developmental dysplasia of the hip represents a spectrum of diseases. All infants should be screened by physical examination; selective imaging based on risk factors is recommended.
- Most cases of congenital muscular torticollis resolve spontaneously. Physical therapy and surgery are reserved for recalcitrant cases.
- A variety of foot deformities are common and can be encountered in the neonate. Stretching, casting, or surgery may be required for resolution.
- Torsional and angular deformities of the lower extremities must be differentiated from physiologic variants. Asymmetry and rapid progression are the hallmarks of pathologic variants.
- Congenital vertebral anomalies result from failures of formation or segmentation of spinal elements. Spinal deformities such as scoliosis or kyphosis may ensue.
- Although orthopedic afflictions of the newborn are generally not life threatening, they do have the potential to significantly impair functional performance, even when diagnosed and treated early. This chapter discusses the most commonly encountered of these orthopedic problems.

Developmental Dysplasia of the Hip

The term *developmental dysplasia of the hip* (DDH) encompasses a spectrum of diseases from acetabular dysplasia to hips that are located but unstable (femoral head can be moved in and out of the confines of the acetabulum), to frankly dislocated hips in which there is a complete loss of contact between the femoral head and acetabulum. Although geographic and racial variations have been reported, DDH occurs in 11.5 of 1000 infants, with frank dislocations occurring in 1 to 2 per 1000.[1] Studies have suggested that breech positioning, family history of DDH, limited hip abduction, talipes, female sex, swaddling, large birth size, and first-born infants have all been associated with a higher probability of finding DDH.[2] The left hip alone is affected in 60% of infants, the right hip alone is affected in 20% of infants, and both hips are affected in 20% of infants.[3]

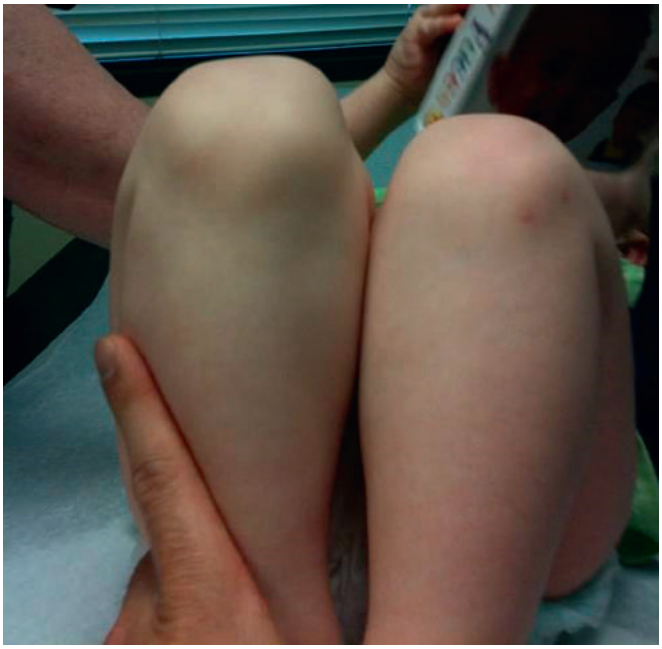
With regards to dislocated hips, they can be divided into two groups: syndromic and typical. *Syndromic* dislocations are most frequently associated with neuromuscular conditions such as myelodysplasia and arthrogryposis or with syndromes such as Larsen syndrome. Syndromic dislocations probably occur between week 12 and week 18 of gestation.[1] *Typical* dislocations occur in otherwise healthy infants in the third-trimester prenatal period or postnatally.

Congruent reduction and stability of the femoral head are necessary for normal growth and development of the hip joint. The natural history of untreated DDH is controversial as newborn hip instability may resolve or progress to painless dislocation. In cases that progress to subluxation, individuals have significantly increased risk of developing precocious arthritis with moderate to severe hip pain as young adults.[4,5] This pain can be debilitating and the reconstruction difficult. Early detection and treatment of DDH are thus important in avoiding the devastating sequelae of a late diagnosis.

While the physical exam of an infant hip is paramount to the diagnosis of DDH, there are no pathognomonic signs of a dislocated hip. The physical examination requires patience on the part of the examiner and may be facilitated by having the baby feed from a bottle or swaddling the arms. Communication between providers is encouraged if the practitioner examining the newborn in the hospital is different from the 2-week follow-up examiner. The presence of asymmetric hip abduction is suggestive of a unilateral dislocation. Limitation of hip abduction in babies older than 12 weeks is the most reliable examination finding suggestive of DDH. Hip abduction of 75 degrees should be possible in most newborns. The Galeazzi sign is elicited with the baby placed supine on an examining table so that the pelvis is level, with the hips and knees flexed to 90 degrees. With the baby's hips in neutral abduction, the examiner determines if the knees are at the same height. If one femur appears shorter than the other, the hip may be dislocated posteriorly (Fig. 89.1). Each of these signs, individually or in combination, may serve to increase the index of suspicion of the examiner and lower the threshold for further diagnostic studies or referral to a pediatric orthopedist. A unilateral dislocated hip may result in asymmetric thigh folds; however, extra thigh folds are a normal variant and do not necessarily indicate hip dislocation. *It is important to note that in an infant with bilateral hip dislocations, the Galeazzi sign will be negative and the hip abduction symmetric.*

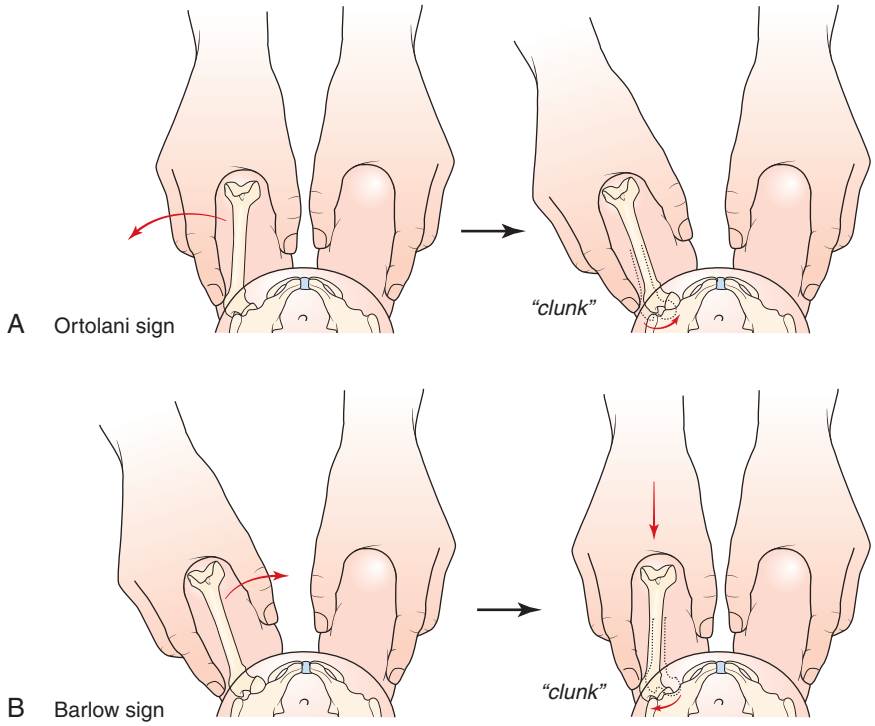
There are two common methods of assessing hip stability in the newborn (Fig. 89.2). The Ortolani test aims to reduce a dislocated hip. This is performed on one leg at a time, with the infant supine on the examining table. The index and middle fingers of the examiner are placed along the greater trochanter, while the thumb is placed on the medial aspect of the thigh. The pelvis is stabilized

by the placing of the thumb and ring or long finger of the opposite hand on top of both anterior iliac crests simultaneously. The hip is flexed to 90 degrees and gently abducted while the leg is lifted with the hip in neutral external/internal rotation. A palpable clunk is felt as the dislocated femoral head reduces into the acetabulum. This finding is reported as the Ortolani sign (positive result on the Ortolani test). The Barlow test is an attempt to dislocate or sublux-ate a located but unstable hip. The thigh is held, and the pelvis stabilized in the same manner as for the Ortolani test. With the hip in neutral external/internal rotation and at 90 degrees of flexion, the leg is then gently adducted with a mild posteriorly directed pressure applied to the knee. A palpable clunk or sensation of posterior movement constitutes a positive result (i.e., the Barlow sign). High-pitched clicks are frequently elicited with hip range of motion. These sounds are most frequently attributed to snapping of the iliotibial band over the greater trochanter and are not associated with dysplasia.[6] With progressive soft tissue contractures and loss of ligamentous laxity, both the Ortolani test and the Barlow test become unreliable after approximately 3 months of age.



• **Fig. 89.1** Presence of Galeazzi Sign.

Imaging of the immature hip can be a valuable adjunct to the physical examination. An anteroposterior (AP) radiograph of the pelvis can be difficult to interpret before the age of 4 to 5 months as the femoral head is composed entirely of cartilage until the secondary center of ossification appears. Before the appearance of the secondary center, ultrasound examination is the method of choice for visualizing the cartilaginous femoral head and acetabulum. Static ultrasound images allow visualization of acetabular and femoral head anatomy, while the complementary dynamic images give information on the stability of the hip joint.[7,8] The primary limitation of hip ultrasonography is that the results are dependent on the experience and skill of the operator, especially when performed within the first 3 weeks after birth.[9] For these reasons, ultrasonography is recommended as an adjunct to clinical evaluation rather than as an independent screening tool.[1] Studies conducted before 6 weeks after birth may be useful for confirming equivocal physical examination findings and for monitoring treatment of hips with known dislocations. Clinicians must be aware, however, that ultrasound images in this age group often reveal minor degrees of dysplasia (physiologic immaturity) that usually resolve spontaneously and may lead to overtreatment of physiologic hip variations. Ultrasonography is the technique of choice for assessment of infants at high risk of DDH after 4 to 6 weeks of age and again is useful in following up the results of



• **Fig. 89.2** Assessing Hip Stability. (A) Ortolani-positive hips are those where the dislocated hip can be relocated. (B) Barlow-positive hips are reduced but can be dislocated.

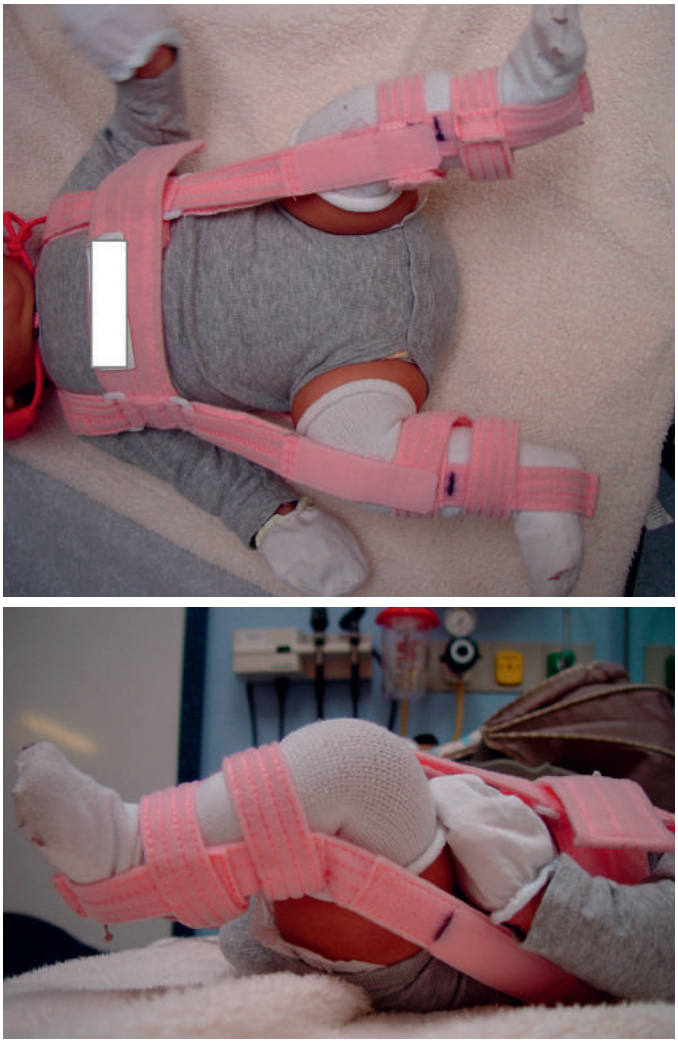
intervention. After 6 months of age, the gold standard remains the AP radiograph of the pelvis.

All newborns should be screened for DDH by a properly trained healthcare provider by physical examination. Risk factors for DDH should be determined by the treating physician. A Cochrane review found that neither universal nor targeted ultrasound screening strategies have been demonstrated to improve clinical outcomes, including the incidence of late-diagnosed DDH and need for surgery.[10] Adding further confusion to the debate over the approaches to optimal DDH screening procedure is a report by the US Preventive Services Task Force, which found "insufficient evidence" to recommend *any* routine DDH screening, including physical examination.[11] This recommendation was based on the lack of clear evidence for the efficacy of infant screening to reduce the incidence of late-presenting DDH. In response to these findings, the American Academy of Orthopedic Surgeons (endorsed by the American Academy of Pediatrics, the Pediatric Society of North America, and the Society for Pediatric Radiology) has published a revised clinical practice guideline to aid in the early diagnosis of and initiation of appropriate intervention for DDH.[12] These recommendations are summarized as follows:

1. Universal ultrasound screening. Moderate evidence supports not performing universal ultrasound screening of newborn infants.
2. Evaluation of infants with risk factors for DDH. Moderate evidence supports performing an imaging study before 6 months of age in infants with one or more of the following risk factors: breech presentation, family history, or history of clinical instability.
3. Imaging of the unstable hip. Limited evidence supports that the practitioner might obtain an ultrasound image in infants younger than 6 weeks of age with positive instability examination findings to guide the decision to initiate brace treatment.
4. Imaging of the infant hip. Limited evidence supports the use of an AP radiograph of the pelvis instead of an ultrasound image to assess DDH in infants beginning at 4 months of age.
5. Surveillance after normal findings from an infant hip examination. Limited evidence supports that a practitioner reexamine infants previously screened as having normal hip examination findings on subsequent visits before 6 months of age.
6. Stable hip with ultrasound imaging abnormalities. Limited evidence supports observation without a brace for infants with a clinically stable hip with morphologic ultrasound imaging abnormalities.
7. Treatment of clinical instability. Limited evidence supports either immediate or delayed (2 to 9 weeks) brace treatment for hips with positive instability examination findings.
8. Type of brace for the unstable hip. Limited evidence supports use of the von Rosen splint over Pavlik, Craig, or Frejka splints for initial treatment of an unstable hip.
9. Monitoring of patients during brace treatment. Limited evidence supports that the practitioner perform serial physical examinations and periodic imaging assessments (ultrasound or radiograph depending on age) during management for unstable infant hips.

If there are no risk factors, then serial examinations are recommended according to a standard periodicity schedule until the child is 6 months old. If during these periodic visits physical findings raise suspicion of DDH, or if a parental concern suggests hip disease, confirmation is recommended by an expert physical examination, by referral to a pediatric orthopedist (or other practitioner with expertise in medical and surgical management of newborn hip disease), or by age-appropriate imaging. When a positive Ortolani or Barlow test is present at birth and persists beyond the usual age of spontaneous resolution (2 to 9 weeks), the infant should be referred to an orthopedist for management. However, if the positive Ortolani or Barlow test disappears, then age-appropriate imaging (ultrasonography at 6 weeks or radiograph by 6 months) is warranted. If the infant has positive risk factors, such as breech positioning at birth or a family history but stable hip examination findings, then age-appropriate imaging is recommended (ultrasonography at 6 weeks or radiograph at 6 months).

Treatment of DDH is dependent on the age at presentation. Although previously recommended, double diapering is not an accepted form of treatment in DDH. For children aged 0 to 6 months, a reducible hip is treated in a Pavlik harness or another appropriate orthosis. The Pavlik harness is a dynamic orthosis that allows the infant to actively move the hips through a sphere of motion that encourages deepening and stabilization of the acetabulum (Fig. 89.3). The harness is applied as soon as possible after the diagnosis of DDH is made. The length of treatment is dependent on the age at presentation and severity of dysplasia. Progress



• **Fig. 89.3** The Pavlik Harness. Lightweight orthotic, useful in treatment of neonatal developmental dysplasia of the hip (DDH). The device holds the hip in flexion and abduction, promoting optimal positioning of the femoral head in the acetabulum. Excessive flexion and abduction should be avoided.

is judged by serial physical examinations and ultrasonography. In the case of a frankly dislocated hip, treatment is abandoned if the hip is not reduced within 4 weeks of harness application. The success of Pavlik harness treatment is variable and correlates with the severity of the hip dysplasia. Treatment is successful in nearly 100% of stable hips, greater than 90% of dislocatable (Barlow-positive) hips, 61% to 93% of dislocated but reducible (Ortolani-positive) hips, and only 40% of irreducible dislocations.[13–17]

For a persistently irreducible dislocation, or a child that presents late with a dislocated hip (after 6 months of age), either closed or open reduction of the hip under general anesthesia, with subsequent spica casting, is often required.[18]

Foot Deformities

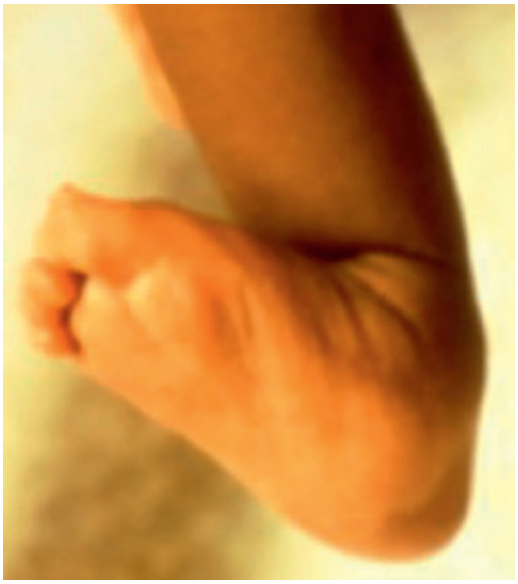
Congenital deformities of the foot are relatively common but often overlooked in newborns. Consequently, the true incidence of the milder, self-limited deformities is unknown. For identification purposes, congenital foot abnormalities can be divided into those that result in the toes pointing upward (calcaneovalgus, congenital vertical talus), those that result in the toes pointing inward (metatarsus adductus, clubfoot), and those with too many toes or toes stuck together (polydactyly, syndactyly).

Calcaneovalgus is thought to be a postural deformity secondary to intrauterine positioning in which the dorsum of the foot is, or can be, directly opposed to the anterior aspect of the leg (Fig. 89.4). Plantar flexion of the foot is often limited from contracture of the anterior ankle and lateral soft tissues. The estimated incidence of calcaneovalgus is 0.4 to 1 per 1000 live births.[19,20] It appears to be more common in girls and after breech deliveries.[20] There may be an increased association with hip dysplasia, so a thorough hip examination is warranted, as outlined in Developmental Dysplasia of the Hip.[21] Complete resolution with gentle stretching exercises conducted by the parents can be achieved, although generally occurs spontaneously by 3 to 6 months of age. In the more severe calcaneovalgus feet where the ankle cannot be plantar flexed past the neutral position, serial casting to facilitate correction is often required. Calcaneovalgus may be seen in conjunction with external rotation of the tibia and posteromedial bowing of the tibia. A deformity that fails to resolve mandates referral to a pediatric orthopedist.

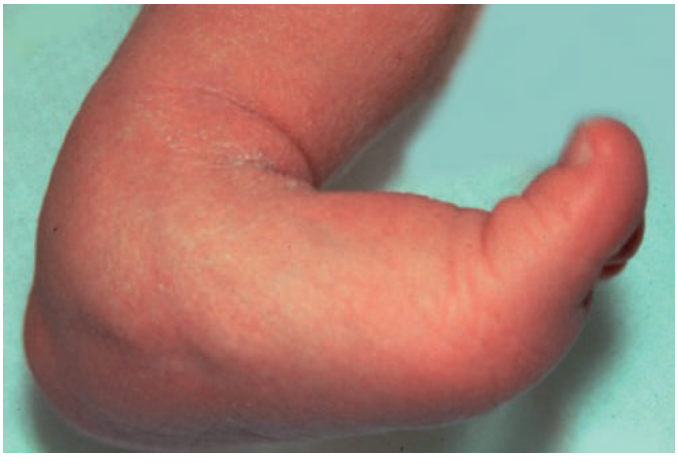
Calcaneovalgus should be differentiated from congenital vertical talus (CVT), a rarer condition that is frequently associated with neuromuscular conditions and syndromes such as arthrogryposis and spina bifida.[22] In CVT the hindfoot is fixed in equinus (plantar flexion), giving the sole of the foot a characteristic "rocker bottom" appearance because of dorsal dislocation of the midfoot though the talonavicular joint (Fig. 89.5). Treatment during infancy consists of serial casting to stretch dorsal soft tissues and reduce the midfoot, followed by limited surgical release if needed, pinning of the talonavicular joint, and Achilles tenotomy.[23] Most children require surgery between 6 and 12 months of age, and best outcomes are achieved when surgery is performed before age 2 years.[24] When casting fails to reduce the midfoot, more extensive surgical releases are required.

The two common neonatal foot deformities resulting in medial deviation of the toes are metatarsus adductus and talipes equinovarus (clubfoot). Metatarsus adductus is present at birth but frequently diagnosed later during the first year of life. It has been estimated to occur in 1 in 100 births[25] and is thought to result from intrauterine crowding.

Characteristic features include a concave medial border of the foot with a curved lateral border, a "bean-shaped" sole of the foot, a higher-than-normal-appearing arch, and a neutral heel (Fig. 89.6).[26] Metatarsus adductus can be classified into cases that undergo passive correction and those that do not. Feet which passively correct are best left alone and will improve spontaneously. Feet in which passive correction is not possible (the curved lateral border cannot be straightened) should be treated with manipulation and serial casting by age 6 to 9 months. The corrections can then be maintained with reverse or straight last shoes if necessary. Operative treatment should be considered only in children older than 3 years who have a rigid deformity and have failed to respond to a casting program.[27]



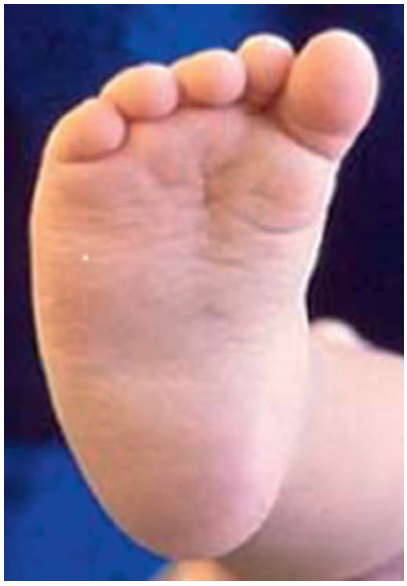
• **Fig. 89.4** Calcaneovalgus Foot. (From *Pediatric Pes Planus JAAOS* Oct. 2015, Bouchard M, Mosca VS. Flatfoot deformity in children and adolescents: surgical indications and management. *J Am Acad Orthop Surg*. 2014;22:623–632.)



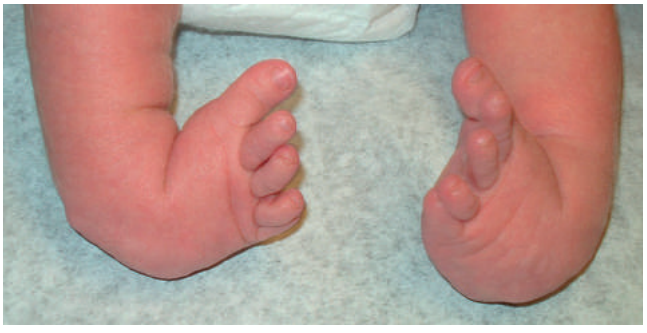
• **Fig. 89.5** Congenital Vertical Talus. (From *Pediatric Pes Planus JAAOS* Oct. 2015, Bouchard M, Mosca VS. Flatfoot deformity in children and adolescents: surgical indications and management. *J Am Acad Orthop Surg*. 2014;22:623–632.)

The term *clubfoot* describes a foot with hindfoot equinus, heel varus, and adduction and supination of the forefoot (Fig. 89.7). Clubfoot deformities range from mild to severe and occur in 1 in 1000 to 2 in 1000 live births.[28] A risk factor for clubfoot is early amniocentesis (11 to 13 weeks' gestation), which is hypothesized to cause decreased fetal movement during a critical phase of foot development.[29] Although the cause of clubfoot remains unproven, there appears to be dysplasia of all osseous, muscular, tendinous, cartilaginous, skin, and neurovascular tissues distal to the knee in the affected limb.

The mild, "postural" clubfoot appears to represent a packaging problem due to intrauterine positioning. This deformity is passively correctible, demonstrates minimal or no calf atrophy, and resolves spontaneously or responds quickly to a stretching and casting regimen. At the opposite end of the spectrum is the arthrogrypotic or neuromuscular clubfoot that demonstrates severe rigidity. Between these two extremes lies the classic, idiopathic clubfoot deformity. Idiopathic clubfeet demonstrate a deep, single medial skin crease, curved lateral border with a high arch, and rigid varus and equinus of the heel with a deep, single, posterior skin crease.[30] This gives the foot its "down and in" position and toes pointing to the midline. In unilateral cases the affected limb has a smaller foot and calf circumference (see Fig. 89.7).



• **Fig. 89.6** The Appearance of the Foot with Metatarsus Adductus. (Courtesy Dr. Vincent S. Mosca, Seattle Children's Hospital, Seattle.)



• **Fig. 89.7** The Appearance of an Untreated Newborn Clubfoot. (Courtesy Dr. Vincent S. Mosca, Seattle Children's Hospital, Seattle.)

All clubfoot deformities should be referred to a pediatric orthopedist for treatment. Initial treatment for all cases of congenital clubfoot is nonoperative. Untreated clubfoot has a poor natural history, with development of early degenerative changes in the foot joints. Historically, clubfeet were treated with early and extensive surgical correction. The long-term results, however, are poor, with high recurrence rates.[27] Consequently, this approach was abandoned, and surgeons began advocating nonoperative methods of clubfoot correction.[31–33] Although many forms of nonoperative clubfoot treatment exist, the Ponseti method of cast correction has achieved preeminence in this regard. Studies show excellent mid-term to long-term results with decreased stiffness.[34]

The Ponseti method uses a specific set of manipulations and serial corrective long-leg casts, followed by a prolonged period of bracing. Treatment is ideally commenced within the first few weeks after birth, but successful treatment is commonly achieved when treatment is initiated up to 1 year of age.[35] We prefer to initiate treatment 1 to 2 weeks after discharge from the hospital to allow parental adjustment for the new infant at home. Every 5 to 7 days, manipulation of the foot is performed with passive stretching, and the correction is maintained with a new long-leg cast, with an average of four to five casts in the idiopathic clubfoot.[23] This is followed by percutaneous Achilles tenotomy in most patients and a further 3 weeks of casting. Children are then placed into a foot abduction orthosis full-time for a period of 3 months and then part-time, while sleeping, until approximately age 4 years.

The "French functional method" has also been successfully duplicated in at least one US hospital with good results.[28,36] This method necessitates daily manipulations by a trained physical therapist for 8 weeks, with the addition of continuous passive motion during the first 4 weeks. This is followed by strapping and continued bracing.

The Ponseti and the French "nonoperative" methods both frequently use Achilles tenotomy and, at times, tendon transfers to attain the ultimate desired result. Recurrences of deformity are common (16% to 37%), requiring further casting. A smaller percentage of patients (8% to 16%) require surgical release of the hindfoot to various degrees.[36,37]

Torticollis

Congenital muscular torticollis (CMT) manifests itself at birth or soon, thereafter, and is the most frequent cause of wryneck. However, other conditions, some more serious, may cause torticollis.

1. Bony anomalies of the vertebra and skull (e.g., Klippel-Feil syndrome, hemivertebrae, basilar invagination, craniosynostosis)[38–40]
2. Abnormalities of the central nervous system (e.g., syringomyelia, tumors)[41]
3. Chiari malformations[42]
4. Ocular abnormalities[43]
5. Pharyngeal abscess
6. Gastroesophageal reflux (e.g., Sandifer syndrome).[44]

Patients with CMT can be divided into those who demonstrate a sternocleidomastoid muscle (SCM) "pseudotumor," those with tightness or fibrosis of the SCM without pseudotumor (termed *muscular torticollis*), and those with all the characteristic features of congenital torticollis without evidence of contracture or fibrosis of muscle (termed *postural torticollis*).[45]

CMT has been estimated to occur in 0.3% to 2.0% of live births.[45] It is usually discovered between 6 and 8 weeks after birth. Infants present with a cock robin appearance, with the head tilted toward and the chin rotated away from the affected SCM. 20-30% of patients will have a palpable pseudotumor present in the middle to inferior aspect of the affected SCM, which spontaneously regresses with time, leaving a fibrous band.[46] More than half will have facial asymmetry or plagiocephaly. The left and right SCMs are affected in equal proportions. CMT probably results from ischemia within the SCM, leading to fibrosis.[47] The cause of the ischemia is unknown, but intrauterine crowding may play a role, in as much as some authors have reported an association of torticollis with other deformations, such as DDH and metatarsus adductus.[48,49]

Excellent results with a manual stretching program can be attained in children first seen before 1 year of age.[48,50] Initially, the parents are instructed in the technique of stretching the contracted SCM by rotating the infant's chin toward the affected SCM while simultaneously tilting the head away from it. This is completed 10 times each session and held for a count of 10 and done at least 10 times per day. Unfortunately, adherence may be an issue. If the child fails to improve substantially within 3 to 4 weeks, a physical therapist is enlisted to see the child two or three times weekly to supervise the program and reinforce the home therapy. Additionally, parents are instructed to configure the infant's crib and toys in such a manner as to encourage active rotation toward the involved side.

Surgery is not warranted in any child younger than 1 year or in any child who has not completed a minimum of 6 months of therapy.[45,48] In a prospective study of 821 children with muscular torticollis, only 8% of patients with a history of a pseudotumor, and 3% of those without, required surgical intervention following a well-structured stretching program.[45] Because of the difficulty of monitoring exercise programs, because parental adherence is always in question, and because surgical intervention is infrequent, it is possible that in many patients the resolution is spontaneous. No patients with postural torticollis require surgery. Risk factors for surgery include late initial presentation, presence of a pseudotumor, and rotation deficit of greater than 15 degrees.

The timing of surgical intervention remains controversial. In patients with significant plagiocephaly and facial asymmetry, surgery should be considered just before 2 years of age to maximize the chance for complete remodeling. For those with either no or mild facial asymmetry, good to excellent results can be expected with surgery up to 6 years of age.[51] Acceptable results are reported as late as 12 years of age, but the ability to remodel facial asymmetry appears diminished.[52] More recent literature suggests good surgical outcomes in neglected CMT even after 15 years of age.[53] Surgery entails bipolar release or lengthening of the SCM through cosmetically pleasing incisions.[54] The use of a molded helmet to promote facial and skull remodeling is common. Prospective studies that establish the effectiveness of helmets are lacking, and in at least one study was found to be possibly detrimental.[55]

A less frequent cause of congenital torticollis is osseous fusion between bones in the cervical spine. These fusions may be between the skull and C1 and/or C2 or in the lower cervical spine. They result from failure of the bones to properly segment during embryogenesis. These abnormalities, in combination with a low posterior hairline and a short, webbed neck with limited range of motion and head tilt, constitute the triad referred to as Klippel-Feil syndrome (KFS).[56] These congenital bone fusions can range from involvement of two segments to involvement of the entire cervical spine. Colloquially, KFS has come to refer to any congenital malformation in the cervical spine with or without other elements of the triad. Most cases of KFS do not have a genetic etiology, however additional forms of KFS include autosomal recessive KFS2 caused by mutation in the MEOX1 gene, autosomal dominant KFS3 caused by mutation in the GDF3 gene, and autosomal recessive KFS4 caused by mutation in the MYO18B gene.

In infants and young children, the neck may remain quite flexible despite the bone abnormalities. In a newborn with torticollis who does not improve with passive stretching exercises, radiologic evaluation is mandatory. Cervical spine radiographs are not recommended in all patients initially presenting with neonatal torticollis, as these radiographs are quite difficult to interpret in this age group because of the predominance of cartilage in the bones of the neck. Furthermore, many neonates would be subjected to unnecessary ionizing radiation.

The natural history of KFS in most cases is quite favorable, requiring nothing more than periodic observation. In patients with severe involvement, however, the consequences of this disorder can include early spondylosis with the development of pain or stenosis, the development of progressive torticollis and scoliosis, and the occurrence of neurologic compromise and sudden death secondary to even minimal trauma.[46] Despite these potentially devastating sequelae, the greatest advantage of early detection of KFS is in being alerted to commonly associated disorders, including congenital heart disease (14% to 29%), renal anomalies (25% to 35%), scoliosis (60%), audiologic anomalies (80%), including deafness (15% to 35%), synkinesis (15% to 20%), and less commonly, posterior fossa desmoid tumors.[46,57] The recognition of a Klippel-Feil anomaly should prompt a thorough evaluation for these associations. Treatment of KFS most often involves periodic observation with activity modification. In the face of progression of deformity or severe deformity, surgical intervention may be warranted.

Sandifer syndrome (gastroesophageal reflux) can also cause a torticollis. With this syndrome the torticollis is intermittent and may change direction, and there is no tightness of the SCM, with normal findings on radiographs.[44]

Hemiatlas, or the failure of formation of a portion of the first cervical vertebra, is also a rare cause of torticollis.[39] In an infant the neck may be quite flexible and the torticollis passively correctable. An open-mouth (odontoid view) cervical spine radiograph reveals this deformity. If the torticollis is progressive or severe, gradual correction of the deformity with a halo vest followed by posterior occiput to cervical spine fusion is necessary. Other potential causes of torticollis in the neonate include central nervous system tumors and syringomyelia. If radiographs appear normal, a thorough neurologic examination and referral to a neurologist are recommended.

Torsional and Angular Deformities of the Lower Extremities

Torsional and angular deformities of the legs constitute the most frequent nontraumatic reason for referral to a children's orthopedist. Torsional deformities of the lower extremities rarely come to the attention of the physician before the child reaches walking age. Neonates often demonstrate bowing of the legs, or genu varum, but are rarely concerning to parents prior to walking age. Internal tibial torsion imparts an appearance of bowing to the tibia,[58] which is often concerning to both the parent and the physician. The true

incidence of genu varum is unknown, but in our experience, it is extremely common. Both genu varum and internal tibial torsion are nearly universal in neonates. Both should spontaneously resolve between 2 and 3 years of age, with a small minority of affected children manifesting a pathologic condition.

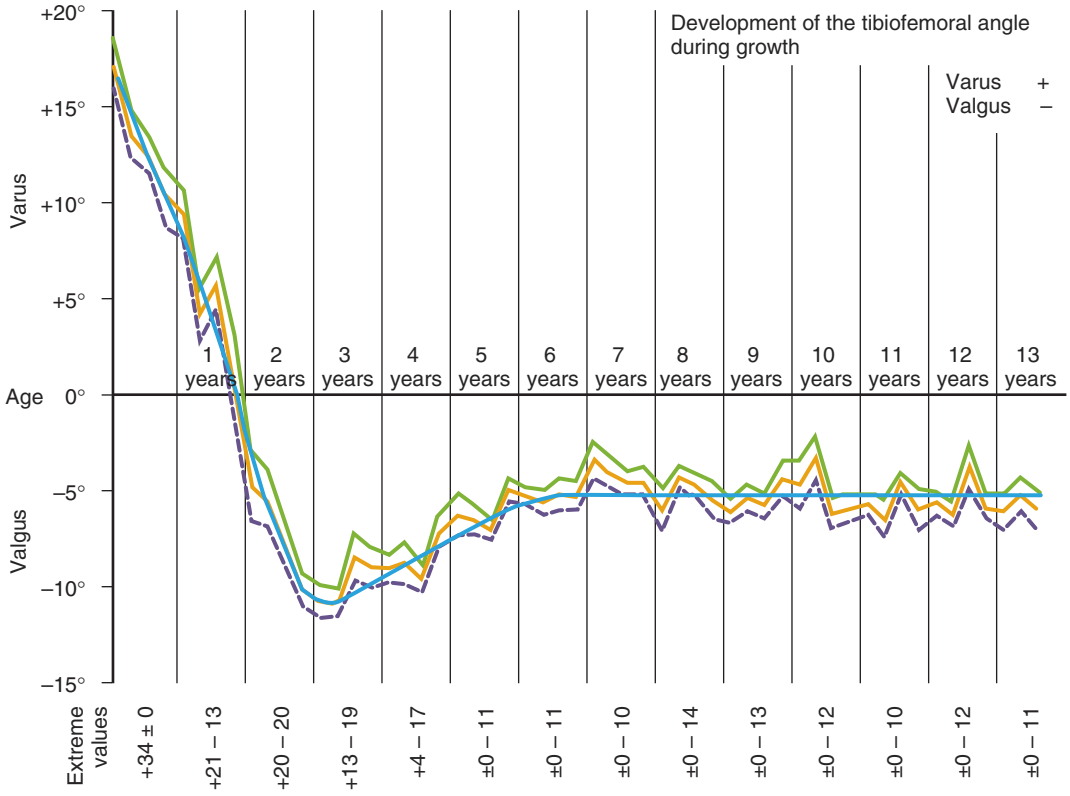
Genu varum is physiologic up to the age of 2 years. In 1975, Salenius and Vankka[59] documented the tibiofemoral angles both clinically and radiographically in 979 children based on 1408 examinations between birth and 16 years of age. They noted that newborns demonstrate a mean varus alignment of 15 degrees, which increases and becomes maximal at 6 months of age and then decreases to neutral at approximately 18 months. The maximum valgus (knock knees) of 12 degrees is then achieved by 3 to 4 years. By age 7 years, normal adult valgus alignment is achieved (Fig. 89.8). Natural history studies have demonstrated that physiologic genu varum is a self-limited process, and even with an angulation greater than 30 degrees has been shown to undergo correction spontaneously with growth.[60] Management of physiologic genu varum and tibial torsion consists of serial observation, parental reassurance and education. Treatment with an orthosis is not recommended.

Physical examination should include evaluation of the torsional profile,[61] which includes measurements of internal and external rotation of the hips, the thigh–foot angle, and the patient's foot progression angle when walking. Measurement of the thigh–foot angle is performed with the child in the prone position comparing the axes of the sole of the foot with the thigh and is an indicator of tibial torsion.

It is important to note whether onset of the varus of the lower extremities is gradual or abrupt, and if the deformity can be localized to the distal part of the femur, the proximal part of the tibia, or the midportion of the tibia. Radiographs are indicated only with asymmetric deformities, with short stature, persistent varus past 2 years of age, or in infants with progressive deformities. The examiner should also look for evidence of rhizomelic shortening and genu varum, which may herald a diagnosis of achondroplasia or other skeletal dysplasia.

Considerations in the differential diagnosis of genu varum include focal fibrocartilage dysplasia, skeletal dysplasias, posttraumatic physeal growth arrests, osteogenesis imperfecta (OI), and metabolic bone disease such as vitamin D–resistant rickets, renal osteodystrophy, and tibia vara (infantile Blount disease). Blount disease is bilateral in 80% of affected children and does not occur before walking age. Most clinicians agree that this diagnosis cannot be made before 2 years of age.

Tibial bowing can also occur in the sagittal plane. There are two major types of bowing distinguished by the direction of the apex of the bow. Posteromedial bowing has been previously described in conjunction with calcaneovalgus foot position in the neonate. Its cause is unknown, but numerous hypotheses have been proffered, including intrauterine fracture with malunion and in utero malpositioning with subsequent growth retardation and soft tissue contractures.[62] The deformity is unilateral and evident at birth. Other features include shortening of the tibia and a smaller calf circumference and smaller foot relative to the contralateral side. Frequently there is a skin dimple at the apex of the deformity. Radiographic examination of the entire extremity from hip to ankle should be performed. Radiographs demonstrate the degree of bowing and in some cases thickening and sclerosis of the diaphyseal cortices on the compression side of the deformity with obliteration of the intramedullary canal. There is no increased fracture risk associated with the deformity.



• **Fig. 89.8** Development of the Tibiofemoral Angle During Growth. (Data from Salenius P, Vankka E. The development of the tibiofemoral angle in children. J Bone Joint Surg Am. 1975;57:259–261.)

Posteromedial bowing tends to resolve with growth, such that much of the deformity resolves by 2 years of age, with continued gradual correction beyond that. The shortening of the tibia and fibula persists, however, and progressively worsens during growth. Leg length inequality at skeletal maturity averages 4.1 cm.[63] Early referral to and serial follow-up assessments by a pediatric orthopedist are necessary to appropriately time epiphysiodesis surgery of the normal longer leg to allow equal leg lengths at skeletal maturity.

The second and more serious type of tibial bowing is anterolateral. It is usually identified at the newborn examination. It is unilateral and can be associated with congenital pseudoarthrosis of the tibia. Although its cause is unknown, congenital pseudoarthrosis of the tibia is associated with neurofibromatosis type 1 (NF1) in 40% to 80% of cases.[62,64,65] It is arguably the most challenging congenital malformation to treat in orthopedics. It is estimated to occur in 1 in 140,000 live births.[66] Cutaneous signs of NF1 may be evident. Early referred to pediatric orthopedics and genetics are recommended.

If fracture has occurred, motion at the pseudoarthrosis site will be apparent. The foot may be normal or slightly small. The ankle may be in slight valgus to compensate for the bowing. The natural history of congenital pseudoarthrosis of the tibia is that of fracture with nonunion and repeated surgical attempts at obtaining union. In the perambulatory child, a total-contact (clamshell) ankle–foot orthosis should be fabricated and worn full-time except for bathing, to diminish the chance of fracture. Bracing is continued until skeletal maturity is attained. Although definite proof that long-term bracing affects the natural history of this condition is lacking, most orthopedists consider that bracing is warranted. Many treatment options exist once a documented pseudoarthrosis occurs. Long-term immobilization, external fixation, internal fixation, bone transport, bone grafting, microvascular bone transfer, and electric stimulation have been attempted.[66] High failure rates are commonly reported. Amputation has been advocated as a salvage procedure after failed attempts at union and typically has good outcomes.[67,68] Herring et al.[68] reported that children who underwent Syme amputation had better psychologic and orthopedic functioning than those children who underwent numerous corrective surgical procedures. A more recent cross-union technique has been described with the goal of producing a synostosis between the tibia and fibula and has shown excellent union rates.[69]

Tibial bowing in the anteromedial direction rarely occurs and is typically seen in children with fibular hemimelia, a condition with multiple lower limb anomalies. In addition to a deficient or absent fibula, there is a strong association with absent lateral rays of the foot, a bowed tibia, knee deformities, a short femur, hip dysplasia, and leg length discrepancy. Orthopedic referral is indicated.

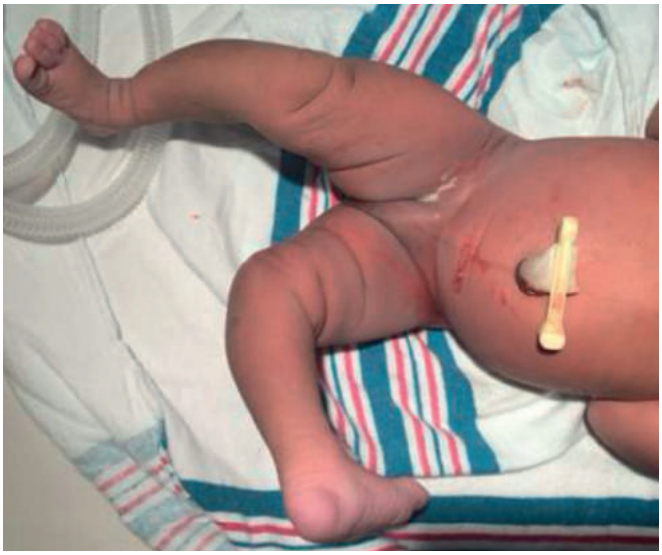
Tibial bowing may also be confused with a congenital knee dislocation. This is a rare condition, noted at birth, with a reported incidence of 0.017 per 1000.[70] The cause is unknown but most likely related to contracture of the quadriceps muscle. Congenital knee dislocations can be associated with clubfoot, arthrogryposis, myelodysplasia, and Larsen syndrome, with ipsilateral hip dysplasia occurring in 70% to 100% of cases.[71] The knee can be hyperextended so severely that the foot might even reach the child's face, and the knee cannot be flexed. Nonsurgical treatment, consisting of manipulation and serial casting, should be started promptly after radiographic diagnosis. Surgery is reserved for children who do not respond to nonsurgical treatment and is best performed at the age of approximately 6 months (Fig. 89.9).[72]

Congenital Vertebral Malformations

Congenital vertebral malformations occur in 0.5 in 1000 to 1 in 1000 live births. Although a minority of cases may be due to genetic inheritance, there are no established gene defects that solely account for these disorders. The syndromes associated with them include Klippel-Feil syndrome (KFS), Goldenhar syndrome, VATER (VACTERL) sequence, and spondylocostal dysostosis. Likewise, many congenital vertebral malformations occur in isolation and may be due to intrauterine exposures such as maternal hyperglycemia, exposure to carbon monoxide, or exposure to antiepileptic drugs. The ultimate concern with congenital vertebral anomalies is their potential to result in significant spinal deformity; namely, scoliosis or kyphosis, or a combination of the two. Many, however, remain asymptomatic throughout life.

Defects can be attributed to a failure of formation, a failure of segmentation, or both.[73] Failures of formation result from asymmetric vertebral body formation and ensuing development of a hemivertebra. Hemivertebrae can be incomplete, with partial retention of the affected side, or complete. When partial retention of the pedicle occurs, a wedge vertebra develops. Complete hemivertebra can be further categorized. Radiographically, the presence of open disk spaces signifies the presence of growth plates and therefore growth potential. Unsegmented hemivertebrae, in which the segment is fused to one vertebra or both adjacent vertebrae, have less growth potential and therefore less deformity potential. Fully segmented hemivertebrae retain full growth potential from both the cranial end and the caudal end and consequently demonstrate a much greater propensity to result in significant deformity. Failures of segmentation are characterized by bony fusions (bars) between adjacent vertebrae. Bilateral bars result in "block vertebrae" that, because of their symmetry, have minimal potential for deformity.

The propensity to result in a clinically significant deformity depends on the location of the defect, the type of defect, and the age of the patient.[74] Curves at the lumbosacral and cervicothoracic junctions may result in more clinically apparent deformities. Prediction of progression is largely driven by the presence of unbalanced defects.[73] In order of severity, the risk of progression in congenital spinal deformities is associated with the following



• **Fig. 89.9** Congenital Knee Dislocation.

defects: unilateral bar with contralateral hemivertebra, unilateral bar, hemivertebra, wedge vertebra, and block vertebra (Fig. 89.10). Additionally, the presence of multiple anomalies at multiple levels (e.g., multiple hemivertebra) can result in additional risk of progression when they are on the ipsilateral side or, conversely, may result in balanced growth when they are on contralateral sides of the spine.

All patients with known congenital spinal deformities should be evaluated for associated cardiac and renal anomalies. Cardiac anomalies are found in approximately 15% of these children and are usually evident on physical examination. Routine screening with an echocardiogram is not recommended unless clinical findings are suggestive.[75] Renal anomalies, on the other hand, are often clinically silent and have been reported in up to 37% of children with known congenital spinal anomalies.[76] Thus, routine sonography of the urinary tract system is recommended for all children with congenital spinal malformations.

Occult intraspinal anomalies are found in up to 30% of children with congenital spinal malformations. These include Chiari malformations, syringomyelia, tethered cord, reduced spinal cord diameter, and diastematomyelia. Associated physical examination findings are those consistent with occult dysraphism, such as dimpling of the skin, pigmentation changes, or the presence of hairy patches or skin tags in the lower back or intergluteal cleft. Changes to the lower extremities such as atrophy, foot deformities, and asymmetric or pathologic reflexes are also suggestive of intraspinal defects.

Infants with congenital spine anomalies should initially be evaluated with dedicated plain radiographs of the whole spine. Coned-down views of affected parts of the spine may offer additional information about the anatomy of interest. Rib anomalies should be noted, because they are commonly associated with thoracic spine malformations and may have significant long-term implications with regard to restrictive lung disease.[77] The position of the scapula should also be evaluated, because Sprengel deformity is found in up to 50% of children with congenital cervical spine anomalies.[38] The use of magnetic resonance imaging (MRI) is reserved for those children preparing to undergo surgical intervention or those with clinical evidence of neurologic abnormality.[74] Cutaneous anomalies of the lumbar spine in the newborn may be evaluated by ultrasonography. This is a particularly effective method for determining the level of the conus medullaris and thus the presence of tethered cord. Computed tomography is typically not indicated in the newborn owing to concerns of unneeded radiation exposure, but if done for other reasons, it can give additional detail on spinal anatomy.

Obstetric Trauma

Birth trauma can be divided into two categories: fractures and neurologic injuries. Birth fractures most commonly involve the clavicle, with clavicular fractures occurring in 2 to 3 per 1000 births.[78] Birth fractures may also occur in the proximal part of the humerus[79,80] the femur (0.13 per 1000 births),[81] and even the thoracic spine. It is important to note that clavicular fracture can be seen in combination with a proximal humeral physeal separation or in combination with a brachial plexus injury. Reported risk factors for upper extremity birth fractures include:

1. Large size of the baby
2. Limited experience of the obstetrician
3. Midforceps delivery[78,82]

Risk factors for femoral fracture include:

1. Twin gestation
2. Breech presentation
3. Prematurity
4. Osteoporosis[81]

Nadas et al. have reported an association of long-bone fractures with cesarean delivery, breech delivery with assistance, and low birth weight.[83]

The natural history of isolated birth fractures to the extremities is that of uneventful rapid healing without untoward sequelae. Clavicle fractures may be difficult to diagnose, because the neonate may be asymptomatic. Newborns with either a clavicle fracture or a proximal humeral physeal separation often have pseudoparalysis of the upper extremity. Considerations in the differential diagnosis include an obstetric brachial plexus palsy and hematogenous metaphyseal osteomyelitis of the humerus with septic glenohumeral arthritis. Pain with direct palpation of the clavicle may be present with obvious deformity. Pain with motion of the shoulder joint and with palpation of the proximal part of the humerus may be caused by either fracture or infection. Elicitation of neonatal reflexes such as the Moro reflex and asymmetric tonic neck reflex (ATNR) may be helpful in evaluating active upper extremity muscle function.[84] Radiographs should be obtained. Ultrasound evaluation of the proximal part of the humerus may be helpful because the proximal humeral epiphysis is entirely cartilaginous at birth and thus radiolucent. Ultrasound examination can detect proximal physeal separation, metaphyseal osteomyelitis, and septic shoulder arthritis.[79,80]

Asymptomatic birth fractures of the clavicle and humerus in neonates may be observed. The fracture will unite in short order, with remodeling of bone occurring with growth. Symptomatic fractures in which the child exhibits pseudoparalysis of the upper

Site of curvature	Type of congenital anomaly: Block vertebra	Wedge vertebra	Hemivertebra: Single	Hemivertebra: Double	Unilateral unsegmented bar	Unilateral unsegmented bar and contralateral hemivertebrae
Upper thoracic	<1°—1°	★—2°	1°—2°	2°—2.5°	2°—4°	5°—6°
Lower thoracic	<1°—1°	2°—3°	2°—2.5°	2°—3°	5°—6.5°	6°—7°
Thoracolumbar	<1°—1°	1.5°—2°	2°—3.5°	5°—★	6°—9°	>10°—★
Lumbar	<1°—★	<1°—★	<1°—1°	★	>5°—★	★
Lumbosacral	★	★	<1°—1.5°	★	★	★

No treatment required; May require spinal surgery; Require spinal fusion; ★ Too few or no curves

Ranges represent the degree of derotation before and after 10 years of age.

• **Fig. 89.10** Types of Congenital Spinal Anomaly and Risk of Progression. (From McMaster MJ, Ohtsuka K. The natural history of congenital scoliosis. A study of two hundred and fifty-one patients. *J Bone Joint Surg Am*. 1982;64:1128–1147.)

extremity should be treated with 7 to 10 days of immobilization in a soft dressing or until symptoms subside. Femoral birth fractures can be treated with a soft Pavlik harness with good results.[81] This device provides a simple means of immobilization that is accepted well by new parents. Excellent outcomes with no residual deformities or limb length inequalities can be expected.

The presence of multiple long-bone and rib fractures at birth may herald the presence of osteogenesis imperfecta (OI). Prenatal diagnosis is commonly made by ultrasonographic screening, as early as 13 to 14 weeks' gestation, based on deformity.[85] OI is typically classified by the Sillence classification, with type II and type III being the most common types identified in the perinatal period.[86] Type II OI is generally considered to be lethal in the neonatal period, whereas most children with type III OI survive into adulthood with considerable short stature and fracture-related morbidity. Thus, prompt genetic consultation is critical to establish a diagnosis and prognosis for an affected child. A diagnosis of Bruck syndrome should be considered when clinical and radiographic findings of OI are coupled with joint contractures.[87] Infants with type III OI often require substantial respiratory support and pain management because of rib fractures, with respiratory failure being identified as the most common cause of death in the neonatal period. Treatment of long fractures is primarily to support pain management and can be achieved by custom splints or merely soft supports, such as pillows or blankets. Patients with multiple fractures at birth who are expected to survive the neonatal period should be considered for bisphosphonate treatment.[88]

Brachial plexus injuries represent the second category of birth trauma afflicting newborns. The mechanism of injury is a separation of the head from the shoulder by lateral bending of the neck with simultaneous shoulder depression during vaginal delivery resulting in a stretching or avulsion of the brachial plexus. These injuries occur in 1 per 1000 to 4 per 1000 live births.[89,90] Risk factors include maternal diabetes, large birthweight, prolonged labor, forceps delivery, and shoulder dystocia during a vertex delivery.[91] They are rarely seen in cesarean deliveries. Brachial plexus palsies are associated with clavicle and humerus fractures, as well as torticollis.[91] Risk factors for brachial plexus injury include:

1. maternal diabetes
2. large birthweight
3. prolonged labor
4. forceps delivery
5. shoulder dystocia during vertex delivery

The brachial plexus receives contributions from the anterior spinal nerve roots of C5 through T1, which combine and divide to form the peripheral nerves that supply the motor innervation to the upper extremity. Three major injuries are encountered. The most frequent injury is to the upper trunk that involves the C5 and C6 nerve roots primarily and results in Erb palsy. Affected infants lack external rotation and abduction of the shoulder. Hand function is preserved. The next most frequently occurring injury is a global plexus palsy involving the C5 through T1 nerve roots. This results in flaccid paralysis of the involved upper extremity, including the hand. An isolated lower plexus injury involving the C8 and T1 nerve roots, termed Klumpke palsy, is the least common and may be a manifestation of a recovered global plexus injury.[92]

Physical examination has proved to be the most reliable method of assessing the level and severity of the neural injury and thereby predicting the potential for spontaneous recovery.[92,93] Myelography, computed tomographic myelography, magnetic resonance imaging, and electrodiagnostic studies have not proved useful in predicting recovery.[92] Active shoulder, elbow, wrist, and finger motion need to be assessed.[91] Frequently, such assessment can be facilitated by elicitation of the primitive reflexes that are transiently present in normal newborns. The hand grasp reflex is normal in all newborns and disappears between 2 and 4 months. The examiner's little finger is placed on the ulnar aspect of the infant's palm, and the infant's fingers reflexively flex and grasp the examiner's finger. The Moro reflex begins to fade at 3 months of age. It is elicited by the examiner holding the newborn's hands while raising the baby off the table and then suddenly releasing them. In response, the newborn extends the spine, abducts and extends all four limbs and digits, and then subsequently adducts and flexes the limbs and digits. Last, the ATNR, or fencing reflex, can be elicited in a normal newborn until the age of 4 months. With the infant lying supine on an examining table, the head is rotated to one side by the examiner. The infant should respond by extending the elbow on the side toward which the face is looking and by flexing the opposite elbow. In newborns with a brachial plexus injury, some of these reflexes will be abnormal because of lack of motor control. For instance, the newborn with Erb palsy will, most notably, not be able to actively flex at the elbow during the ATNR or the Moro reflex. The presence or absence of Horner syndrome (contracted pupil, drooping eyelid, and decreased sweating on the affected side) must also be noted.

Affected infants need repeated serial examinations until 6 months of age. Return of biceps function by 3 months is the most important indicator of brachial plexus recovery.[94] When biceps recovery is combined with the return of shoulder abduction, wrist extension, and finger extension, there is a 95% chance of normal function.[94] When biceps function recovers later than 3 months, it is rare for the child to have complete recovery of normal function.[95] A total plexus palsy or the presence of Horner syndrome also heralds a poor prognosis.[92,94]

The initial treatment of obstetric brachial plexus injury is aimed at avoiding contractures of the shoulder, elbow, forearm, and hand with occupational or physical therapy during the observation-for-recovery phase. Fortunately, as few as 1 in 10 infants with brachial plexus palsies at birth will require surgical intervention.[91] With the decision for surgery being based on muscle function recovery, prompt referral to a specialist is recommended to initiate monthly neurologic examinations.

Brachial plexus exploration with subsequent reconstruction is indicated for infants with total plexus involvement, Horner syndrome, and no return of biceps function at 3 months and for infants with a C5 to C6 (Erb) plexopathy and no return of biceps function at 3 to 6 months.[92] Surgery is undertaken between 3 and 6 months of age. Using this algorithm prospectively,[95] operated on six infants at 6 months and found that their results were better than those for the 15 patients with biceps recovery at 5 months but worse than those for the 11 patients with biceps recovery at 4 months. Despite treatment as outlined, some children will have residual deficits. Secondary reconstruction, for chronic brachial plexopathy resulting in a dysfunctional shoulder, can be achieved with a tendon transfer of the latissimus dorsi and teres major to the rotator cuff or by derotational osteotomy of the humerus. These procedures and others designed to correct limitations in hand and forearm function are undertaken after the true scope of the disability has been assessed. Some infants with no biceps recovery by 3 months will eventually achieve adequate biceps and shoulder function without surgery.[96] The optimal timing of

surgical intervention remains controversial. Surgical exploration after 18 months is of little benefit.

Neonatal Osteomyelitis and Septic Arthritis

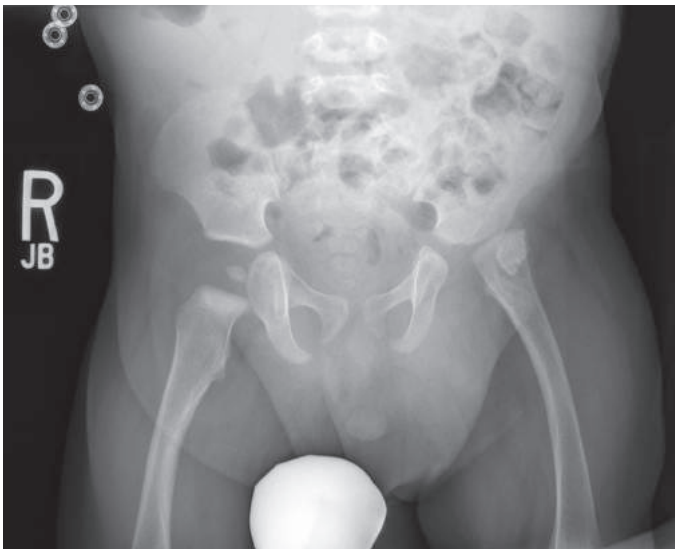
Osteomyelitis is a bacterial infection of bone, and septic arthritis is a pyogenic infection of a joint. Incidence rates of 0.12 per 1000 live births and 0.67 per 1000 neonatal intensive care unit admissions[97] have been reported for septic arthritis. The mortality rate is reported to be 7.3%.[98] The hip, knee, and shoulder joints are involved most frequently. Neonates are particularly susceptible to osteomyelitis and septic arthritis because of an immature immune response, resulting in vulnerability to organisms that are not ordinarily virulent in infants and children, and because of delays in expressing the classic physical findings associated with these conditions.[99]

Two subgroups of neonates are affected: premature neonates requiring prolonged hospitalization and otherwise healthy newborns in whom presentation occurs within 2 to 4 weeks after delivery.[99] Most cases of neonatal osteomyelitis and septic arthritis result from hematogenous spread, but some occur from direct inoculation during percutaneous arterial blood sampling (especially if obtained from the femoral artery). Acute hematogenous osteomyelitis (AHO) and septic arthritis in hospitalized neonates usually occur in premature infants with multiple peripheral and central vascular catheters.[100] These infections are frequently caused by *Staphylococcus aureus* or gram-negative organisms.[101,102] Up to 40% of these patients may demonstrate multiple areas of involvement and these patients can be systemically ill.[103,104]

This presentation contrasts with that of the typical out-of-hospital newborn with AHO and septic arthritis, in whom 2 to 4 weeks after birth, swelling, pseudo paralysis (lack of active movement of affected limb) and tenderness of the extremity present, but the infant otherwise feeds well and is not systemically ill.[99] *S. aureus* and group B streptococcus are the most common organisms encountered in this latter population. Because of the immature immune response, neonates frequently do not demonstrate fever, leukocytosis, or elevation in erythrocyte sedimentation rate (ESR).[105] However, C-reactive protein (CRP) is a reliable indicator of AHO and septic arthritis, with a negative predictive value of 95%.[100] Blood culture findings are positive in only 50% of patients; cultures of synovial aspirates identify an organism in only 30% of cases.[106]

Prompt diagnosis is imperative, both to decrease the risk of systemic infection and sepsis but also decrease the chances of long term local musculoskeletal issue such as avascular necrosis of the hip. Imaging studies are useful in diagnosing neonatal osteomyelitis and septic arthritis. Plain radiographs are usually the first study performed although they do not always yield a diagnosis in early infections. Bone changes on plan radiographs are not present for 1 week after the onset of symptoms.[107,108] Ultrasound examination is the first line radiologic method for evaluating the neonate with suspected AHO and/or septic arthritis, as it detects joint effusions and subperiosteal and soft tissue fluid collections and can guide aspiration. If an infection is still suspected despite negative findings after radiographic and ultrasound examination, MRI is warranted. MRI provides accurate regional information on both the soft tissues and bones and is highly sensitive in identifying early infections[109] but requires sedation in infants.

In neonates, before the appearance of the secondary ossification center, the epiphysis receives blood directly from metaphyseal blood vessels. When the infection occurs in these metaphyseal veins causing osteomyelitis, it can traverse the growth plate into the epiphysis, cause abscess formation, and rupture into the joint.[110] Thus, septic arthritis is a sequela of adjacent osteomyelitis in up to 76% of neonates.[103,111] The hip and shoulder are two of the more common sites of neonatal septic arthritis as they have intra-articular metaphysis, allowing a more direct subperiosteal route of decompression for pus into the joint. Because of this unique ability to spread from the metaphysis through the growth plate into the joint, early detection and treatment are necessary to avoid permanent damage to each of these structures. Infection can result in destruction of the growth plate and articular cartilage in short order, resulting in growth disturbances and precocious arthritis. When an area of involvement is suspected, aspiration should be undertaken,[99] which can confirm the diagnosis and provide fluid for culture to better direct antibiotic treatment. Osteomyelitis without a pus collection in bone or soft tissue can typically be managed with antibiotic therapy; septic arthritis and subperiosteal and bone abscesses should always be drained surgically in the operating room (Fig. 89.11).



• **Fig. 89.11** Unrecognized neonatal hip sepsis can result in complete dissolution of the femoral head as demonstrated in this child's left hip radiograph.

Suggested Readings

Cheng JC, Wong MW, Tang SP, et al. Clinical determinants of the outcome of manual stretching in the treatment of congenital muscular torticollis in infants. A prospective study of eight hundred and twenty-one cases. *J Bone Joint Surg Am*. 2001;83:679–687.

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Skeletal Dysplasias and Heritable Connective Tissue Disorders

GEORGE E. TILLER AND GARY A. BELLUS

KEY POINTS

- Many skeletal dysplasias, as well as many connective tissue disorders, look similar in the newborn period, especially in premature infants. Arriving at the most accurate diagnosis possible is essential to proper medical decision-making and counseling the family. Utilizing all resources available (medical consultants, texts, online resources, and molecular diagnostics) contributes to this process.
- With respect to molecular diagnostics, (1) it is not essential in all cases, especially where diagnoses can be made clinically by physical examination and/or radiographs, (2) particular mutations within a gene do not always determine prognosis, with notable exceptions (i.e., *FGFR3*, *COL1A1*), (3) diagnostic molecular testing is commercially available in all disorders for which a gene has been identified, and prenatal diagnosis is available if the particular gene mutation has been previously identified in an affected individual, and (4) medical geneticists and genetic counselors, as well as genetics laboratory directors, can aid in choosing the best approach to a molecular diagnosis (which may take several weeks to complete), so as to accommodate disorders exhibiting genetic heterogeneity.
- As in all cases, the family should be kept updated on the current clinical status, what is known, what remains unknown, and the timeline for receiving additional diagnostic and prognostic input.

Skeletal dysplasias, or osteochondrodysplasias, are disorders of the development and growth of cartilage and bone. Connective tissue disorders involve abnormalities of the cells' supporting and connecting components in the extracellular matrix. In a Boston series of greater than 100,000 deliveries monitored postnatally for 15 years, the incidence of skeletal dysplasias was 2.14 in 10,000.[1] With the growing use and accuracy of ultrasonography in prenatal care, a greater number of osteochondrodysplasias and connective tissue disorders are diagnosed prenatally.

The most recent classification of skeletal dysplasias into 42 groups is based on radiologic, clinical, and/or molecular criteria.[2] This chapter focuses on several of the more "common" skeletal dysplasias (Tables 90.1 and 90.2 for an expanded list) and connective tissue disorders (Table 90.3) that manifest themselves prenatally or perinatally, but the discussion is not exhaustive. The osteochondrodysplasias have been reviewed extensively elsewhere.[3,4]

There are many different connective tissue molecules, including collagens (more than 24 types), elastin, fibrillin (two types), and microfibril-associated glycoproteins. These molecules are components of tissues such as bone, cartilage, skin, vascular media, tendon, ligament, and basement membrane in many organs. The heritable disorders of connective tissue are varied, may be very dissimilar clinically, and may manifest themselves in utero or at any age postnatally. Those that may manifest themselves at birth include the early-onset (neonatal) form of Marfan syndrome, congenital contractural arachnodactyly (CCA; Beals syndrome), cutis laxa, some forms of Ehlers–Danlos syndrome (EDS), and Menkes disease.

Clinical Spectra of Disorders With Common Molecular or Cellular Bases

The number of clinically distinguishable skeletal dysplasias and connective tissue disorders is extensive. With advances in molecular knowledge, several different dysplasias have been recognized to have mutations in the same genes. In some of these disorders, clinical similarities noted previously suggested a common cause. One such clinical spectrum includes achondroplasia, hypochondroplasia, severe achondroplasia with developmental delay and acanthosis nigricans (SADDAN), and thanatophoric dysplasia, all of which are caused by mutations in the fibroblast growth factor receptor 3 gene, *FGFR3*.[5–8] Another spectrum of disorders includes Stickler syndrome, Kniest dysplasia, some forms of spondyloepimetaphyseal dysplasia, spondyloepiphyseal dysplasia congenita (SEDC), hypochondrogenesis, achondrogenesis type II, and recessive multiple epiphyseal dysplasia, all of which are caused by mutations in the gene encoding collagen type II, *COL2A1*.[9] With other disorders, the common cause is not as obvious clinically: diastrophic dysplasia, atelosteogenesis type II, and achondrogenesis type IB are all caused by mutations in the *SLC26A2* gene.[10–12] The obverse is also evident, wherein a specific clinical entity (e.g., multiple epiphyseal dysplasia) may be caused by a mutation in one of several genes—a concept known as *genetic heterogeneity*.

Genetic heterogeneity can sometimes be explained when (a) the functions of a group of genes are similar, (b) they share a common cellular pathway, or (c) they all impact a common subcellular process. This is the case for ciliopathies, which are disorders affecting the primary cilia of cells. Cilia are microtubule-based organelles that provide a cellular "antenna" for receptors involved in chemosensory, mechanosensory, and osmoregulatory signaling. The short rib thoracic dysplasias are ciliopathies that disproportionately affect the development of the fetal skeleton (see Table 90.1).[13]

TABLE 90.1 Lethal Skeletal Dysplasias Manifesting Themselves Prenatally or Perinatally

Dysplasia	Skeletal Features	Nonskeletal Features	Radiographic Features	Inheritance and Genes
Achondrogenesis type IB (OMIM 600972)	Soft cranium; short, round chest; very short limbs	Round face; polyhydramnios	Poorly ossified calvarium; short ribs with fractures and beading; nonossified vertebrae; short broad femurs with metaphyseal spikes, short broad tibiae, and fibulae	AR; *SLC26A2*
Achondrogenesis type II, hypochondrogenesis (OMIM 200610)	Large head, flat face, cleft palate; short trunk; very short limbs (micromelia)	Fetal hydrops; distended abdomen	Lack of vertebral mineralization; short limbs (all segments); enlarged cranium with normal ossification	AD; *COL2A1*
Atelosteogenesis type II (OMIM 256050)	Narrow chest, short limbs, joint dislocations, equinovarus deformities, gap between first and second digits	Cleft palate, laryngeal stenosis; patent foramen ovale	Occasional coronal and sagittal vertebral clefts; short ribs; short "dumbbell" humeri and femurs, small fibulae; large second and third metacarpals; small round midphalanges	AR; *SLC26A2*
Campomelic dysplasia (OMIM 114290)	Large cranium; small face, flat nasal bridge, cleft soft palate; small, narrow chest; angulated thighs and legs; dimples on legs	Polyhydramnios, congenital cardiac abnormalities; female external genitalia in XY males	Large dolichocephalic calvarium with shallow orbits; short and wavy ribs, often 11 pairs; hypoplastic scapula; small, flat vertebrae; tall, narrow pelvis; relatively long, thin limbs with bent femurs and short tibiae	AD (most are new mutations); *SOX9*
Chondrodysplasia punctata, rhizomelic type 1 (OMIM 215100)	Flat face; very flat nasal bridge and tip; proximal shortening of limbs	Cataracts; joint contractures; ichthyosiform erythroderma	Wide coronal vertebral clefts; short humeri and femurs; stippled epiphyses of long bones, pelvis, and periarticular areas; trapezoid ilia	AR; *PEX7*
Short rib thoracic dysplasias ± polydactyly (including asphyxiating thoracic dystrophy [a.k.a. Jeune] and Ellis-van Creveld syndromes) (OMIM 613091, 611263, 225500, others)	Variable chest narrowing, variable limb shortening; severe cases: round flat face, hydropic appearance, micrognathia, very narrow chest, very short limbs ± postaxial polydactyly	Usually lethal pulmonary insufficiency; variable cardiac, renal, and/or anal malformations	Very short, horizontal ribs; flat, wide intervertebral disk spaces; small pelvis; short limbs with lateral and medial metaphyseal spurs	AR; *DYNC2H1*, *IFT80*, *IFT140*, *IFT172*, *WDR19*, *WDR34*, *TTC21B*, *EVC*, and >12 others
Thanatophoric dysplasia (OMIM 187600)	Large cranium, proptosis, flat nasal bridge, narrow chest, very short limbs (all segments)	Polyhydramnios, hydrocephalus, brain anomalies, congenital cardiac abnormalities	Large calvarium, small foramen magnum, cloverleaf skull (type 2); short, splayed, cupped ribs; very flat U-shaped vertebrae; short, flat pelvis; short, bowed limbs; metaphyseal flare with spike	AD (most are new mutations); *FGFR3*

AD, Autosomal dominant; *AR*, autosomal recessive; *OMIM*, Online Mendelian Inheritance in Man (www.ncbi.nlm.nih.gov/omim).

Approach to Diagnosis

An early and precise diagnosis is important for prognosis, optimal immediate-term and long-term management, accurate genetic counseling about recurrence risk, and identification of other possibly affected family members or disease carriers. An example is the group of disorders with punctate calcifications ("stippling") in epiphyses, called *chondrodysplasia punctata*. There are several types, with three possible modes of inheritance: autosomal recessive, X-linked recessive, and X-linked dominant (see Table 90.1). As in any uncommon genetic condition, multiple components may be required to arrive at the correct diagnosis: a complete physical examination, three-generation family history, radiologic studies, and biochemical and/or molecular tests.

Most skeletal dysplasias cause short stature, which can be proportionate or disproportionate. The disproportion may be evident as a short-limbed or short-trunk form of dwarfism. If the limbs are affected, there may be segmental shortening of the upper arms

TABLE 90.2 Nonlethal Skeletal Dysplasias Manifesting Themselves Prenatally or Perinatally

Dysplasia	Skeletal Features	Nonskeletal Features	Radiographic Features	Inheritance and Gene
Achondroplasia (OMIM 100800)	Large cranium; frontal bossing, flat nasal bridge, short neck; slightly narrow chest; proximal limb shortening (rhizomelia), short trident hands; brachydactyly; joint laxity	Hypotonia: delayed motor milestones; spinal stenosis causing spinal compression; small foramen magnum may cause hydrocephalus and/or apnea	Large calvarium, small foramen magnum; diminished lumbosacral interpedicular space, short pedicles; short ribs with anterior cupping; short humeri and femurs; relatively long fibulas; metaphyseal flare; small squared iliac wings	AD (most are new mutations); *FGFR3*
Chondrodysplasia punctata, X-linked recessive (OMIM 302950)	Distal phalangeal hypoplasia; severe hypoplasia of the nose; short stature	Cataracts; hearing loss; congenital ichthyosis; anosmia, and hypogonadism (in contiguous gene deletion patients)	Distal phalangeal hypoplasia; stippled epiphyses of long bones; paravertebral stippling	XLR; *ARSE*
Chondrodysplasia punctata, X-linked dominant (Conradi–Hünermann syndrome) (OMIM 302960)	Asymmetric rhizomesomelia	Congenital cataracts; ichthyosis; patchy alopecia	Stippled epiphyses of long bones; paravertebral stippling; tracheal calcifications	XLD; *EBP*
Diastrophic dysplasia (OMIM 222600)	Cleft palate; micrognathia; normal chest at birth; very short limbs; thumbs proximally placed and adducted (hitchhiker's thumb); equinovarus; limited joint movement	Cystic masses in auricles (cauliflower ears) during infancy; deafness caused by lack or fusion of ossicles; narrow external auditory canal	Premature ossification of rib cartilage; narrow L1–L5 interpedicular spaces; scoliosis; short limbs; short ulnae and fibulae (mesomelia); broad flared metaphyses; ovoid first metacarpals; variable symphalangism of proximal interphalangeal joints	AR; *SLC26A2*
Kniest syndrome (OMIM 156550)	Large cranium; flat face with large eyes, flat nasal bridge, cleft palate; proximal limb shortening, enlarged joints, flexion contractures	Infancy: tracheomalacia childhood: myopia and retinal detachment, hearing loss, delayed motor development	Frontal and maxillary hypoplasia, shallow orbits; slightly short ribs; flat vertebrae with coronal clefts; irregular acetabular roof; short limbs with dumbbell metaphyses; lateral bowing of femurs and tibiae	AD; *COL2A1*
Spondyloepiphyseal dysplasia congenita (OMIM 183900)	Flat face, cleft palate, short limbs	Infancy: tracheomalacia childhood: myopia and retinal detachment, hearing loss	Frontal and maxillary hypoplasia, flat vertebrae, small pelvis with irregular acetabular roof, short limbs; normal hands and feet	AD; *COL2A1*

AD, Autosomal dominant; *AR*, autosomal recessive; *XLD*, X-linked dominant; *XLR*, X-linked recessive; *OMIM*, Online Mendelian Inheritance in Man (www.ncbi.nlm.nih.gov/omim).

and thighs (rhizomelia), forearms and legs (mesomelia), and/or hands and feet (acromelia). Most skeletal dysplasias that manifest themselves at birth involve short limbs. Accurate measurements of length (on a firm surface) and head and chest circumferences should be plotted on standard growth curves, with measurement of arm span and calculation of upper body/lower body segment ratios to objectively assess disproportion.

Other skeletal characteristics can give important clues for specific disorders:

- Children with achondroplasia and thanatophoric dysplasia have large heads (macrocephaly). Cloverleaf skull deformity is present in some forms of thanatophoric dysplasia.
- A relatively long, narrow chest is seen in asphyxiating thoracic dystrophy and other short rib thoracic dystrophies.
- In achondroplasia, the hand is short, and the fingers form a trident configuration. In diastrophic dysplasia, there are distinctive "hitchhiker" thumbs.
- Clubfeet may occur in diastrophic dysplasia, Kniest dysplasia, spondyloepiphyseal dysplasias, and osteogenesis imperfecta (OI) type II.
- Postaxial (and occasionally preaxial) polydactyly may be present in some of the short-rib thoracic dysplasias.
- Multiple joint dislocations can manifest themselves at birth in Larsen syndrome, EDS type VII, atelosteogenesis, and Desbuquois syndrome.

The presence of extraskeletal abnormalities may provide additional clues to diagnosis, as follows:

- Cleft palate may occur in campomelic, Kniest, spondyloepiphyseal, short-rib polydactyly (Majewski), atelosteogenesis types I and II, hypochondrogenesis, and diastrophic dysplasia.
- Congenital cataracts are frequent in some forms of chondrodysplasia punctata.
- Congenital cardiac defects occur in some short-rib thoracic dysplasias, such as Ellis-van Creveld syndrome.

Clinical and Molecular Evaluation

Radiographs of the entire skeleton, including the skull, thorax (with rib technique), long bones, hands, feet, pelvis, and lateral spine, are essential for accurate diagnosis. Atlases dedicated

TABLE 90.3 Heritable Connective Tissue Disorders Manifesting Themselves Perinatally or in Childhood

Connective Tissue Disorders	Inheritance	Genes	Key Clinical Features
Marfan syndrome (OMIM 154700)	AD; congenital Marfan syndrome, usually sporadic	*FBN1*	Aortic dilation, joint laxity, arachnodactyly, ectopia lentis, dural ectasia
Loeys–Dietz syndrome (OMIM 609192, 610168, 601366, 613795, 614816, and 615582)	AD	*TGFBR1*, *TGFBR2*, *TGFB2*, *TGFB3*, *SMAD2*, *SMAD3*	Arterial tortuosity, cardiac anomalies, joint laxity, aneurysms, arachnodactyly
Congenital contractural arachnodactyly/distal arthrogryposis type 9 (OMIM 121050)	AD	*FBN2*	Kyphoscoliosis, joint contractures, crumpled ears, cardiac anomalies
Ehlers–Danlos Syndromes			
Classic type (type I) (OMIM 130000)	AD	*COL5A1*, *COL5A2*, *COL1A1*	Joint laxity, atrophic scarring, easy bruising, premature birth, skin hyperelasticity
Vascular type (type IV) (OMIM 130050)	AD	*COL3A1*	Aortic and medium-sized arterial aneurysm, intestinal rupture
Kyphoscoliotic type (type VI) (OMIM 225400)	AR	*PLOD1*	Scoliosis, joint laxity, congenital hip dislocation, ocular globe rupture
Arthrochalasis type (types VIIA and VIIB) (OMIM 130060)	AD	*COL1A1*, *COL1A2*	Congenital hip dislocation, joint laxity
Dermatosparaxis type (type VIIC) (OMIM 225410)	AR	*ADAMTS2*	Fragile skin, joint laxity
Cutis Laxa			
Autosomal dominant (OMIM 123700 and 130160)	AD	*ELN*, *FBLN5*	Loose redundant skin
Autosomal recessive (OMIM 219100, 219150, 614437, 219200, 278250, 612940, 613177, and 613075)	AR	*FBLN5*, *EFEMP2*, *ATP6V0A2*, *LTBP4*, *PYCR1*	Cutis laxa, musculoskeletal, genitourinary, vascular, and other systemic features
Gerodermia osteodysplasticum (OMIM 231070)	AR	*GORAB*	Prematurely aged appearance, camptodactyly, bowed legs
De Barsy syndrome (OMIM 179035)	AR	*PYCR1*	Aged appearance, intrauterine growth restriction, cutis laxa
Menkes syndrome (OMIM 309400)	XLR	*ATP7A*	Skin laxity, joint laxity, kinky sparse hair, neurologic degeneration

AD, Autosomal dominant; *AR*, autosomal recessive; *R*, X-linked recessive; *OMIM*, Online Mendelian Inheritance in Man (www.ncbi.nlm.nih.gov/omim).

to skeletal dysplasias are essential for this purpose,[4,14] even for the experienced radiologist or neonatologist. Ultrasound images of the brain, heart, and kidneys may be helpful if anomalies in those organs are suspected. Detailed family history and measurements of family members may be helpful; disorders in more mildly affected members may have gone undiagnosed. Molecular investigations may be necessary to arrive at the proper diagnosis; given their complexity, such analyses should be considered after consultation with a clinical geneticist. The advent of "next-generation" DNA sequencing has led to more widespread availability of multigene diagnostic panels, which can be used when the diagnosis cannot be determined solely by clinical and radiographic means (see the Genetic Testing Registry at https://www.ncbi.nlm.nih.gov/gtr/). Rapid whole exome sequencing may also be employed in the newborn period when an accurate diagnosis is critical to clinical decision-making. The molecular definition is also helpful in the cases of autosomal recessive and X-linked disorders, as this information may be useful for counseling with respect to recurrence risk and prenatal diagnosis in subsequent pregnancies.

If the infant or fetus dies with the disorder undiagnosed, cord blood for DNA as well as specimens of cartilage and skin fibroblasts can be obtained for histochemical tests, biochemical assays, and/or molecular analyses; these can be used to make or confirm diagnoses and permit accurate future prenatal diagnosis. Even if the molecular or enzymatic basis of the condition is not understood at the time, the tissue may be useful in the future. If photographs and skeletal radiographs were not obtained before death, they should be obtained after death. Additional information for diagnosing challenging cases may be obtained from the International Skeletal Dysplasia Registry at the University of California, Los Angeles (https://www.uclahealth.org/departments/ortho/isdr/about-isdr).

Disorders of Bone Fragility

Osteogenesis Imperfecta Types II and III

OI is characterized by increased bone fragility. There are classically four major clinical types; of these, types II and III are the most severe and manifest prenatally and perinatally.[15] However, fractures at birth can also occur in the mildest form, OI type I. Further heterogeneity in OI has recently been described, especially in severe autosomal recessive forms.

Presentation

OI type II (perinatal lethal type) is estimated to affect 1 in 20,000 to 1 in 60,000 infants. Affected infants may be born prematurely, with low birth weight and disproportionately short stature. The limbs are short and bowed with extra circular skin creases; the hips are abducted and flexed. The head is soft and boggy, and minimal calvarial bone can be felt. The sclerae are dark blue, and the chest is narrow. The infant cries with handling because there may be many fractures at different stages of healing. Sixty percent of affected babies are stillborn or die during the first day of life, and 80% die by 1 month. With the growing use of ultrasonography, affected fetuses may be detected in the second trimester because of short and bowed or angulated limbs and narrow thoraces (Fig. 90.1).

OI type III (progressive deforming type) can manifest itself prenatally, perinatally, or during the first 2 two years after birth. Prenatal and perinatal clinical features resemble those in OI type II but are less severe (Fig. 90.2), and perinatal death is not uncommon. The highest prevalence of fractures in OI, up to 200 in a lifetime, occurs in type III. Extremely short stature, with an adult height of 92 to 108 cm, can result from microfractures in growth plates. The head may be large because the calvarium is soft with a large anterior fontanel. The sclerae may be blue initially but are white by puberty. The head assumes a triangular shape, with a bossed, broad forehead and a tapered, pointed chin. Later in childhood, dentinogenesis imperfecta and hearing loss may develop. Severe kyphoscoliosis may occur, leading to cardiopulmonary compromise, which is the major cause of early death.

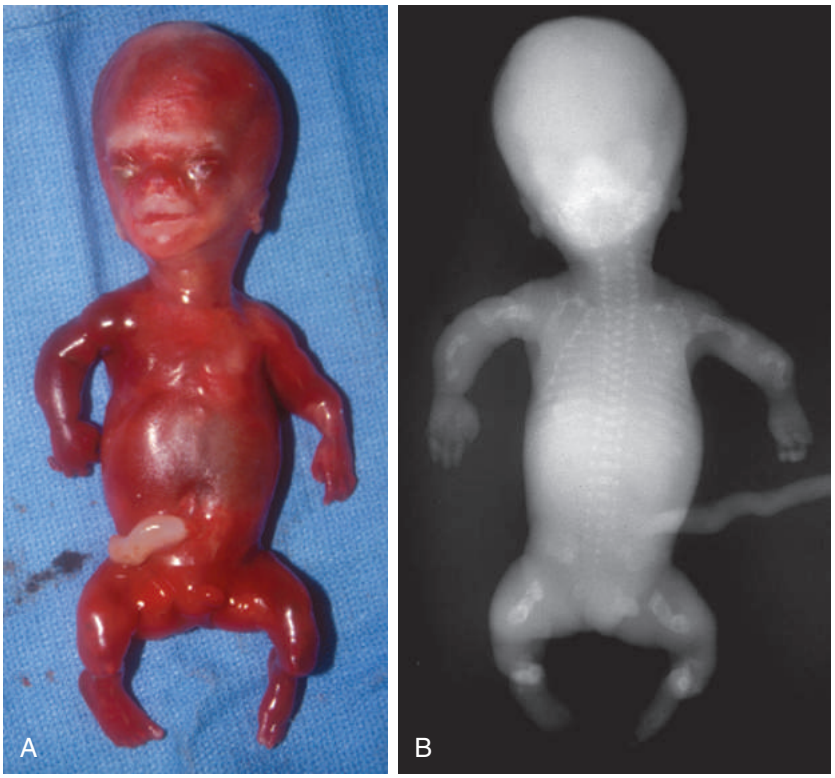
Radiographic Features

Radiographs show the femurs in OI type II to be short, broad, and "telescoped" or "crumpled." The tibiae are short and bowed or angulated, and the fibulae may be thin (see Fig. 90.1B). There is minimal to no calvarial mineralization. The acetabular and iliac wings may be somewhat flattened. The ribs are short, wavy, and thin or broad, with "beading" from callus formation at fetal fracture sites.

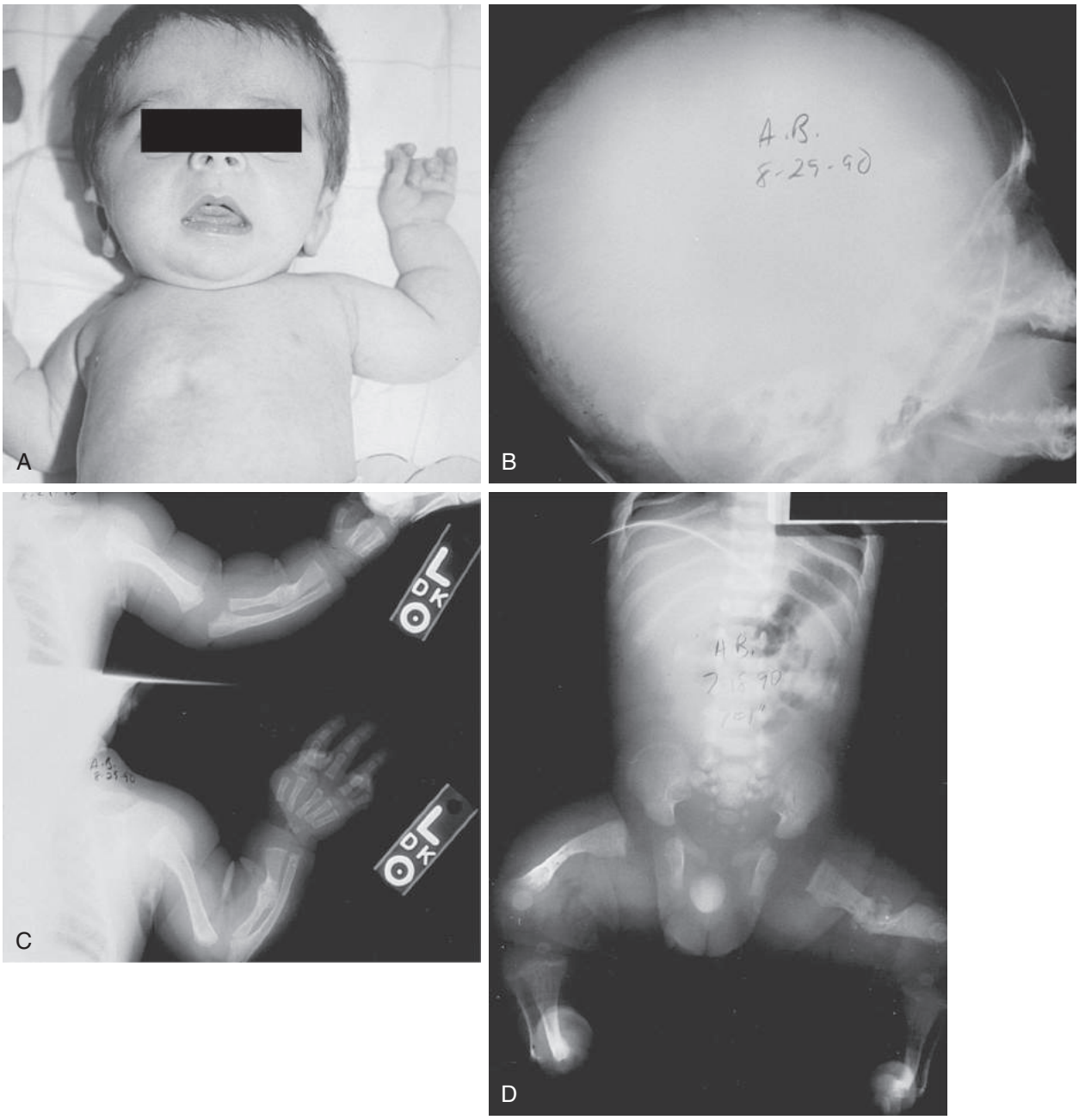
In OI type III, the femurs are short and deformed but not crumpled as in OI type II (see Fig. 90.2B–D). The other long bones are thinner than usual, with healing fractures incurred in utero, bowing, and deformations. The calvarium is undermineralized with a large anterior fontanel, and there are many Wormian bones (small islands of bone in the suture spaces; see Fig. 90.2D). The ribs are thin and gracile.

Etiology

OI is most commonly caused by mutations in one of the two genes encoding collagen type I (*COL1A1* and *COL1A2*), the predominant protein building block of bone. More clinically severe forms of OI are the result of qualitatively abnormal collagen synthesis rather than decreased production,[15] as well as the result of numerous recessive types affecting noncollagen proteins.[16]



• **Fig. 90.1** Osteogenesis Imperfecta Type II. (A) A 20-week fetus. The limbs are angulated and deformed from multiple fractures. (B) Radiograph of a fetus (20 weeks' gestation) showing an absence of ossification in the calvarium, short telescoped or crumpled humeri and femurs, and short and wavy ribs with fractures.



• **Fig. 90.2** Osteogenesis Imperfecta Type III. (A) Neonate with a normal face, short neck, and slightly short limbs. (B) Radiograph showing that the calvarium is undermineralized with Wormian bones. (C) Radiograph showing the upper limbs, which have bowed humeri and callus in the ulnae. (D) Radiograph showing lower limbs with moderately short, thick femurs and angulated tibiae and fibulae. (Courtesy of Paige Kaplan, Children's Hospital, Philadelphia.)

Inheritance

A fetus or infant with OI type II or III is usually the result of a spontaneous dominant-acting gene mutation, but there is a small risk of recurrence (approximately 6%) in subsequent siblings because of possible parental somatic or gonadal mosaicism. The parent is usually asymptomatic but may have minimal manifestations, such as short stature. Prenatal diagnosis is available if the particular gene mutation has been identified in the affected individual. Most cases of OI are inherited as autosomal dominant traits, although rare recessive forms have been shown to be caused by mutations in genes encoding, for example, cartilage-associated protein (*CRTAP*), prolyl 3-hydroxylase (*P3H1*), and cyclophilin B (*PPIB*).[16]

Differential Diagnosis

Other lethal skeletal dysplasias may have abnormalities similar to those in OI type II and may be difficult to distinguish by prenatal ultrasonography; however, in experienced hands, they can be differentiated on the basis of several ultrasound findings.[17] Krakow et al.[18] published a retrospective analysis of 1500 prenatally diagnosed cases of skeletal dysplasias. The three most common

prenatal-onset skeletal dysplasias were OI type II, thanatophoric dysplasia, and achondrogenesis type II, which together accounted for almost 40% of all cases. Postnatal radiographs clearly reveal distinctive differences among thanatophoric dysplasia, campomelic dysplasia, achondrogenesis, and perinatal hypophosphatasia, among other disorders.

Management

If the diagnosis of OI is made prenatally, cesarean delivery has *not* been shown to decrease the fracture rate or increase the survival rate of severely affected fetuses.[19] Those severely affected with OI type II are not expected to survive the neonatal period. In OI type III, the neonate needs careful handling to minimize pain and prevent further fractures. Analgesia alleviates pain. Consideration can be given to treatment with bisphosphonates (with the use of intravenously administered pamidronate), which increase bone density, reduce the frequency of fractures and pain, possibly prevent short stature and deformations, and permit ambulation.[16] It is prudent to treat only severely affected children in whom the clinical benefits outweigh potential long-term effects.

Handling an Infant With Osteogenesis Imperfecta

When the diapers of an infant with OI are being changed, a hand should be placed behind the infant's buttocks with the forearm supporting the legs. Similarly, when the infant is lifted, the buttocks, head, and neck must be supported. The infant can be laid on a pillow to be carried. To transport the infant, an infant seat that reclines as much as possible and allows easy placement or removal should be used. The seat can be padded with egg crating or 1-inch foam. A layer of foam can be placed between the seat's harnesses and the child for extra protection. The car seat must always be placed in the back seat. Sling carriers and "umbrella" strollers should not be used for infants with OI because they do not give sufficient leg, head, and neck support.

Perinatal Hypophosphatasia

Presentation

Perinatal hypophosphatasia is a lethal condition characterized by short, deformed limbs, a soft skull, blue sclerae, and undermineralization of the entire skeleton, such that many bones cannot be visualized and may seem absent in radiographs. In the skull, only the base can be visualized radiologically. There may be rachitic changes and fractures. Seizures that are responsive to pyridoxine may occur. There is polyhydramnios during pregnancy, and death can occur in utero. The disorder affects approximately 1 in 100,000 live births, and neonatal death is common.[20,21]

Radiographic Features

The radiographic features of perinatal hypophosphatasia include polyhydramnios (prenatal); underossification, especially of the calvarium and long bones (with marked variability); small thoracic cavity; short, bowed limbs; spurs in the middle portion of the forearms and lower legs; and dense vertebral bodies.

Etiology

Mutations in the *ALPL* gene are responsible for deficiency of the tissue-nonspecific isoenzyme of alkaline phosphatase (TNSALP), thus causing perinatal hypophosphatasia. The serum alkaline phosphatase value is low. Serum values of inorganic pyrophosphate and pyridoxal 5′-phosphate (putative natural substrates for TNSALP) may be elevated, and urinary phosphoethanolamine level is elevated.[20]

TNSALP acts on multiple substrates: the essential function of TNSALP is in osteoblastic bone matrix mineralization. TNSALP hydrolyzes inorganic pyrophosphate to phosphate, which is thought to be critical in promoting osteoblastic mineralization. If TNSALP is deficient, there is an extracellular accumulation of inorganic pyrophosphate, which inhibits hydroxyapatite crystal formation and mineralization of the skeleton. TNSALP is also needed for the delivery of pyridoxal 5′-phosphate into cells, where it is a cofactor (vitamin B_6).

Inheritance

Perinatal hypophosphatasia is inherited as an autosomal recessive trait, with a 25% recurrence risk in future pregnancies. Prenatal diagnosis is optimized with the use of ultrasonography, an assay of TNSALP activity in amniocytes, DNA mutation analysis if the previously affected infant's mutations were identified, or a combination of these methods.

Differential Diagnosis

Differential diagnoses include OI type II and achondrogenesis.

Management

Until very recently, treatment was primarily supportive and directed toward minimizing pain and discomfort. Clinical trials with a bone-targeted human recombinant enzyme replacement therapy have been shown to be effective in nonlethal cases.[22]

FGFR3 Spectrum

Achondroplasia

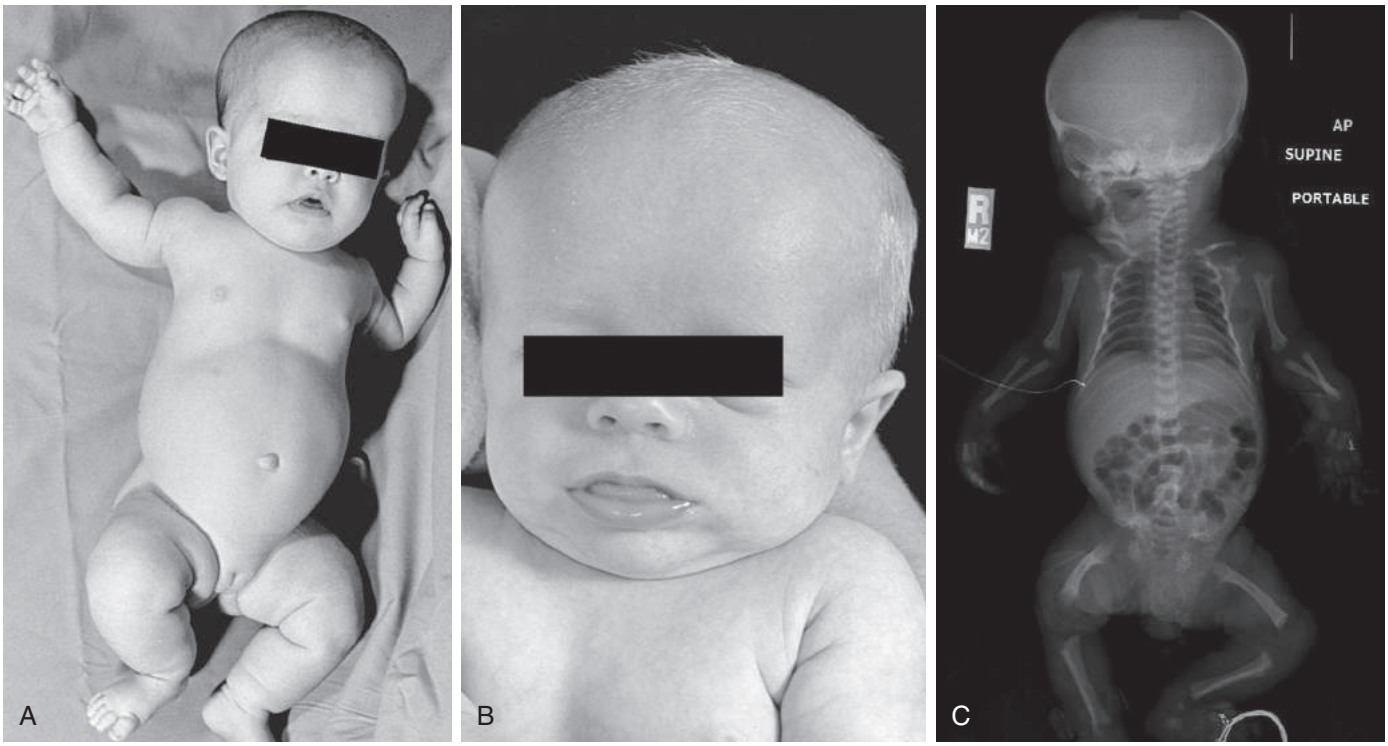
Presentation

Achondroplasia is the most common of the nonlethal chondrodysplasias; it affects 1 in 25,000 live births. It is characterized by short stature with short limbs, particularly rhizomelic (proximal) and acromelic (hands) shortening with trident hand configuration, large head with frontal prominence ("bossing"), flat nasal bridge and midface, long narrow trunk, joint laxity, and development of thoracolumbar kyphosis ("gibbus") in infancy (Fig. 90.3).[5]

The foramen magnum and cervical spinal canal may be narrow and can cause compression of the spinal cord. Standards have been published for foramen magnum size in achondroplasia.[23] Compression of the lower brainstem and cervical spinal cord can lead to hypotonia, central apnea, retardation, quadriparesis, and (rarely) sudden death.[24]

Radiographic Features

The calvarium is large with a relatively small foramen magnum and a short base. The lateral cerebral ventricles may be large, but hydrocephalus is not a common complication. The proximal long bones (humeri and femurs) are short, including the femoral neck. Fibulae are longer than tibiae. There is metaphyseal flaring. The hand is short with a trident configuration of the fingers, with short proximal and middle phalanges. Vertebrae are small and cuboid with short pedicles, and there may be anterior beaking of the first or second lumbar vertebrae; there is a lack of widening of the interpedicular distance in the lumbar vertebrae. The pelvis has squared iliac wings ("elephant ear" appearance), a narrow greater sciatic



• **Fig. 90.3** Achondroplasia. (A) Infant with achondroplasia who has macrocephaly and proximal limb shortening (rhizomelia). (B) Infant with achondroplasia who exhibits frontal bossing and flat nasal bridge. (C) Neonatal radiograph of achondroplasia illustrating a large skull, a somewhat narrow chest, short vertebral bodies with a lack of lumbar interpediculate flare, and rhizomelia. (B courtesy Charles I. Scott, Nemours/Alfred I. du Pont Hospital for Children, Wilmington, DE.)

notch, and flat acetabular roofs (see Fig. 90.3C). Compression of the cervical cord, if present, can be ascertained with magnetic resonance imaging with cerebrospinal fluid flow studies in flexion and extension.

Etiology

The cause of achondroplasia is a mutation of the *FGFR3* gene, which encodes fibroblast growth factor receptor 3, a membrane-spanning tyrosine kinase receptor. More than 99% of individuals with achondroplasia have a common recurrent mutation in the transmembrane domain of the *FGFR3* gene, in which arginine is substituted for glycine (Gly380Arg). The same gene is mutated at different sites in hypochondroplasia, thanatophoric dysplasia, SADDAN, Muenke craniosynostosis, and Crouzon craniosynostosis syndrome with acanthosis nigricans.[5] Histopathologic examination demonstrates a defect in the organization and maturation of the cartilage growth plates of long bones because of differing degrees of constitutive activation of the receptor.

Inheritance

The inheritance pattern in achondroplasia is autosomal dominant. Approximately 80% of cases are sporadic occurrences in a family, representing new mutations. Cases may be associated with advanced paternal age, and molecular studies have confirmed that new mutations are of paternal origin.[25] Rare recurrences due to gonadal mosaicism in a parent have been reported. Affected individuals are fertile, and achondroplasia is transmitted as a fully penetrant autosomal dominant trait, meaning that each person who inherits the mutant gene will manifest the condition.

Differential Diagnosis

Differential diagnoses include SADDAN[5,8] and hypochondroplasia.[6]

Management

The infant with achondroplasia is often hypotonic; together with the large head, the hypotonia leads to delayed motor milestones. Development of thoracolumbar kyphosis may be exacerbated by unsupported sitting before truncal muscle strength is adequate; therefore, infants should not be carried in flexed positions (including soft sling carriers and umbrella strollers). Rear-facing car safety seats should always be used. Physical therapy in the first year of life may strengthen core musculature at a faster rate. Most infants lose their kyphosis and develop the typical exaggerated lumbar lordosis when they begin walking.

Hydrocephalus may occasionally develop during the first 2 years, so the head circumference and body length should be carefully measured and plotted on standard achondroplasia growth charts.[26] Routine imaging of the skull and brain is not recommended; however, the development of hyperreflexia, hypotonia, or apnea may herald the development of clinically significant cord compression in infancy or early childhood and requires prompt evaluation. Surgical decompression at the foramen magnum or the upper cervical spine may prevent neurologic damage, although most patients usually gain motor milestones late but spontaneously because the foraminal diameter expands faster than the cord.

The upper airway in individuals with achondroplasia is small, often leading to obstructive apnea, snoring, and chronic serous otitis media beyond infancy. Treatment may consist of tonsillectomy, adenoidectomy, and placement of myringotomy tubes. Parents

should be counseled about the clinical and hereditary aspects of the disorder and given a copy of the guidelines for health supervision of children and adults with achondroplasia issued by the American Academy of Pediatrics.[26]

Drugs designed to impede the overexpression of the FGFR3/tyrosine kinase pathway are currently in clinical trials and thus far look promising.[27] However, trials as yet have not included newborns and infants.

Thanatophoric Dysplasia

Presentation

Thanatophoric dysplasia is one of the most common lethal dysplasias, occurring once in 45,000 births.[7] It is characterized by extremely short limbs, long narrow trunk, large head with a bulging forehead, prominent eyes, flat nasal bridge, wide fontanel, and occasionally cloverleaf skull deformity (Fig. 90.4). It is differentiated into types I and II based on radiologic features and mutation specificity. Death typically occurs in the neonatal period from respiratory insufficiency, although rare survivors have been reported with multiple chronic problems. Polyhydramnios is common during pregnancy.

Radiographic Features

Femurs are short, flared at the metaphyses with a medial spike, and are bowed (type I) or straight (type II); other long bones are also short and bowed (see Fig. 90.4). The calvarium is large with a short base and small foramen magnum; the cloverleaf skull is sometimes present in type I thanatophoric dysplasia and is severe in type II. Vertebrae are strikingly flat (platyspondyly) with a U shape or an H shape in anteroposterior projection and uniform interpediculate narrowing. Ribs are short, cupped, and splayed anteriorly.[4,14]

Etiology

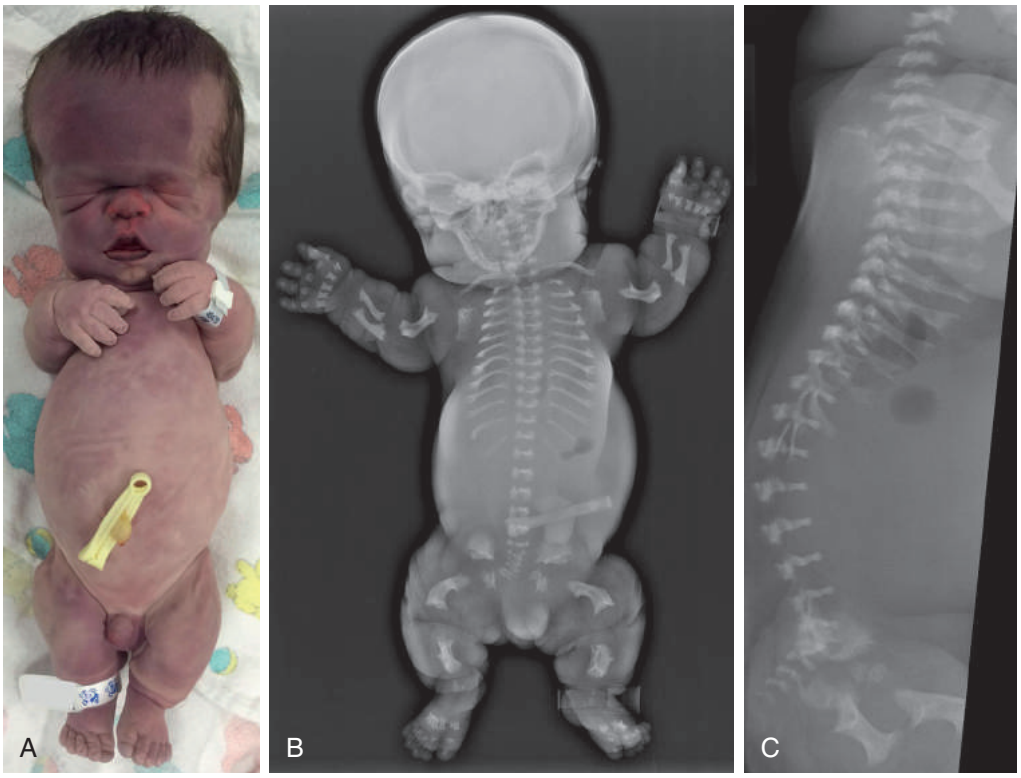
Thanatophoric dysplasia represents the severe end of the *FGFR3* spectrum. In thanatophoric dysplasia type I, the most common mutation is a substitution of cysteine for arginine at position 248 (Arg248Cys) in the receptor's extracellular domain, but other mutations have been described throughout the gene. In all studied cases of thanatophoric dysplasia type II, there is a substitution of glutamate for lysine at position 650 (Lys650Glu).[7] These mutations have been shown to constitutively activate FGFR3 to a greater extent than the common mutation seen in achondroplasia, thus resulting in a more severe phenotype.

Inheritance

All cases of thanatophoric dysplasia, as with most cases of achondroplasia and hypochondroplasia, occur sporadically and result from new autosomal dominant-acting mutations. Nevertheless, there may be a small risk of recurrence in siblings of an infant with sporadic thanatophoric dysplasia, possibly due to gonadal mosaicism. Prenatal diagnosis is available if the particular gene mutation has been previously identified in an affected individual.

Differential Diagnosis

Differential diagnoses include OI types II and III, achondroplasia (severe), achondrogenesis, and hypochondrogenesis.



• **Fig. 90.4** Thanatophoric Dysplasia. (A) Stillborn with thanatophoric dysplasia with a large head with frontal bossing, narrow chest, short limbs, and short fingers (brachydactyly). (B) Radiograph of stillborn with thanatophoric dysplasia demonstrating large cranium, narrow chest, with short ribs, shortening of all long bones (micromelia) with bowing, flattened vertebral bodies (platyspondyly), and normal bone density. (C) Lateral spine radiograph of stillborn with thanatophoric dysplasia demonstrating remarkable platyspondyly.

Management

If the condition is suspected prenatally and diagnosed by molecular means (mutation analysis following amniocentesis), the parents should receive genetic counseling and anticipate neonatal death. If the diagnosis is suggested after delivery and radiographically confirmed, management is solely supportive, with death from pulmonary insufficiency usually occurring within hours to days.

COL2A1 Spectrum

Spondyloepiphyseal Dysplasia Congenita

Presentation

SEDC manifests itself with shortened neck, trunk, and limbs, normal-sized hands and feet, flat facial profile, and occasional cleft palate and clubfoot (Fig. 90.5).[28] The name *spondyloepiphyseal dysplasia congenita* (SEDC) is derived from the spinal (*spondylo*) and growth plate (*epiphyseal*) involvement. *Congenita* indicates that the condition is evident at birth.

Radiographic Features

Radiographic features include ovoid or pear-shaped vertebral bodies in infancy, with platyspondyly more evident at a later age; odontoid hypoplasia evident in early childhood; midface hypoplasia; retrognathia; mild rhizomelia and mesomelia (see Fig. 90.5B–D); absent ossification of the os pubis; apparent decreased bone age caused by epiphyseal involvement; and development of coxa vara, variable kyphosis, and scoliosis in childhood.[15]

Etiology

SEDC is caused by mutations in the gene for collagen type II (*COL2A1*), the predominant protein building block of cartilage. Mutations in *COL2A1* are also responsible for Kniest dysplasia, some forms of spondyloepimetaphyseal dysplasia and Stickler syndrome, and the perinatal lethal disorders achondrogenesis type II and hypochondrogenesis.[9,28]

Inheritance

SEDC is inherited in an autosomal dominant pattern. Offspring of affected individuals are at 50% risk of inheriting the disorder. The recurrence risk for unaffected parents is approximately 6%, considering the possibility of parental gonadal mosaicism.

Differential Diagnosis

Differential diagnoses include a mild form of hypochondrogenesis (in neonates), as well as Morquio syndrome (in early childhood).

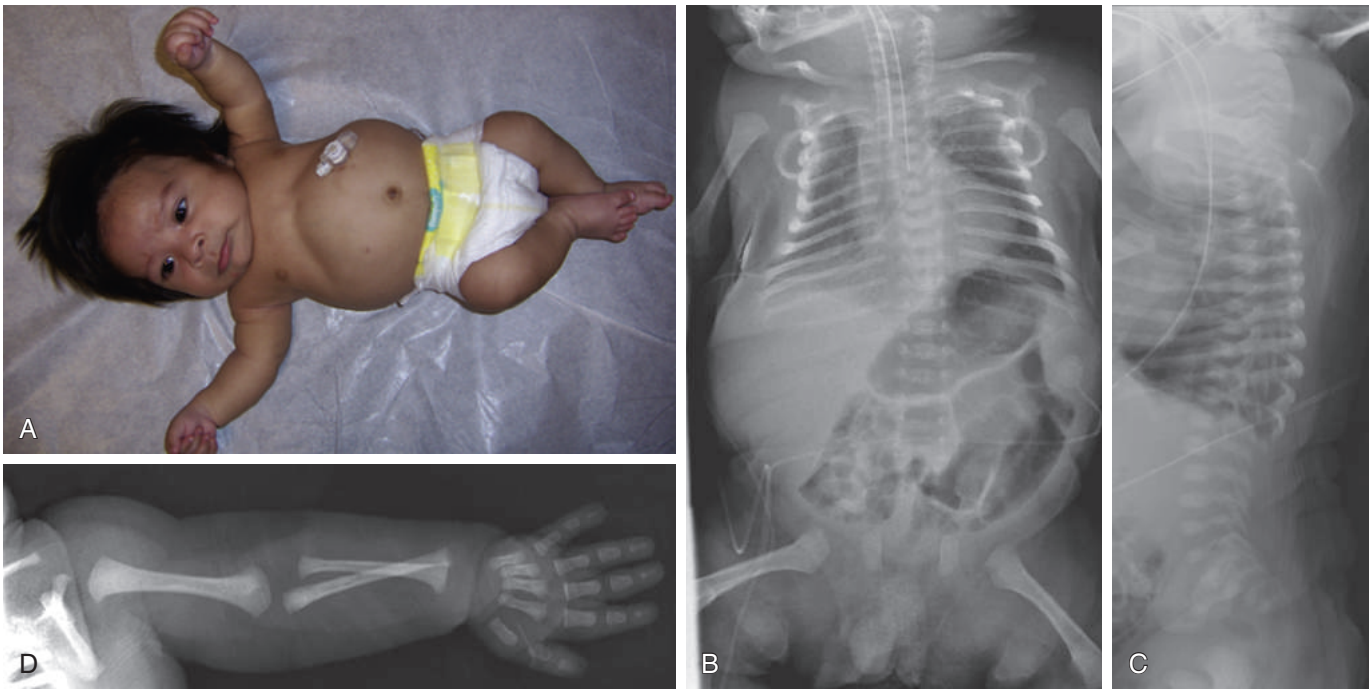
Management

Neonates may require intubation because of upper airway compromise. Care must be given when the cervical spine is manipulated (as in endotracheal intubation) because of odontoid hypoplasia. C1 and C2 fusion may be required in early childhood to stabilize the cervical spine. Annual hearing screens are recommended during childhood. Regular ophthalmologic evaluation (semiannually before school age) is essential to detect the early development of retinal detachment and to manage myopia. Osteoarthritis is a common feature in early adulthood, often requiring hip arthroplasty.

Achondrogenesis Type II–Hypochondrogenesis

Presentation

The severe end of the *COL2A1* spectrum manifests itself with fetal hydrops and maternal polyhydramnios, severely shortened trunk and limbs, and fetal or neonatal death caused by pulmonary hypoplasia.



• **Fig. 90.5** Spondyloepiphyseal Dysplasia Congenita. (A) A 2-month-old infant with a short neck, trunk, and limbs. Note the flat facial profile and normal size of the hands and feet. (B) Anteroposterior radiograph revealing platyspondyly and short chest. (C) Lateral radiograph revealing platyspondyly. (D) Upper limb radiograph revealing rhizomelia, mesomelia, and a normal-sized hand.

Radiographic Features

Radiographic features include prenatal polyhydramnios, a large calvarium with normal ossification, midface hypoplasia, retrognathia, platyspondyly with underossification of the vertebral bodies (achondrogenesis type II), short chest with a protuberant abdomen, marked shortening of all tubular bones (Fig. 90.6), and small iliac wings.

Etiology

Achondrogenesis type II–hypochondrogenesis is caused by mutations in the gene for collagen type II (*COL2A1*), the predominant protein building block of cartilage.[9,28]

Inheritance

All cases of achondrogenesis type II–hypochondrogenesis are caused by spontaneous dominant-acting mutations in *COL2A1*. The recurrence risk has been reported to be as high as 6% because of parental gonadal mosaicism.[29] Prenatal diagnosis is available if the particular gene mutation has been previously identified in an affected individual.

Differential Diagnosis

Differential diagnoses include achondrogenesis type I and OI types II and III.

Management

If the condition is diagnosed prenatally, the couple should receive genetic counseling and anticipate neonatal death. If the diagnosis is suggested after delivery and radiographic confirmation is obtained, management is solely supportive, with death from pulmonary insufficiency usually occurring within hours to days.

SLC26A2 Spectrum

Diastrophic Dysplasia

Presentation

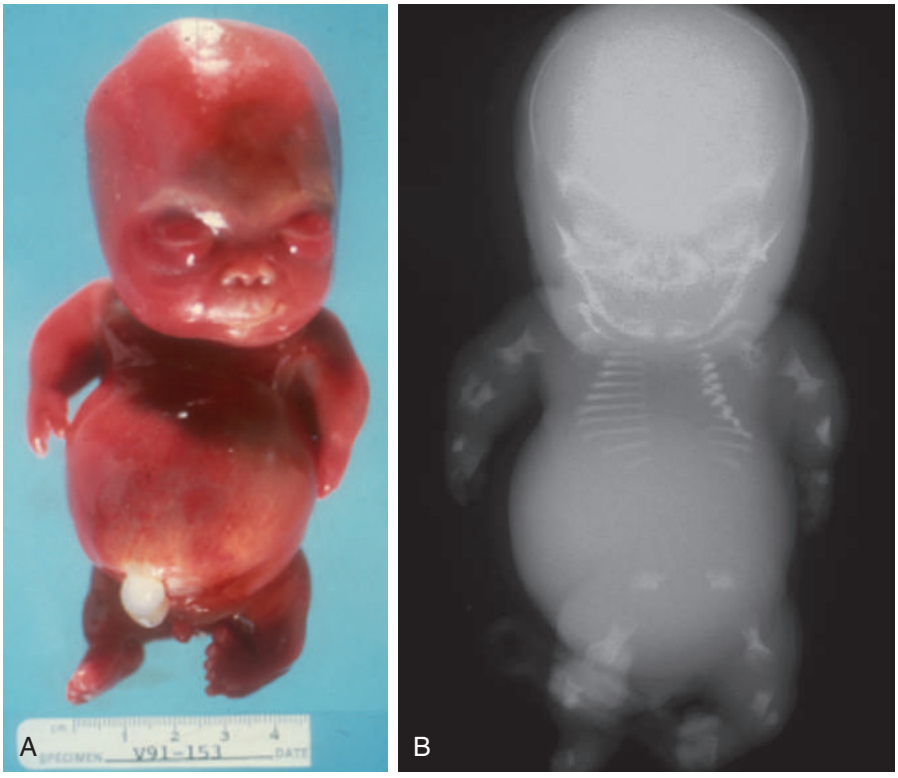
Newborns with diastrophic dysplasia exhibit limb shortening, hitchhiker thumbs, spinal deformities (especially cervical kyphosis), and contractures of the large joints (Fig. 90.7). Clubfoot and ulnar deviation of the fingers may also be present. Cystic ear swelling, with subsequent inflammation and calcification, may develop in infancy. On occasion, the disease can be lethal at birth, but most individuals survive the neonatal period.[10]

Radiographic Features

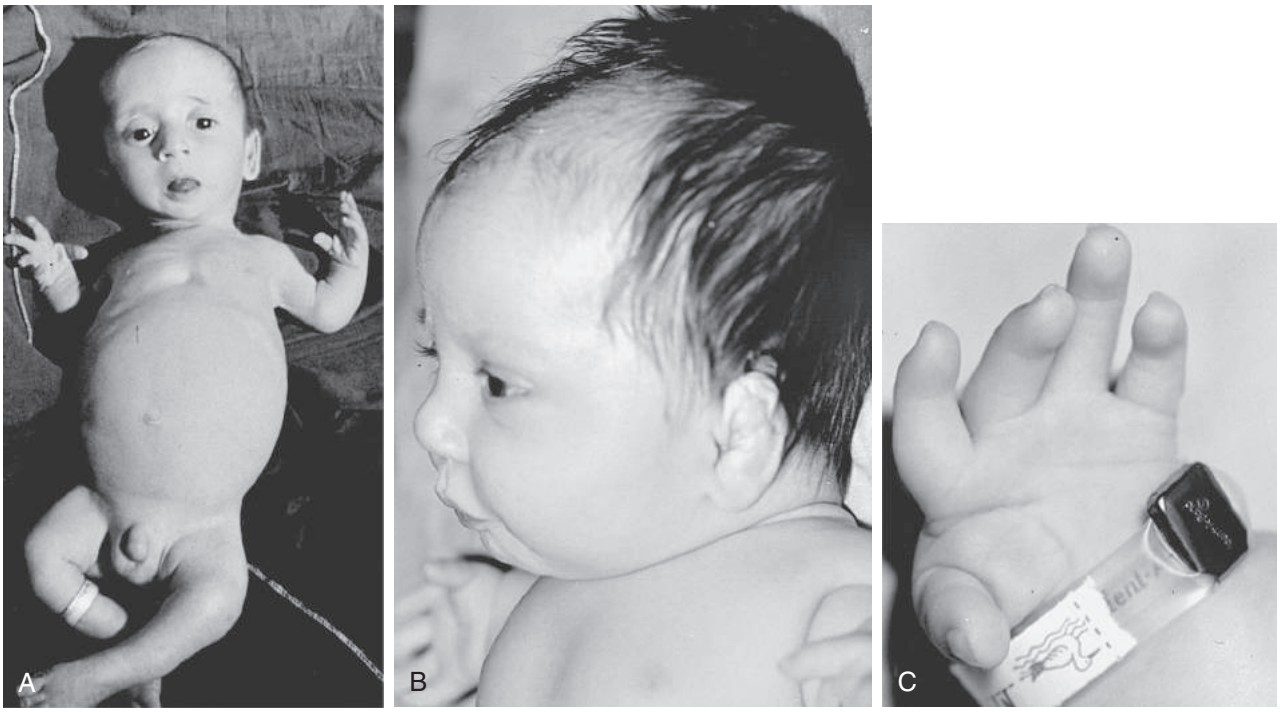
The most characteristic clinical and radiographic feature is the proximally placed hitchhiker thumb, with ulnar deviation of the fingers. Cervical kyphosis is a frequent finding. Long bones are moderately shortened and thick, with mild metaphyseal flaring, rounding of the distal part of the femur, and bowing of the radius and tibia. Severe talipes equinovarus may be present. Iliac wings are hypoplastic, with flat acetabular roofs. The chest can be narrow, bell-shaped, or both. Narrowing (lack of flare) of the interpedicular distance in the lumbar spine is reminiscent of achondroplasia.

Etiology

Diastrophic dysplasia is caused by mutations in the *SLC26A2* gene (formerly referred to as the diastrophic dysplasia sulfate transporter gene, *DTDST*), which also cause the lethal disorders achondrogenesis type IB and atelosteogenesis type II, as well as a rare recessive form of multiple epiphyseal dysplasia. The gene product is a sulfate–chloride exchanger of the cell membrane[10]; this affects



• **Fig. 90.6** Achondrogenesis Type II. (A) A 20-week fetus with a small chest and short limbs. (B) Radiograph demonstrating poor ossification of vertebral bodies and short limbs.



• **Fig. 90.7** Diastrophic Dysplasia. (A) Infant with prominent eyes, small chin, slightly narrow chest, proximally placed angulated thumbs and short limbs. (B) Neonate profile showing small chin, swollen ears, and short neck. Note the proximally placed angulated thumb. (C) View of the neonate's hand showing the proximally placed angulated thumb and mild syndactyly. (Courtesy of Paige Kaplan, Children's Hospital, Philadelphia.)

the incorporation of sulfate into proteoglycans (mucopolysaccharides), especially chondroitin sulfate B–containing proteoglycans, which are prevalent in the cartilage extracellular matrix.

Inheritance

Diastrophic dysplasia is inherited in an autosomal recessive pattern. Siblings of affected individuals are at 25% risk of inheriting an abnormal allele from both carrier parents.

Differential Diagnosis

Differential diagnoses include atelosteogenesis type II (part of the *SLC26A2* spectrum),[11] spondyloepiphyseal dysplasia, and arthrogryposis.

Management

Mechanical ventilation may be required because of the small chest circumference and a floppy airway. Maintenance of joint mobility and proper positioning through physical therapy is essential. Serial casting and/or surgical correction of clubfeet may be required. Cervical kyphosis can impede endotracheal intubation and can result in cord compression but may resolve spontaneously during infancy. The preferred treatment of auricular cysts is an application of a compression mold rather than incision and drainage.[30]

Achondrogenesis Type IB

Presentation

Achondrogenesis type IB is characterized by short stature, extremely short limbs, a relatively large head with a round face, short nose, small mouth, soft skull, and a very short neck.[12] Polyhydramnios during pregnancy, premature delivery, and hydrops are common. The affected infant is stillborn or dies within hours of birth.

Radiographic Features

The long bones are extremely short, with square, globular, or triangular shapes and medial spikes in the metaphyses of the femurs (Fig. 90.8). The calvarium and vertebrae are poorly ossified (type IB), and the ribs are short.

Etiology

Achondrogenesis type IB is caused by mutations in the *SLC26A2* gene (see Diastrophic Dysplasia earlier).

Inheritance

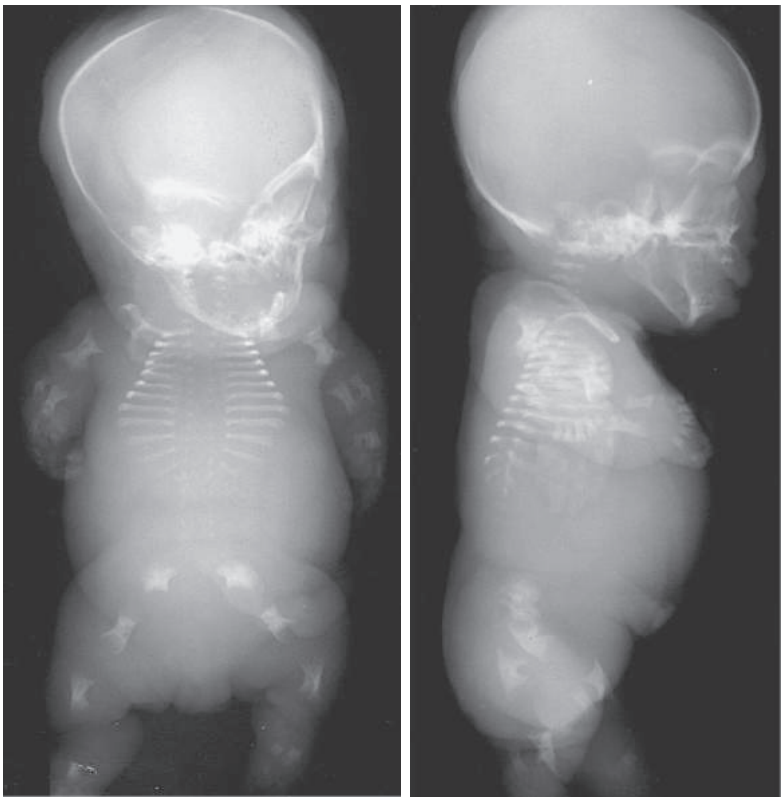
Achondrogenesis type IB is inherited in an autosomal recessive pattern. Siblings of affected individuals are at 25% risk of inheriting an abnormal allele from both carrier parents.

Differential Diagnosis

Differential diagnoses include atelosteogenesis type II (part of the *SLC26A2* spectrum), achondrogenesis type II, and hypochondrogenesis.

Management

If the condition is diagnosed prenatally, the couple should receive genetic counseling and anticipate neonatal death. If the diagnosis is suggested after delivery and radiographic confirmation is obtained, management is solely supportive, with death from pulmonary insufficiency usually occurring within hours to days.



• **Fig. 90.8** Achondrogenesis Type IB. Cervical, thoracic, and lumbar vertebral bodies are not ossified, the sacrum is not ossified, the ribs are short, and the limbs are extremely short with medial femoral metaphyseal spikes. (Courtesy Elaine Zackai, Children's Hospital, Philadelphia.)

Other Skeletal Dysplasias

Campomelic Dysplasia

Presentation

Campomelic dysplasia is characterized by short stature (birth length of 35 to 49 cm), large narrow (dolichocephalic) skull, large anterior fontanel, high forehead, flat face, widely spaced eyes with short palpebral fissures, low-set ears, cleft soft palate, micrognathia, relatively long and slender thighs, and upper arms, somewhat shortened bowed legs with dimples in the midshaft (in most cases), narrow chest, and kyphoscoliosis (Fig. 90.9). Sex reversal or varying degrees of ambiguous genitalia affects 75% of chromosomal (46, XY) males. The absence of the olfactory bulbs and tracts, as well as cardiac and renal malformations, may occur. Death, usually in infancy, results from pulmonary hypoplasia, tracheomalacia, or cervical spinal instability. Rare survivors are usually globally developmentally delayed. A few more mildly affected people without bowed limbs have been reported.[31]

Radiographic Features

The most characteristic finding is midshaft angulation (campomelia) of the femurs, although it is not a constant finding. Other features include hypoplastic, undermineralized cervical vertebrae and thoracic pedicles; narrow iliac wings with dislocated hips; brachydactyly; clubfeet; anterior bowing of tibiae; bell-shaped chest with thin, wavy ribs (with only 11 pairs); and scapular hypoplasia (see Fig. 90.9C).

Etiology

Campomelic dysplasia is caused by mutations in or near the sex-determining region Y–box 9 gene, *SOX9*.[31] *SOX9* is homologous to the *SRY* gene and encodes a transcription factor involved in both bone formation and testis development.

Inheritance

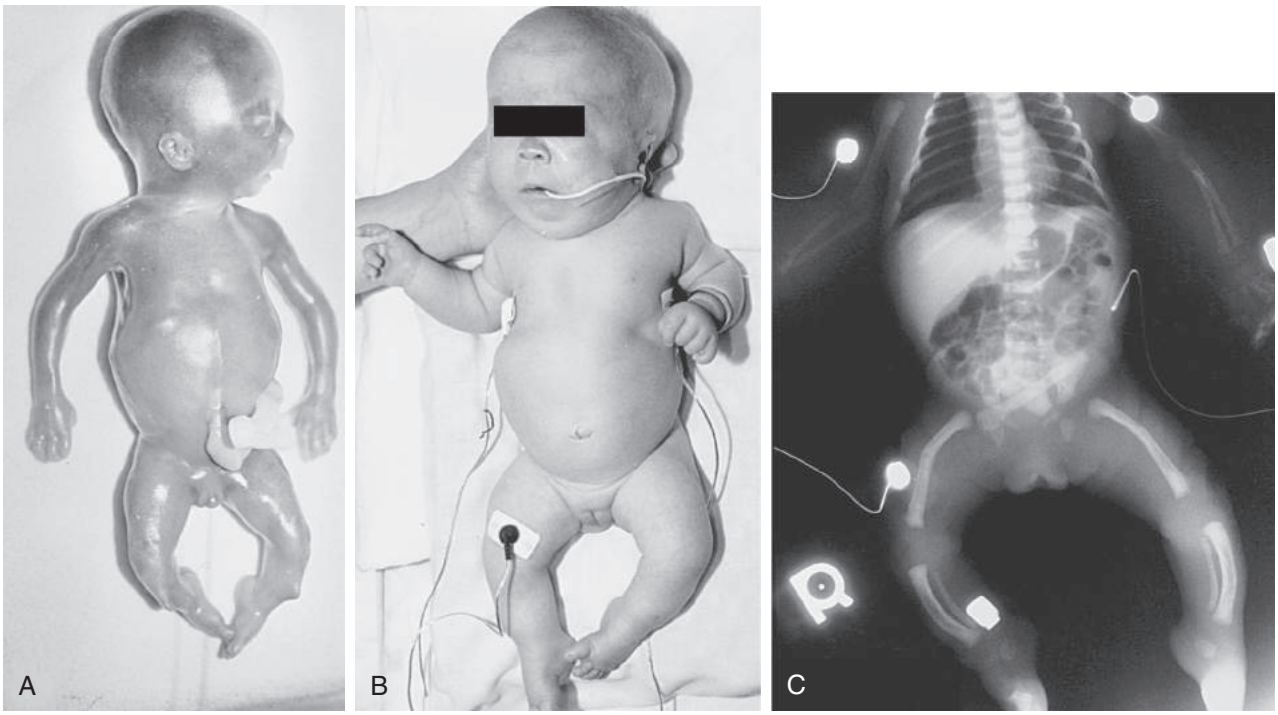
Campomelic dysplasia is due to spontaneous dominant-acting SOX9 mutations. Most cases are new sporadic occurrences in a family; recurrence caused by gonadal mosaicism has been reported.[31]

Differential Diagnosis

Differential diagnoses include OI types II and III, diastrophic dysplasia, kyphomelic dysplasia, thanatophoric dysplasia, and SEDC (severe).

Management

Survival beyond the newborn period is rare; therefore, support is primarily directed toward comfort measures. In survivors, care must be given to the cervical spine, which may be unstable. Chromosomal studies to determine sex and pelvic ultrasonography to examine internal genitalia may be performed. For survivors, cleft palate may be repaired in those able to feed orally, and clubfeet may require casting or surgical correction.



• **Fig. 90.9** Campomelic Dysplasia. (A) A 22-week-old 46, XY female fetus with normal head, long philtrum, micrognathia, low-set ears, mild narrowing of the chest, proximally placed thumbs, and bowed or angulated lower limbs resembling those of osteogenesis imperfecta type II but less shortened. The external genitalia is female. (B) Neonate with the long-limb form of the disorder who has a relatively large head, micrognathia, a narrow chest, and bowing of the lower limbs with characteristic dimpling of the lower leg. (C) Radiograph showing the narrow chest, the relatively long, thin limb bones with bowing of the femurs and tibiae, and a long, narrow pelvis. (Courtesy of Paige Kaplan, Children's Hospital, Philadelphia.)

Heritable Connective Tissue Disorders

Genetic disorders of connective tissue represent a large group of pleiotropic diseases that may involve the musculoskeletal, cardiovascular, and pulmonary systems as well as the eyes and skin. These diseases are caused by pathogenic variants in genes that function in extracellular matrix assembly and/or homeostasis and may result in serious clinical consequences.[32,33] Several of these disorders present in the newborn period with dramatic joint hypermobility and/or abnormal skin findings (see Table 90.3). Diagnosis of many of these conditions can be established by prenatal or neonatal testing via multigene NGS panels[34] or whole exome sequencing.

Early-Onset/Rapidly Progressive (Congenital; Neonatal, Infantile) Marfan Syndrome

Presentation

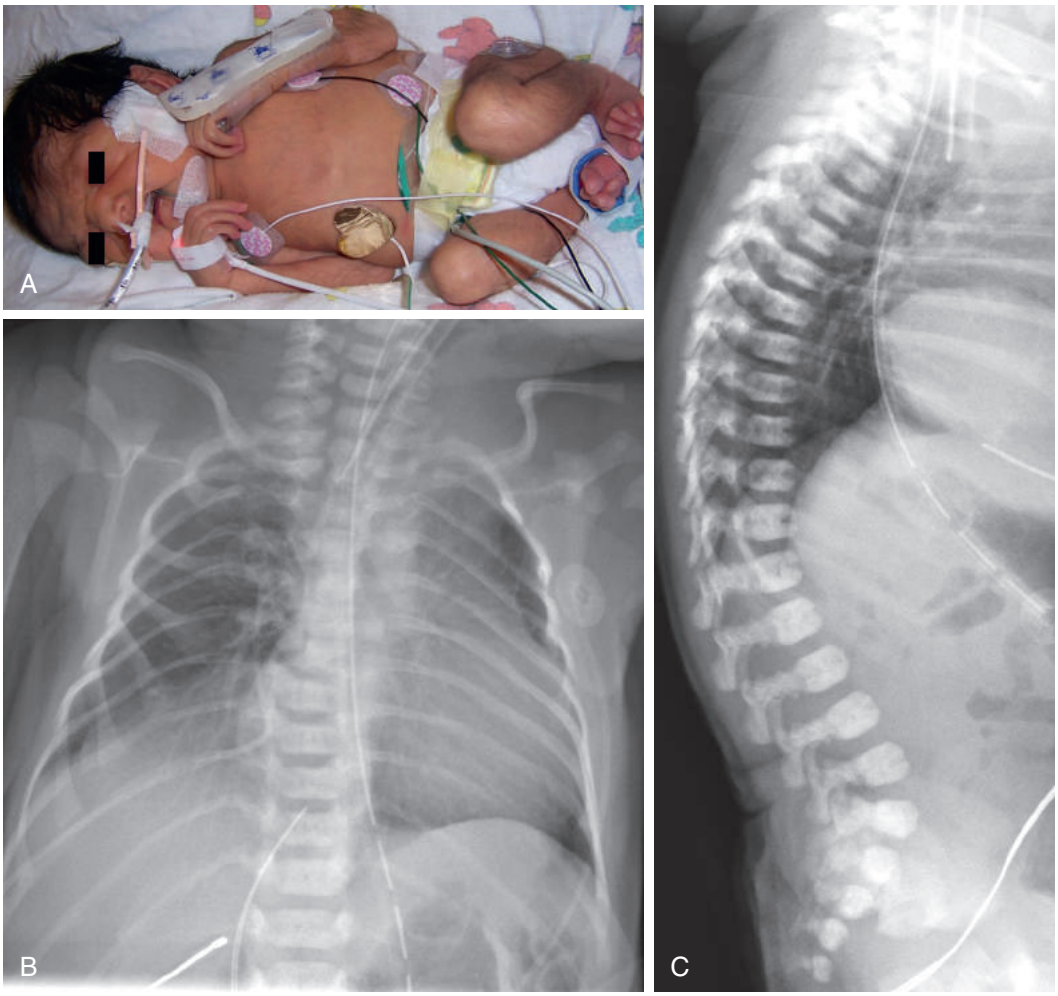
Infants with early-onset/rapidly progressive Marfan syndrome have a long, thin body and can have an aged appearance because of a lack of subcutaneous tissue and wrinkled, sagging skin (Fig. 90.10).[35] The craniofacial features include dolichocephaly (narrow head), deep-set eyes with large or small corneas (and occasionally cataracts), high nasal bridge, high palate, small pointed chin with a horizontal skin crease, and large, simple or crumpled ears. The fingers and toes are long and thin (arachnodactyly). Some joints are hyperextensible, and others have flexion contractures causing clubfoot, dislocated hips, or adducted thumbs. Infants tend to exhibit hypotonia with low muscle mass. Lenses are usually not subluxated at birth. The most important cause of morbidity and mortality is severe cardiovascular disease, which affects almost every neonate with congenital Marfan syndrome (cMS)—namely, mitral and tricuspid valve prolapse and insufficiency, as well as aortic root dilation. The ascending aorta may be dilated and tortuous. Many infants die of congestive heart failure in the first year of life. Survivors have chronic hypotonia and contractures, are unable to walk, and require many surgical procedures.

Radiographic Features

Radiographic features include pectus deformity, spontaneous pneumothorax, dural ectasia, aortic root dilation, and mitral valve prolapse. Many of these features may not be present in the newborn period.

Etiology

Congenital Marfan syndrome is caused by mutations in the gene encoding fibrillin 1 (*FBN1*).[36] Fibrillin is a glycoprotein associated with microfibrils, which form linear bundles in the matrices of many tissues, such as aorta, periosteum, perichondrium, cartilage, tendons, muscle, pleura, and meninges. There are two regions in *FBN1* in which many mutations cause cMS to occur; these lie among exons 24 to 27 and exons 31 to 32.[36] Molecular analysis does not yield mutations in all cases.



• **Fig. 90.10** Congenital Marfan Syndrome. (A) Neonate with furrowed brow and lax facial skin, thin, simple ears, small chin, short neck with redundant skin (not shown), multiple joint flexion contractures, and striking arachnodactyly of fingers and toes. The neonate also had megalocornea, pectus carinatum, mitral valve prolapse, aortic root dilation, and an *FBN1* gene missense mutation. (B) Chest radiograph revealing thin ribs and serpentine clavicles. (C) Lateral spine radiograph demonstrating exaggerated thoracic kyphosis.

Inheritance

Marfan syndrome is an autosomal dominant disorder. In most neonates with cMS, an occurrence is sporadic within a family.[35] However, there is one well-documented neonate with cMS whose father had classic Marfan syndrome except for average height.[37] There are also reports of familial cMS due to parental gonadal mosaicism.[38]

Differential Diagnosis

Differential diagnoses include CCA, autosomal recessive cutis laxa, and Loeys–Dietz syndrome.

Management

Patients require annual ophthalmologic and cardiac evaluation throughout childhood. The use of cardioselective beta-blockers, such as atenolol, is often implemented at the first signs of aortic root dilation. The angiotensin II antagonist losartan has also shown promise in this regard and may be used in combination with beta-blockers.[39,40] Children should be screened for the development of scoliosis.

Congenital Arachnodactyly (Beals Syndrome; Distal Arthrogryposis Type 9)

Presentation

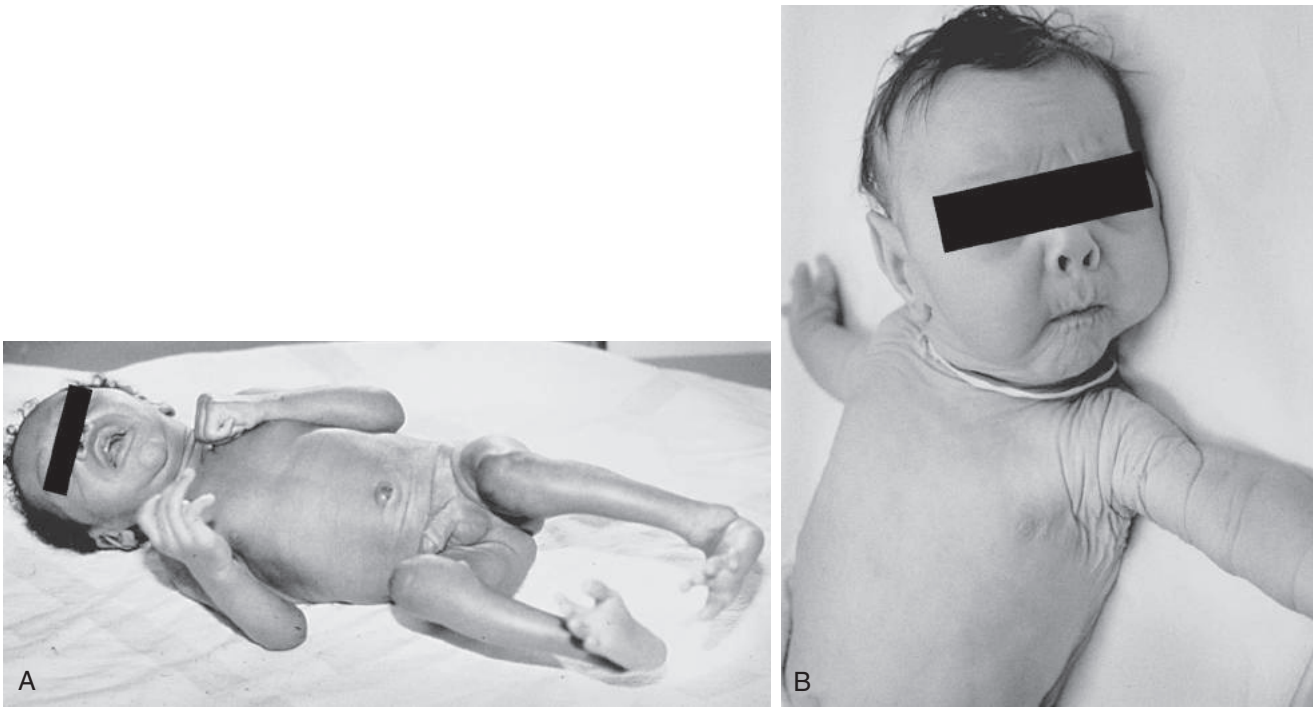
CCA (Beals syndrome; distal arthrogryposis type 9) is characterized by a thin, wasted appearance with minimal muscle and fat mass (similar to neonatal Marfan syndrome). Distinctive features include arachnodactyly with contractures of the large and small joints (Fig. 90.11A), as well as crumpled, overfolded helices of the external ear. Cardiovascular involvement is usually limited to mitral valve prolapse, but aortic root dilation may occasionally develop.[41]

Radiographic Features

Features are nonspecific and include elongated proximal phalanges; contractures of digits, ankles, knees, and hips; thin, gracile tubular bones; and gradual development of kyphoscoliosis.

Etiology

CCA is caused by dominant-acting mutations in the gene encoding fibrillin 2 (*FBN2*).



• **Fig. 90.11** (A) Congenital contractural arachnodactyly (Beals syndrome). This infant has a long, thin trunk and limbs, contractures of the joints, and crumpled ears. (B) Infant with cutis laxa. (Courtesy Montreal Children's Hospital.)

Inheritance

CCA is inherited in an autosomal dominant manner, with most patients representing the result of spontaneous mutations. Offspring of affected individuals are at 50% risk of inheriting the condition. Gonadal mosaicism has been described in CCA.[42]

Differential Diagnosis

Differential diagnoses include cMS, Stickler syndrome, homocystinuria, and distal arthrogryposis.

Management

Proper nutrition is essential to ensure adequate weight gain. Joint contractures respond to physical therapy, but occasionally surgical release may be required. Surveillance for the development of spinal curvature and aortic root dilation, although rare, is essential throughout childhood.[41]

Ehlers–Danlos Syndromes

Presentation

The EDSs are a clinically and genetically heterogeneous group of connective tissue disorders that are characterized by various degrees of joint and skin hypermobility, excessive bruising, abnormal wound healing, and fragility of tissues.[32,43] The types that are more likely to present in the newborn period include the classical type (formerly type I), the kyphoscoliotic type (formerly type VI), the dermatosparaxis type (formerly type VIIc), and the arthrochalasis type (formerly types VIIa and VIIb). The classical type often presents with premature delivery of an affected fetus as a result of rupture of the fragile amniotic membranes. The infant may be floppy and in the breech position, and there may be joint laxity and joint instability.[44] Among the rarer types of EDS, the kyphoscoliotic type often presents with severe hypotonia and scoliosis with a risk of subsequent ocular involvement.[45] In the arthrochalasis type, the major involvement is of the ligaments and joint capsules. Large and small joints are hypermobile and dislocated; severe congenital dislocation of hips occurs. The findings in the dermatosparaxis type include soft, fragile, and redundant skin with some joint hypermobility.

In vascular (formerly type IV) EDS, the greatest danger is to the pregnant affected woman, for whom there is a high risk of uterine and arterial rupture.[46] Although there is a 50% risk that the fetus will be affected, the problems of blood loss and prematurity are more important in the newborn period than the disorder itself.

Radiographic Features

Radiographic features are dependent on the particular type of EDS. Congenital hip dislocation may be evident on plain films. Hydronephrosis, bladder diverticula, and spontaneous pneumothorax may occur occasionally. Aortic dilation and arterial aneurysms may be evident by echocardiography and other imaging modalities but occur only in patients of school age or older.

Etiology

Mutations in two of the genes for collagen type V (*COL5A1* and *COL5A2*) are demonstrable in some cases of classical EDS.[44] The vascular type is caused by mutations in the gene for collagen type III (*COL3A1*). The arthrochalasis type is caused by mutations in either of the collagen type I genes (*COL1A1* or *COL1A2*), which result in loss of the N-proteinase cleavage site of the protein. The kyphoscoliotic type is caused by mutations in the gene for a procollagen cross-linking enzyme (*PLOD1*). The dermatosparaxis type is caused by mutations in a gene for a procollagen proteinase (*ADAMTS2*).

Inheritance

Most types of EDSs are inherited as autosomal dominant traits. Each child of an affected person has a 50% chance of inheriting and manifesting the disorder, although there can be marked intrafamilial variability.[44] The kyphoscoliotic and dermatosparaxis types of EDSs are inherited in an autosomal recessive manner.

Differential Diagnosis

Differential diagnoses include cMS, CCA (distal arthrogryposis type 9), Larsen syndrome, and other rare variant forms of EDS.[32,33]

Management

Trauma should be avoided because of skin fragility. The effective closure of surgical wounds is challenging in some types because of a tendency for dehiscence.

Cutis Laxa

Presentation

Cutis laxa is a group of genetically heterogeneous disorders that primarily affect the assembly and homeostasis of elastic fibers. As such, the presentation can be highly varied. Infantile forms may exhibit loose, furrowed skin, a large anterior fontanel, hypotonia, hernias, and congenital hip dislocation (see Fig. 90.11B).[33,47]

Radiographic Features

Radiographic features are, in part, dependent on the genetic form of the disorder. Nonspecific features include a large anterior fontanel, congenital hip dislocation, and hernias. The X-linked form may exhibit occipital horns of the skull. Arterial tortuosity, aortic root dilation, and cortical and cerebellar anomalies may be seen in some forms, as well as the gastrointestinal tract and urinary tract diverticula.

Etiology

The relatively mild, autosomal dominant form of cutis laxa is caused by mutations in the elastin gene (*ELN*). The X-linked recessive form (occipital horn syndrome) is caused by mutations in the *ATP7A* gene (allelic with Menkes syndrome). Autosomal recessive forms may be caused by mutations in the fibulin 4 gene (*FBLN4*), the fibulin 5 gene (*FBLN5*), or the gene that encodes the A2 subunit of vacuolar H^+-ATPase (*ATP6V0A2*). Biochemical clues as to the cause in a particular patient may include decreased serum copper and ceruloplasmin levels (X-linked form), or an abnormal serum sialotransferrin isoelectric focusing pattern in cases caused by *ATP6V0A2* mutations.

Inheritance

Because cutis laxa is genetically heterogeneous, the modes of inheritance include autosomal dominant, autosomal recessive, and X-linked recessive. The latter two modes are usually responsible for forms with a neonatal and infantile presentation.

Differential Diagnosis

Differential diagnoses include EDS, Menkes syndrome, generodermia osteodysplastica, and de Barsy syndrome.

Management

Serious childhood complications include developmental delay, pulmonary emphysema, aortic root dilation, and arterial tortuosity. Annual ophthalmologic and cardiac examinations are essential, and referral to special education programs may be indicated.

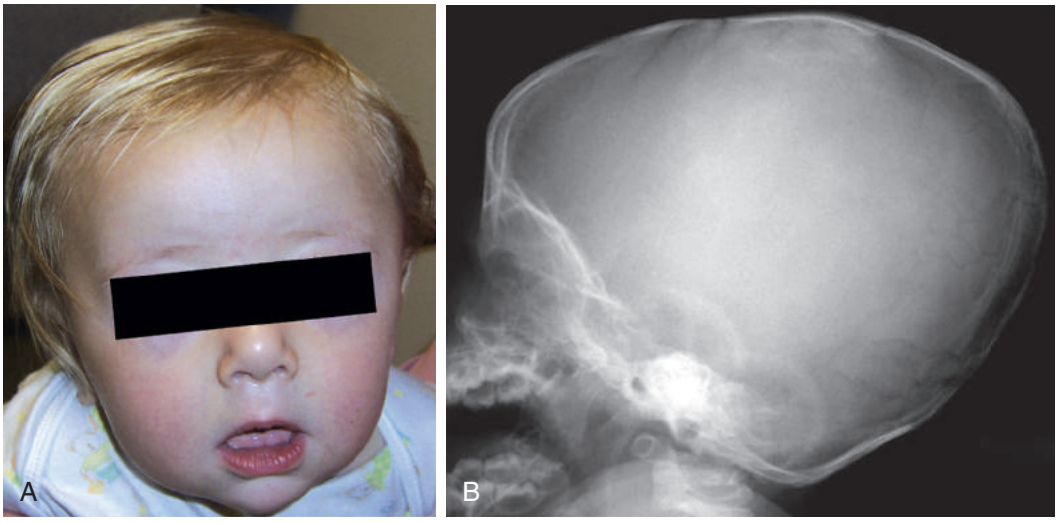
Menkes Syndrome

Presentation

Menkes syndrome often appears in the newborn period with nonspecific neurologic manifestations. Typically, developmental delay is evident in the first 2 to 3 months of life, with failure to thrive, seizures, and severe ocular manifestations. Changes in the appearance of the hair include hypopigmentation, brittleness, patchy alopecia, and twisted shafts seen by light microscopy (i.e., pili torti; Fig. 90.12A). Early death is common and may occur in infancy. Serum copper and ceruloplasmin concentrations are low, and the plasma dopamine-to-norepinephrine ratio may be elevated.[48]

Radiographic Features

Features may evolve during infancy and may include bladder diverticula (seen on bladder ultrasonography and voiding cystourethrogram), tortuous vessels (on echocardiogram or magnetic resonance



• **Fig. 90.12** Menkes Syndrome. (A) Note the blonde hair and fair complexion in this 11-month-old Hispanic boy. (B) Note multiple Wormian bones near the occiput.

imaging with contrast agent), gastric polyps (on upper gastrointestinal [GI] contrast study), metaphyseal spurring, osteopenia, and Wormian bones on plain radiographs (see Fig. 90.12B).[14]

Etiology

Menkes syndrome is caused by mutations in the gene encoding a copper-transporting adenosine triphosphatase (*ATP7A*). This enzyme participates in the final processing of several copper-dependent enzymes, including dopamine β-hydroxylase, tyrosinase, lysyl oxidase, superoxide dismutase, and cytochrome *c* oxidase. As a result, several physiologic processes and cellular functions are impaired, including collagen cross-linking, pigment production, and neurotransmission.[48]

Inheritance

Menkes syndrome is an X-linked recessive disorder, and therefore only males are affected. Female carriers may exhibit pili torti in some hair shafts because of lyonization.[48] Sons born to carrier females have a 50% risk of manifesting the disease.

Differential Diagnosis

Differential diagnoses include cutis laxa (the occipital horn form is allelic), EDS, neonatal cMS, biotinidase deficiency, mitochondrial myopathies, nutritional copper deficiency, and some organic acidurias.

Management

Early diagnosis allows copper supplementation therapy, but this is not effective in all patients.[49] Patients should be monitored for the development of seizures, as well as a propensity for bone fragility, poor wound healing, and vascular fragility leading to excessive bleeding, hemorrhagic strokes, and subdural hematomas. Bladder diverticula may result in urinary retention and urinary tract infections and should be surgically corrected. Patients are at risk of moderate to severe developmental delay, and they should be referred to infant stimulation and early intervention programs.

Family Support and Education

Many of the conditions described in this chapter are rare, and parents (and sometimes medical staff) are unfamiliar with or bewildered by such conditions. Providing appropriate educational materials is of utmost importance for making important medical decisions and preparing for the future. Support groups such as the Little People of America (http://www.lpaonline.org) and the Osteogenesis Imperfecta Foundation (http://www.oif.org) are helpful in this regard, as the medical content is reviewed and updated by experts in the field. In addition, individual entries in GeneReviews (https://www.ncbi.nlm.nih.gov/books/NBK1116/) frequently include links to support groups as well as to educational pieces written in lay languages, such as the NIH Genetics Home Reference (https://ghr.nlm.nih.gov/condition).

Suggested Readings

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91 Newborn Skin Development: Structure and Function

ROBERT SIDBURY

KEY POINTS

- Healthy full-term infant skin is well developed and serves as an effective barrier. Premature infants, especially those of very low birth weight, have an ineffective barrier that increases the risk of invasive infection, dehydration, cutaneous injury, and toxic absorption.
- Birthmarks may have neurologic implications that require magnetic resonance imaging. In some cases, newborns and very young infants can be imaged using a "feed and wrap" technique without sedation. Awareness of this window of opportunity may help avoid unnecessary general anesthesia.
- Early application of emollients in certain infants can improve barrier function, decrease risk of infection, and potentially prevent atopic outcomes.
- Blaschko lines are patterns formed by the migration of skin cells during embryogenesis. They are distinct from dermatomes. Cutaneous findings in this distribution are a clue to an underlying mutational event.

The skin serves several critical roles, including as a primary barrier against infection, thermoregulation, and electrolyte homeostasis. Disruption as a result of genetic mutation, injury, or prematurity can be life-threatening. This chapter details the stages of human skin development, the potential challenges of prematurity, and an approach to important clinical presentations.

Cutaneous morphogenesis is complex and incompletely understood. Advances have come from traditional murine model work but also in "reverse" by identifying mutations causing disorders that manifest with developmental defects (e.g., aplasia cutis congenita).[1] Human skin develops from apposed tissue of both mesodermal and ectodermal origin. From the mesoderm arise fibroblasts, vascular cells, adipocytes, and immune-presenting Langerhans cells, which ultimately reside in the epidermis. Ectoderm-derived tissue includes keratinocytes and neural crest-derived melanocytes. The assembly of the epidermis, dermoepidermal junction (DEJ), dermis, and fat, along with epidermal appendages and immigrant cells, is a complex dynamic with the potential for clinically significant disruption at every step. Table 91.1 enumerates these critical stages. The clinician can use this ontogeny to guide assessment: Any newborn with evident cutaneous pathology should prompt additional scrutiny of the hair, nails, teeth (natal teeth can suggest specific disorders), and the central nervous system.

Epidermis

The epidermis derives entirely from ectoderm. A single-layered epithelium spans the embryo from gastrulation. Subsequent development proceeds in discrete stages: embryonic (5 to 8 weeks) and embryonic/fetal transition (9 to 10 weeks) and then to the early (11 to 14 weeks), mid (15 to 20 weeks), and late (20 weeks–birth) fetal periods.[2] Differentiation and formation of appendages occur predominantly during the second trimester. It is during the late fetal stage that the skin first becomes functional. The stratum corneum, or outer epidermis, has been likened to "bricks and mortar."[3] Epidermal keratinization begins first on the head, face, and palms. Anucleate corneocytes flatten and form "bricks," while a mixture of cells, adhesion proteins, and lipids form the semipermeable mortar. One such protein, filament aggregating protein, or filaggrin, has recently been linked to atopy. Patients with filaggrin mutations are at greater risk for eczema, asthma, and allergies.[4] Decreased ability to retain moisture with resulting fissures in the stratum corneum may promote epicutaneous allergen exposure and sensitization. Emerging evidence suggests that early aggressive application of thick emollients to at-risk infants decreases their chance of developing atopic dermatitis.[5]

Dermoepidermal Junction

The DEJ is the interface between basal layer keratinocytes of the epidermis and the dermis and helps the skin resist shearing forces. The critical elements of the DEJ form by 8 to 10 weeks' gestation. The DEJ is composed of hemidesmosomes, anchoring filaments, anchoring fibrils, and type VII collagen, which combine to tether the dermis to the epidermis (Fig. 91.1). Epidermolysis bullosa (EB) is an inherited disorder of cutaneous fragility that results from defective proteins in this complex. The type of EB depends on the affected protein, and outcomes can vary widely.

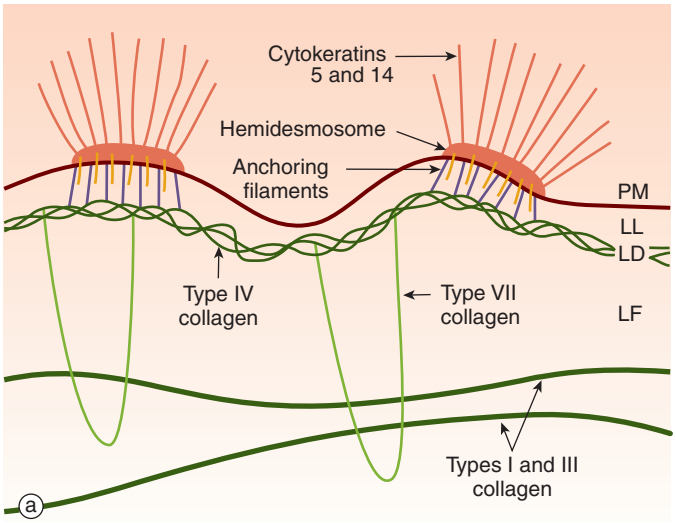
Dermis and Subcutis

The dermis is derived from diverse tissue types. Embryonic mesenchymal cells capable of becoming multiple cell types are enmeshed in a hyaluronic matrix called a *cellular dermis*. At 8 weeks' estimated gestational age (EGA), the dermis is

TABLE 91.1 Milestones in Fetal Skin Embryogenesis

Milestone	Gestational Week
Expression of epidermal keratins	6
Development of specialized cells, including melanocytes, Langerhans	8
Formation of dermoepidermal junction	8–10
Beginning of nail development	10
Beginning of hair development	12
Palmoplantar sweat gland formation	10–12
Formation of fat in subcutis	15
Sweat gland formation in rest of body	24–26

Adapted from Holbrook KA, Odland GF. The fine structure of developing human epidermis: light, scanning, and transmission electron microscopy of the periderm. *J Invest Dermatol.* 1975;65:16–38.



• **Fig. 91.1** Dermoepidermal junction. (Courtesy of Basicmedical key.com.) *a*, Collagen; *LD*, lamina densa; *LF*, lamina fibroreticularis; *LL*, lamina lucida; *PM*, plasma membrane.

distinguishable from underlying tissue. By 15 weeks, collagen fibers accumulate, and by 22 to 24 weeks, elastin fibers are first detectable by electron microscopy.[2] Immature dermis will not scar when traumatized (e.g., with a fetal skin biopsy), whereas the increasing tensile strength that accompanies maturation also connotes potential to scar; this occurs at roughly the end of the second trimester. Blood vessel plexi are discernible by 12 weeks' EGA but do not fully mature until after birth. Vasculogenesis, the formation of endothelial cells and then vessels from angioblasts, is completed by 20 weeks' gestation; angiogenesis follows as existing vessels give rise to new ones. Sprouting angiogenesis may add new vessels to tissue previously lacking vascularity; this involves spouts of endothelial cells growing toward an angiogenic stimulus, such as vascular endothelial growth factor or basic fibroblast growth factor. Intussusceptive angiogenesis is characterized by a splitting process whereby the vessel wall invades the lumen, causing the vessel to divide in two. This type of angiogenesis occurs only in areas of preexisting vascularity. Both types of angiogenesis can occur in virtually all tissues and organs.[6] Neural networks develop toward the end of the first trimester and follow a vascular pattern. Adipose tissue develops beneath the dermis, starting in the second trimester.[7]

Goltz syndrome (focal dermal hypoplasia) is an X-linked dominant disorder caused by mutations in *PORCN*. Ninety percent of affected individuals are female and manifest with striking linear atrophic plaques with distinctive fat herniation due to dermal atrophy.

Appendages

Hair, nail, sweat gland, and apocrine gland development relies on coordinated dermal–epidermal interaction that commences around 10 weeks' gestation. Hair follicle formation, which directs overlying epidermal basal cells to aggregate in the form of a placode, is initiated in the dermis. Hair follicles differentiate during the second trimester, and hair canals are formed by 20 weeks' EGA when scalp hairs are just visible. Hairs cycle between growth (anagen), resting (catagen), and shedding (telogen). The first shedding cycle occurs around 28 weeks' gestation, after which they reenter the anagen phase. The final shedding phase generally occurs postnatally, at the occiput, leading to occipital alopecia that often is misattributed to pressure or friction.

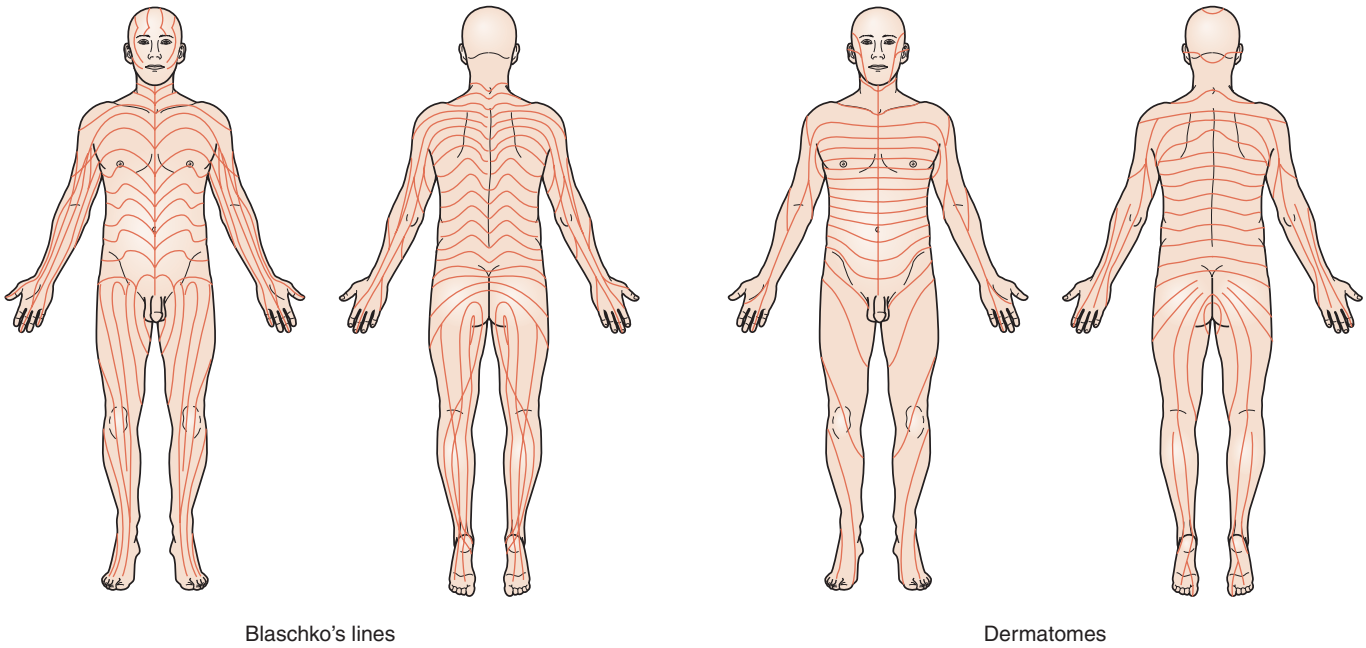
Nails begin development at 8 weeks' EGA, slightly earlier than the hair follicles. A keratinized nail has completely covered the nail plate by 5 months' EGA. Hair and nail keratins have greater structural integrity than those in the epidermis.

Sebaceous glands develop contemporaneously with hair follicles. At 13 to 16 weeks' EGA, characteristic sebaceous gland bulges can be seen with hair follicles, just above the insertion of the arrector pili muscle. Maternal hormones induce sebaceous gland production of sebum during the late second and third trimesters. Sebum and shed corneocytes form vernix, which progressively coats the fetus in a cephalocaudal orientation. Fetal lung maturity parallels sebaceous gland activity, and increased physiologic concentrations of surfactant emulsifies surface vernix.

Palmoplantar eccrine sweat glands begin to develop at 55 to 65 days' gestation and are fully developed by the second trimester. Interfollicular sweat glands and apocrine glands do not form until the middle of the second trimester. Ectodermal dysplasias are a heterogeneous group of disorders with striking abnormalities in skin appendages. Hydrotic ectodermal dysplasia, or Clouston syndrome, is due to mutations in *GJB6* (connexin 30), which directs formation of gap junctions found notably in adnexal structures.

Specialized Skin Cells

Melanocytes, Langerhans cells, and Merkel cells comprise the primary epidermal immigrant cells. Melanocytes derive from the neural crest and migrate along Blaschko lines to populate the entire fetal epidermis. Melanocytes are first identifiable at 50 days' EGA and are fully present at birth, but melanogenesis continues such that skin is not fully pigmented at birth and will darken in the first months. Blaschko lines are distinct from



• **Fig. 91.2** Blaschko's lines or developmental lines of epidermal migration.

dermatomes, and clinical abnormalities in this distribution can be helpful diagnostically (Fig. 91.2). Incontinentia pigmenti, an X-linked dominantly inherited gene dermatosis due to mutations in *NEMO*, presents with a series of cutaneous findings over time, first vesicles and then a warty hyperkeratotic phase, followed by dyspigmentation all along Blaschko lines. Knowledge of this pattern can help discriminate from fundamentally different processes, such as herpes zoster, which would present with vesicles in a dermatomal distribution.

Langerhans cells first appear at 40 days' gestation and serve as antigen-presenting cells. Merkel cells appear at 8 weeks' gestation and serve a mechanoreceptor function.

Epidermal Stem Cells

The skin must constantly repair and renew itself. There are several different types of stem cells within the skin, including basal keratinocytes, hair follicles, and melanocytes. Basal keratinocytes can be grown in sheets and used for therapeutic barrier purposes in burn patients and those with chronic wounds (e.g., in EB). Such grafts are useful but do not contain appendages such as hair follicles or sweat glands. Improved understanding of the interaction among various cutaneous stem cells, their microenvironments, and their pluripotential is driving advances in stem cell technology.[8]

Impact of Prematurity

The stratum corneum reaches functional maturity around 33 weeks' EGA. The combination of a developmentally and functionally incompetent barrier, absence or diminished vernix caseosa, large body-surface-area-to-mass ratio, and an immature immune system places premature infants, in particular those born at less than 33 weeks' gestation, at significant risk for invasive infection and skin injury. A unique consideration in premature infants is the risk of percutaneous toxicity. Topical medications, cleansers, and even emollients (e.g., lactic acid) can lead to dangerous systemic levels in premature infants. A particularly seductive risk is that of cutaneous anesthesia when used for small invasive procedures. Minimizing pain is important, but careful attention to safe limits of topical anesthetic is vital.[9] Table 91.2 compares premature and term infant skin. Table 91.3 summarizes important potential cutaneous absorption risks.

TABLE 91.2 The Impact of Prematurity on Skin Physiology

	Premature	Term
Skin thickness	0.9 mm	1.2 mm
Epidermal thickness	20 μ	40 μ
Epidermal covering	No vernix before 28 weeks	Vernix
TEWL	High (at 26 weeks TEWL = 45 g/m per h)	Low (TEWL = 5 g/m per h)
Acid mantle	Incomplete	Complete
Percutaneous absorption	Very high	Normal

TEWL, Transepidermal water loss.

Care of Preterm Skin

Advances in the understanding of newborn physiology, coupled with technological developments, have contributed to the increased survival of extremely low birth weight (ELBW) infants. The care of such infants poses unique issues across organ systems,

TABLE 91.3 Reported Hazards of Percutaneous Absorption in the Newborn

Compound	Reference	Product	Toxicity
Aniline	Rutter (1987)	Dye used as a laundry marker	Methemoglobinemia,* death
Mercury	Dinehart et al. (1988)	Diaper rinses, teething powders	Rash, hypotonia
Pentachlorophenol	West et al. (1981)	Laundry disinfectant	Tachycardia, sweating, hepatomegaly, metabolic acidosis, death
Hexachlorophene	—	Topical antiseptic (pHisoHex)	Vacuolar encephalopathy, death
Resorcinol	West et al. (1981)	Topical antiseptic	Methemoglobinemia*
Boric acid	Goldbloom and Goldbloom (1953)	Baby powder	Vomiting, diarrhea, erythroderma, seizures, death
Lindane	Rutter (1987) and West et al. (1981)	Scabicide	Neurotoxicity
Salicylic acid	Abidel-Magid and El Awad Ahmed (1994) and West et al. (1981)	Keratolytic emollient	Metabolic acidosis, salicylism
Isopropyl alcohol (underocclusion)	Rutter (1987)	Topical antiseptic	Cutaneous hemorrhagic necrosis
Silver sulfadiazine	Payne et al. (1992)	Topical antibiotic (Silvadene)	Kernicterus (sulfa component), argyria (silver component)
Povidone–iodine	Rutter (1987) and West et al. (1981)	Topical antiseptic (Betadine)	Hypothyroidism, goiter
Neomycin	Rutter (1987)	Topical antibiotic	Neural deafness
Corticosteroids	Rutter (1987) and West et al. (1981)	Topical antiinflammatory (Lotrisone)	Skin atrophy, adrenal suppression
Benzocaine	Gelman et al. (1996)	Mucosal anesthetic (teething products)	Methemoglobinemia*
Prilocaine	Frayling et al. (1990) and Reynolds (1996)	Epidermal anesthetic (EMLA)	Methemoglobinemia*

*Heritable glucose-6-phosphate deficiencies are associated with an increased susceptibility to methemoglobinemia, as is coadministration of several drugs such as sulfonamides, acetaminophen, nitroprusside, phenobarbital, and phenytoin.
EMLA, Eutectic mixture lidocaine anesthetic.

and the skin is no exception. The most immediate issue is thermoregulation. The skin barrier in ELBW infants is ineffective, predisposing them toward dehydration and electrolyte imbalance. At delivery, maintenance of body temperature is critical; gentle drying and the use of alternate heat sources, such as a humidified incubator, will limit evaporative water loss. Barrier immaturity also puts EBLW infants at risk for skin injury from pedestrian exposures such as adhesives or cleaners like alcohol or iodine. Frequent repositioning and appropriate bedding are necessary to avoid pressure necrosis. Specialized dressings, such as those that may be used for infants with mechanobullous diseases like epidermolysis bullosa, can protect vulnerable sites (e.g., umbilical catheter, endotracheal tubes). Examples of such dressings include DuoDerm (ConvaTech, Bridgewater, New Jersey, United States) Mepilex (Molnlycke, Gothenburg, Sweden), and Vigilon (Bard Medical, Covington, Georgia, United States).

Early use of emollients has yielded conflicting results. Proposed benefits include decreased transepidermal water loss, decreased skin fragility, and lower risk of infection. However, a randomized trial demonstrated a higher rate of nosocomial infection in infants using prophylactic emollients compared with a control cohort.[10] Accordingly, the use of prophylactic emollients is not broadly recommended but may be beneficial for some infants at greater risk for skin breakdown.

General Care of Newborn Skin

Conversely, application of cream-based emollients in infants older than 29 weeks' EGA has been shown to enhance skin barrier development.[11] In infants at high risk for atopic dermatitis, early application of an emollient decreased the incidence of that disorder at 6 months of age by 50%,[5] although a more recent randomized trial did not validate these findings.[12] Sunflower seed oil application reduced the incidence of nosocomial infections by 41% when applied to infants less than 33 weeks' EGA in a study from Bangladesh.[13] Oils such as safflower, sesame, coconut, and apricot contain fatty acids that may have antiinflammatory and antibacterial properties. Olive oil can promote *Pityrosporum* growth, so it may not be the optimal choice.[14] Box 91.1 details general principles of neonatal skin care.

• BOX 91.1 Proposed Guidelines for Basic Skin Care in the Newborn

Use adhesives sparingly
- Place protective dressing (e.g., DuoDerm or Tegaderm) at sites of frequent taping (endotracheal tube and nasogastric tube placement)
- Use nonadhesive electrodes, and change them only when they become nonfunctional

Limit bathing
- Defer initial cleansing until body temperature has stabilized
- Avoid cleansing agents for the first 2 weeks
- Use warm water and moistened cotton pledgets in a humid environment
- Surface cleansing is required no more than twice per week
- If antimicrobial skin preparation is required, use short-contact chlorhexidine (except on the face)

Be aware of the composition and quantity of all topically applied agents
- These agents include antimicrobial cleansers, diaper wipes, adhesive removers, and perineal products

Dispense from single-use containers, if possible

Ensure adequate intake of protein, essential fatty acids, zinc, biotin, and vitamins A, D, and B
- Erosive periorificial dermatitis is a sign of nutritional deficiency

Apply a simple cream or ointment emollient every 8 hours

Guard against excessive thermal and ultraviolet exposure
- Use thermally controlled water for bathing
- Avoid surface monitors with metal contacts
- Use Plexiglas shielding over daylight fluorescent phototherapy

Protect sites of cutaneous injury with the appropriate occlusive dressing
- Use a film dressing on nonexudative sites
- Use a hydrogel dressing on exudative wounds
- Maintain appropriate hydration at the skin-dressing interface
- Remove necrotic debris with each dressing change

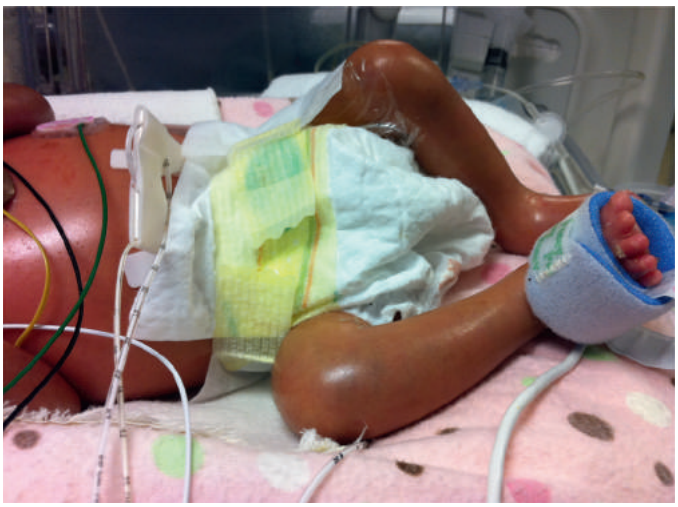
Bathing newborns has myriad potential hygienic, social, and cultural benefits but can potentially be detrimental to newborn skin. The timing of vernix removal varies considerably. Vernix is a complex mixture of water, protein, and lipids. It is hydrophobic and hydrating and contains lysozyme and lactoferrin, which are antimicrobial. Delayed bathing of newborns beyond 6 hours after birth is recommended by the World Health Organization in high-risk settings. The pH of the skin surface of term and preterm infants is more alkaline (6.5 to 7.5) but declines in the first week to values comparable to adult skin (4.0 to 5.5). The establishment of this "acid mantle" is important for many reasons and can be interrupted or delayed by the use of alkaline or irritating soaps. Gentle cleansers, neutral in pH and lacking fragrances and excessive preservatives or abrasives, can be used safely. Caregivers should practice good hand hygiene, preferably with chlorhexidine, for bacterial decontamination, although 10% povidone–iodine is advantageous for *Candida* species. Chlorhexidine may provide the most effective agent for umbilical stump care to prevent omphalitis and sepsis. Chlorhexidine 0.5% is superior to povidone–iodine in reducing peripheral catheter colonization in neonates.[7]

Morphologic Approach to Skin Pathology

Any skin examination should begin by attempting to identify a primary morphology. This helps limit a differential diagnosis and avoid the diagnostic confusion that can arise from secondary findings such as erosions and ulcerations (Table 91.4). Newborns present most commonly with benign, transient skin findings. These and other common neonatal presentations are detailed in subsequent chapters. There are several distinctive presentations that are important for pediatricians and neonatologists to recognize.

TABLE 91.4 Morphologic Approach to Diagnosis

Morphology	Description	Example
Macule/patch	Flat, no epidermal change	Capillary malformation
Papule/plaque	Raised, discrete edge, epidermal change	Superficial hemangioma
Nodule/tumor	Raised, sloping border, no epidermal change	Dermoid cyst
Vesicle/bulla	Clear, fluid filled	Epidermolysis bullosa
Pustule	Turbid, fluid filled	Candidiasis
Wheal	Edematous, red plaque	Neonatal onset multi-inflammatory disease



• Fig. 91.3 A newborn with taut skin representing a collodion membrane.

Collodion Membrane

Collodion membrane (CM) is a congenital cutaneous phenotype that has both immediate and longer term implications.[15] Clinically, a "collodion baby" presents with a tight "plastic wrap–"like encasement on the skin (Fig. 91.3). This taut skin can lead to eversion of the lips (eclabium) and eyelids (ectropion) as well as dysregulation of cutaneous homeostasis, mandating careful monitoring of fluids, electrolytes, and thermoregulation. Affected infants should be kept in the neonatal intensive care unit in a controlled environment as the membrane spontaneously sheds over the first weeks after birth. The majority of CM infants will ultimately have some form of chronic skin disorder, most commonly autosomal recessive congenital ichthyosis; however, other disorders, including Gaucher disease and ectodermal dysplasia, can present as CM. Rarely, CM infants may go on to have normal skin (lamellar exfoliation of the newborn). Diagnosis may be facilitated by skin biopsy and/or genetic testing, but evolution of the skin examination over time may also point toward the ultimate diagnosis noninvasively.

• BOX 91.2 Differential Diagnosis of Cutaneous Vesicles, Bullae, and Erosions in the Neonate

Infectious Vesiculopustular Dermatoses

Herpes simplex virus infection
Varicella infection
Cytomegalovirus
Candida infection
Scabies
Aspergillus infection
Bacterial infection
- Group B streptococcus
- Group A streptococcus
- *Haemophilus influenzae* type B
- *Staphylococcus aureus*
- *Listeria*
- *Treponema pallidum*
- *Pseudomonas*

Noninfectious Transient Conditions With Vesicles and Erosions

Erythema toxicum neonatorum
Transient neonatal pustular melanosis
Miliaria
Neonatal acne
Eosinophilic pustular folliculitis
Acropustulosis of infancy
Sucking blister
Trauma

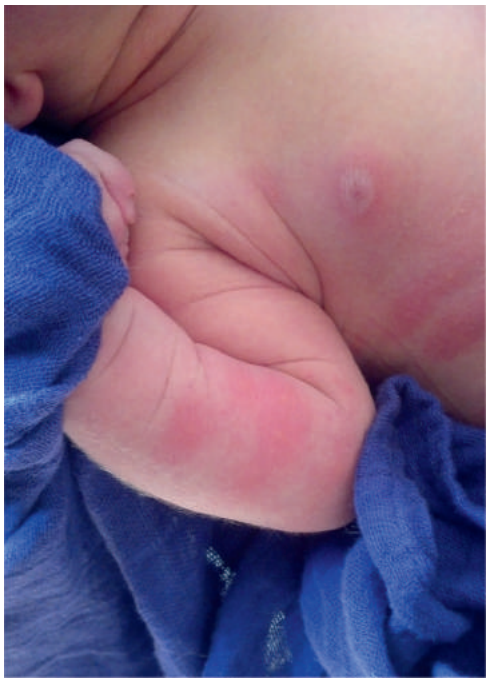
Nontransient Bullous Dermatoses

Epidermolysis bullosa
Incontinentia pigmenti
Epidermolytic hyperkeratosis
Hyper-IgE syndrome
Herpes gestationis
Pemphigus vulgaris
Langerhans cell histiocytosis
Mastocytosis

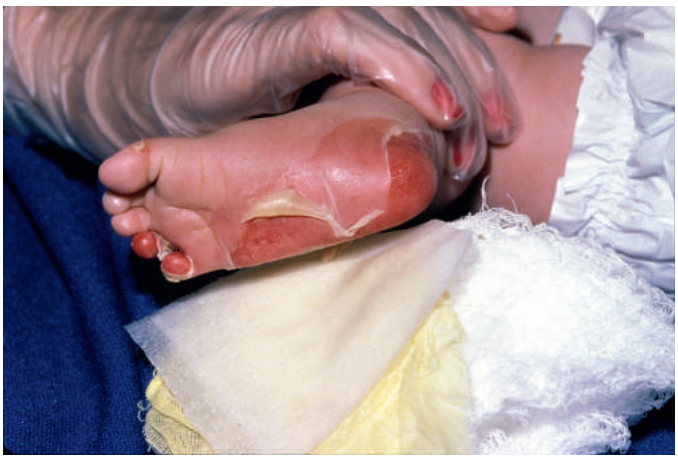
From Avram MM, Gobel V, Sepehr A. Case records of the Massachusetts General Hospital. Case 30-2007. A newborn girl with skin lesions. *N Engl J Med.* 2007;357:1327–1325.

Vesicopustular and Bullous Eruptions

An infant born with widespread vesicles, pustules, bulla, or erosions presents a daunting differential diagnosis (Box 91.2), including infectious, inflammatory, metabolic, and genetic disorders.[16] Treatable infectious etiologies, such as herpes simplex virus (HSV), varicella, *Staphylococcus aureus*, and *Candida* infections should be first ruled out by careful history and cultures. Subsequent consideration may be given to other dermatoses, including mastocytosis and histiocytosis. Transient benign dermatoses that present with a vesicopustular morphology, such as erythema toxicum (Fig. 91.4) or transient neonatal pustular melanosis (TNPM), should be considered and may be identified by simple laboratory studies (e.g., eosinophils on a smear of erythema toxicum vs. neutrophils on a smear from TNPM). Widespread blistering and erosion, in particular at friction-prone areas, such as the hands and feet, raise the uniquely important specter of EB (Fig. 91.5). EB is an inherited disorder of cutaneous fragility, and early awareness can avoid unnecessary iatrogenic exacerbation because of inappropriate handling, adhesives, leads, or monitors.[17] It is impossible to determine EB subtype from clinical examination alone. Relatively mild blistering at birth can occur in infants with severe subtypes, so providers should be cautioned against trying to predict the eventual phenotype on the basis of initial presentation.



• Fig. 91.4 Small eosinophil-rich pustules on an inflamed base consistent with erythema toxicum.



• Fig. 91.5 Blistering on the foot of a newborn with epidermolysis bullosa.

Blueberry Muffin Babies

A so-called "blueberry muffin baby" presents with distinctive red–purple macules, papules, and plaques that have historically prompted consideration of congenital infections denoted by the acronym TORCH: *T*oxoplasmosis, *O*ther, *R*ubella, *C*ytomegalovirus and *H*SV. These entities should be ruled out by history; evaluation; appropriate cultures; and supportive findings, such as dysmorphology. Several noninfectious conditions should be added to the clinical differential. Histiocytosis, neuroblastoma, leukemia cutis, transient myeloproliferative disorder, mastocytosis, and multifocal vascular lesions can all mimic blueberry muffin lesions.[18] A skin biopsy may be necessary to discriminate and appropriately triage such infants.

Erythroderma

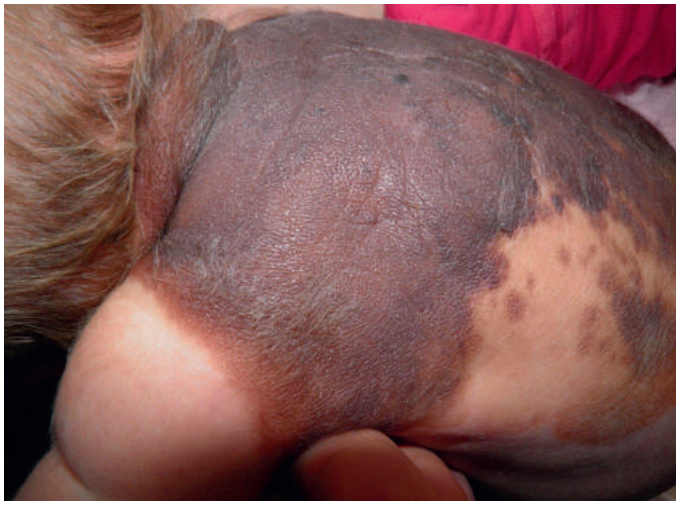
Erythroderma is defined as diffuse erythema with or without associated findings, like scaling. Newborns have extremely reactive skin, so it may take time to discern normal physiologic change, including cutis marmorata, from actual pathology. Diffuse erythema can herald many underlying conditions, and appropriate assessment must be comprehensive, not unlike the approach to a newborn with vesicles and pustules. Although infectious causes of erythroderma are less common, all treatable triggers should be eliminated. More commonly, erythroderma in a newborn may herald genetic or inflammatory conditions, such as ichthyosis, psoriasis, or seborrheic dermatitis. Metabolic disorders, primary immunodeficiency, and autoinflammatory syndromes must also be considered. Timely assessment is essential, even when infectious etiologies have been ruled out, because timely intervention can mitigate morbidity in some disorders (e.g., Menkes disease).[19]

Birthmarks With Neurologic Implications

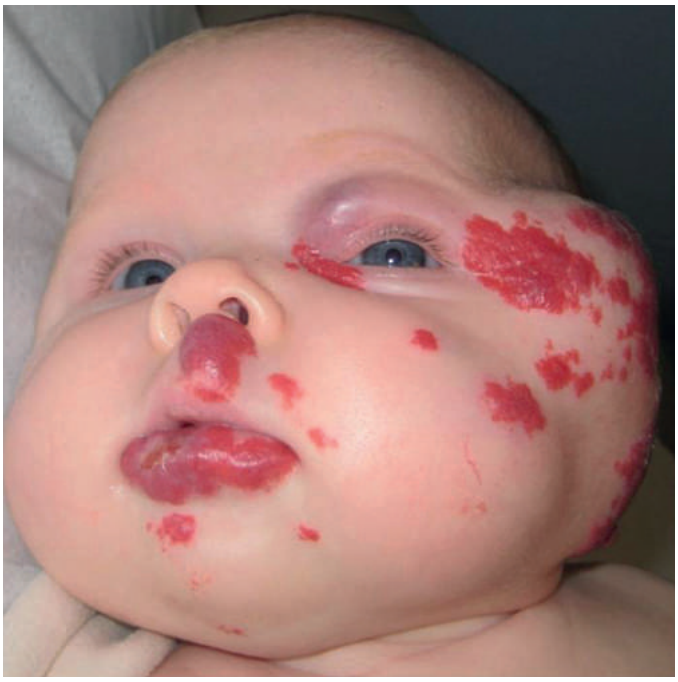
Large congenital melanocytic nevi may be associated with neurocutaneous melanosis (NCM) (Fig. 91.6). The risk of NCM is increased if the nevus is located on the scalp, or anywhere in the midline, or if infants have multiple (>20) satellite nevi. Magnetic resonance imaging (MRI) with and without contrast is necessary to rule out NCM. Early infancy may offer a unique opportunity to obtain an unsedated (feed-and-wrap) MRI, potentially avoiding general anesthesia later.[20]

Cutaneous stigmata, including pigmented or vascular lesions, excessive hair, or abnormal dimpling/clefting in the midline sacrum, can all herald underlying occult spinal dysraphism(OSD). Two or more such lesions, dimples more than 5 mm at the base or farther than 2.5 cm from the anus, all increase the risk of OSD.[21] Ultrasound, before complete vertebral ossification, can permit assessment of the brain and spine; however, the false-negative rate is high.

Facial vascular anomalies, such as capillary malformations, may herald underlying ipsilateral angiomatosis and glaucoma (Sturge-Weber syndrome). An MRI with contrast may show leptomeningeal malformation that can predispose toward seizures and developmental delay. Computed tomography may show "tram-track calcifications" in the temporal and occipital cortex, although these may not be present initially.



• **Fig. 91.6** Giant melanocytic nevus on the back.



• **Fig. 91.7** Large segmental infantile hemangioma in the setting of PHACE syndrome.

Segmental infantile hemangiomas, also typically on the face, can be associated with posterior fossa abnormalities such as Dandy–Walker malformation and cerebral artery dysmorphology, which predispose toward stroke, aortic coarctation, and ocular abnormalities (PHACE syndrome)[22] (Fig. 91.7). An MRI with angiography looking for distinctively tortuous, dysmorphic cerebral vessels can be diagnostic. As noted earlier, the potential for unsedated feed-and-wrap imaging may exist.

Suggested Readings

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92

Congenital and Hereditary Disorders of the Skin

CHERYL BAYART AND HEATHER A. BRANDLING-BENNETT

KEY POINTS

- Genodermatoses are a broad spectrum of heritable disorders that affect the skin and may or may not affect other organ systems.
- Many, but not all, genodermatoses present with cutaneous and sometimes systemic findings in the neonatal period.
- Classification and nomenclature of these disorders is evolving as we learn more about the genetic basis and pathogenesis of these conditions.
- Newborns with signs of genetic skin disease often require multidisciplinary care.

Heritable disorders of the skin, also known as *genodermatoses*, encompass a diverse array of conditions that can affect the color, texture, and structural integrity of the epidermis, epidermal appendages, and connective tissue. Some of these diseases affect only the skin, but many are associated with other organ system anomalies. Knowledge of the molecular genetic basis of the genodermatoses is rapidly evolving, with the hope that this will lead to novel and more efficacious therapies. Table 92.1 provides a list of resources and support groups for patients with genodermatoses and their families.

Genetic mosaicism refers to an organism composed of two or more genetically distinct populations of cells. Mosaicism affecting the skin can lead to unique cutaneous patterns. One example of this is Blaschko lines, which refers to a linear and whorled pattern with midline demarcation, thought to represent migration of embryonic cells (Fig. 92.1; Lombillo and Sybert, 2005). Blaschko lines are different from dermatomes, skin tension lines, or lines of lymphatic drainage. There are various heritable skin disorders that present with skin lesions that follow Blaschko lines, including pigmentary disorders, incontinentia pigmenti (IP), and Goltz syndrome, which will be discussed in this chapter.

Ichthyoses

The ichthyoses, also referred to as *disorders of keratinization or cornification*, are a group of skin disorders that are characterized by scaly skin and/or hyperkeratosis (thickened stratum corneum). Historically they have been classified by their clinical and sometimes histologic features, but nosology is evolving as the molecular basis of these disorders is being discovered. More than 50 genes have been reported to result is various types of ichthyoses.[1] In 2009 an international consensus conference was held to revise the nomenclature and classification of inherited ichthyoses, which were subdivided into two principal groups: nonsyndromic and syndromic forms, summarized in Tables 92.2 and 92.3, respectively, with exceedingly rare or minor variants that are beyond the scope of this chapter.[2,3] The most striking neonatal presentations are discussed in more detail.

Collodion Baby

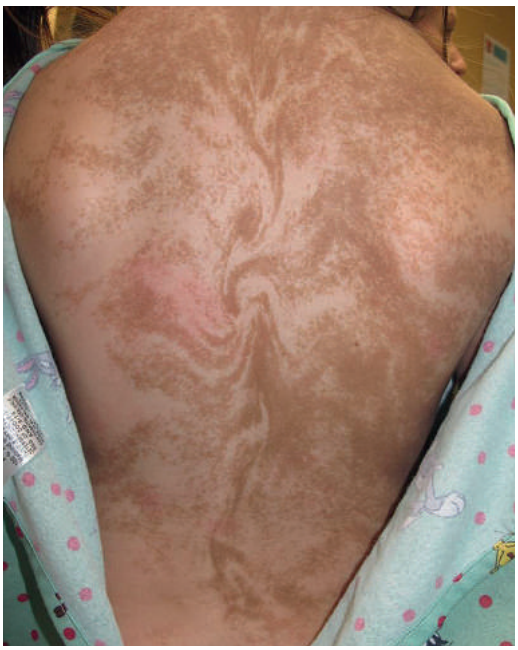
The term *collodion baby* refers to a neonate born encased in shiny, thickened skin that resembles parchment or plastic wrap (Fig. 92.2). The taut skin leads to various distorting features such as ectropion (eversion of eyelids), eclabium (eversion of lips), flattening or hypoplasia of the nose and ears, and pseudocontractures of the digits. These infants have an ineffective cutaneous barrier against transepidermal water loss and invasion of pathogenic organisms and are at risk of multiple complications, including temperature instability, dehydration, electrolyte instability, infections, poor sucking, failure to thrive, keratitis, ear canal obstruction, percutaneous toxicity from topical medications, pneumonia from aspiration of squamous material in amniotic fluid, and distal limb or digital ischemia.[4]

These neonates need diligent supportive care in a humidified incubator with close monitoring of temperature, fluid and electrolyte balance, and caloric intake. Surveillance for signs of cutaneous or systemic infection is important, but prophylactic antibiotics or antifungals are not recommended. Skin care is somewhat controversial, but many advocate application of a bland petroleum-based or water-in-oil emollient at least every 6 to 8 hours. Caution is advised with use of topical medications or keratolytics because of increased percutaneous absorption and risk of toxicity. If there are erosions or fissures, bathing with normal saline may be more comfortable than with plain water. Ophthalmology and otolaryngology consultations should be considered for eye and ear involvement.[4]

Collodion baby refers not to a specific disease but rather to an initial phenotype seen in several forms of ichthyosis

TABLE 92.1 Resources for Patients With Genodermatoses and Their Families

Disorder	Resource(s)	Website
Ectodermal dysplasia/Incontinentia pigmenti	National Foundation for Ectodermal Dysplasias	http://www.nfed.org
Ehlers–Danlos syndrome	The Ehlers-Danlos Society Hypermobility Syndromes Association	http://www.ehlers-danlos.com http://www.hypermobility.org
Epidermolysis bullosa	Dystrophic Epidermolysis Bullosa Research Association EB Research Partnership	http://www.debra.org https://ebresearch.org
Ichthyoses/disorders of keratinization	Foundation for Ichthyosis & Related Skin Types	http://www.firstskinfoundation.org
Neurofibromatosis	Neurofibromatosis Network	http://www.nfnetwork.org
Oculocutaneous albinism	National Organization for Albinism and Hypopigmentation	http://www.albinism.org
Porphyria	American Porphyria Foundation	http://porphyriafoundation.org
Tuberous sclerosis	Tuberous Sclerosis Alliance	http://www.tsalliance.org
Xeroderma pigmentosum	The Xeroderma Pigmentosum Society XP Family Support Group	http://www.xps.org http://www.xpfamilysupport.org



• **Fig. 92.1** Blaschko lines in a girl with extensive epidermal nevi.

TABLE 92.2 Most Common Nonsyndromic Inherited Ichthyoses

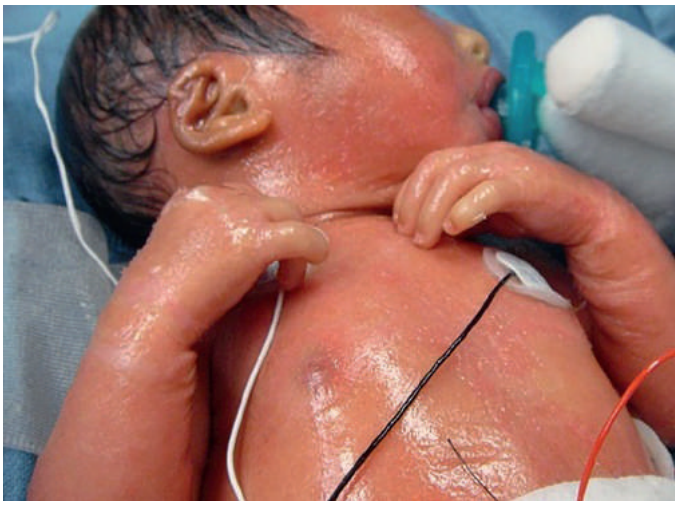
Disorder	Newborn Cutaneous Features	Extracutaneous Features
Common Ichthyoses		
Ichthyosis vulgaris	Onset usually after 2–6 months of age	Association with atopy
Recessive X-linked ichthyosis	May have mild scaling and/or erythroderma or mild collodion membrane	Cryptorchidism (5%–20%); corneal opacities (~50%); prolonged labor; contiguous gene syndromes
Autosomal Recessive Congenital Ichthyosis, Major Subtypes		
Lamellar Ichthyosis	Collodion membrane; ectropion; eclabium	With or without short stature
Congenital ichthyosiform erythroderma	Mild scaling and erythroderma or mild collodion membrane	With or without short stature; failure to thrive
Harlequin ichthyosis	Severe collodion membrane with armor-like scales; ectropion; eclabium; contractures	Contractures; failure to thrive; short stature
Keratinopathic Ichthyosis, Major Subtype		
Epidermolytic ichthyosis	Erythroderma, bullae, or erosions, mild scaling	With or without growth failure

(Table 92.4). Ichthyosis prematurity syndrome, Netherton syndrome (NS), and Sjögren–Larsson syndrome must also be considered in the differential diagnosis. The collodion membrane is typically shed in 3 to 4 weeks, and the eventual phenotype depends on the underlying genetic mutation. More than half of collodion babies have autosomal recessive congenital ichthyosis (ARCI), discussed in more detail below. About 10% have almost normal skin after the collodion membrane sheds, which has been referred to as *self-healing collodion baby*. It is difficult to predict the long-term prognosis during the neonatal period. The diagnostic work-up should include a detailed family history and consideration of microscopic examination of scalp and eyebrow hair to look for tiger-tail banding seen on polarized microscopy in trichothiodystrophy (TTD). The role of skin biopsy before the collodion membrane has shed is controversial, and the underlying diagnosis may become evident with time, which will help determine appropriate genetic testing or the need for biochemical or metabolic assays.

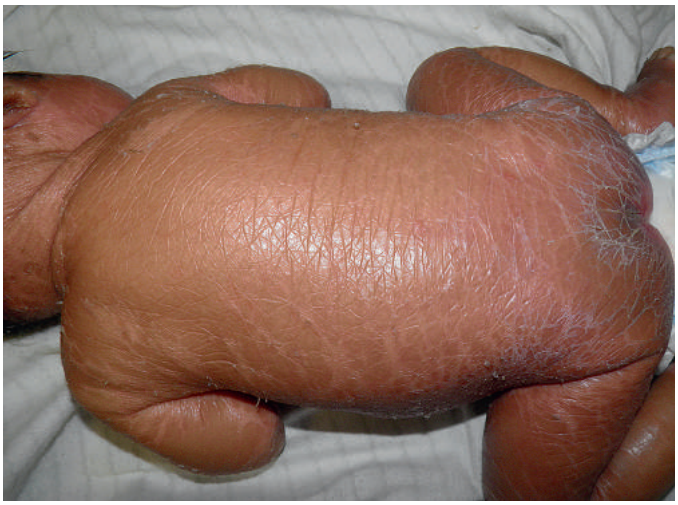
TABLE 92.3 Most Common Syndromic Inherited Ichthyoses

Disorder	Newborn Cutaneous Features	Extracutaneous Features
Prominent Hair Abnormalities		
Netherton syndrome	Erythroderma, scaling	Atopic diathesis with elevated IgE concentration and eosinophilia; failure to thrive
Ichthyosis–hypotrichosis–sclerosing cholangitis syndrome[a]	Mild scaling; jaundice	Sclerosing cholangitis or congenital paucity of bile ducts
Trichothiodystrophy[b]	Collodion membrane or erythroderma and scaling	Developmental delay; short stature; cataracts; facial dysmorphism; bone abnormalities; gonadal abnormalities; recurrent infections
Ichthyosis follicularis–atrichia–photophobia	Mild collodion membrane; congenital atrichia (alopecia)	Severe photophobia (vascularizing keratitis); developmental delay; short stature; atopic diathesis; other
Prominent Neurologic Abnormalities		
Sjögren–Larsson syndrome	Scaling accentuated on scalp and neck, with or without erythema	Spastic paraplegia; mental retardation; ocular findings (retinal glistening white dots)
Mental retardation–enteropathy–deafness–neuropathy–ichthyosis–keratoderma syndrome	EKV-like features, may present at birth or within the first few weeks of life	Congenital sensorineural deafness; peripheral neuropathy; developmental and growth delay; chronic diarrhea
Refsum disease	Usually normal	Tetrad of retinitis pigmentosa, peripheral neuropathy, cerebellar ataxia, and elevated protein levels in the cerebrospinal fluid; anosmia; progressive deafness; other
Prominent Skeletal Abnormalities		
Conradi-Hünermann-Happle syndrome (CDPX2)	Severe scaly erythroderma or collodion membrane	Chondrodysplasia punctata with asymmetric skeletal hypoplasia; cataracts; short stature
Congenital hemidysplasia–ichthyosiform erythroderma or nevus-limb defects[c]	Persistent cutaneous findings may present at birth or within the first few months	Ipsilateral skeletal hypoplasia (x-ray may show punctate epiphyseal dysplasia); with or without renal, cardiac, or CNS anomalies
Fatal Disease Course		
Gaucher syndrome type 2	Mild collodion membrane or CIE-like features	Hydrops fetalis; progressive neurologic deterioration; hepatosplenomegaly
Multiple sulfatase deficiency	RXLI-like (may have mild scaling and/or erythroderma or mild collodion membrane) or presents later	Metachromatic leukodystrophy and mucopolysaccharidosis with progressive neurologic deterioration
Cerebral dysgenesis-neuropathy-ichthyosis-keratoderma	Normal at birth, presents between 5 and 11 months of age	Sensorineural deafness; cerebral dysgenesis; neuropathy; microcephaly; neurogenic muscle atrophy; optic nerve atrophy; cachexia
Arthrogryposis–renal dysfunction–cholestasis syndrome	Scaling within a few days of birth or mild collodion membrane	Arthrogryposis; intrahepatic bile duct hypoplasia with cholestasis; renal tubular degeneration with metabolic acidosis; cerebral malformation; abnormal platelets
Other Abnormalities		
Keratitis–ichthyosis–deafness syndrome	Erythematous, thickened skin; with or without congenital alopecia	Congenital sensorineural deafness; progressive keratitis with photophobia
Neutral lipid storage disease with ichthyosis[c]	Mild collodion membrane or CIE-like or EKV-like features	Vacuolated granulocytes (Jordan anomaly); hepatosplenomegaly; myopathy; cataracts; hearing loss; developmental delay
Ichthyosis prematurity syndrome	Thick, desquamating scale (vernix caseosa-like) with accentuation on scalp and eyebrows	Polyhydramnios with opaque amniotic fluid (from shedding epidermis); respiratory distress at birth; asthma; eosinophilia

[a]Also known as neonatal ichthyosis sclerosing cholangitis syndrome.
[b]Also known as ichthyosis–brittle hair–impaired intelligence–decreased fertility–short stature syndrome; there are forms of trichothiodystrophy not associated with congenital ichthyosis.
[c]Also known as Chanarin-Dorfman syndrome.
CDPX2, Chondrodysplasia punctate (X-linked dominant) type 2; *CIE*, congenital ichthyosiform erythroderma; *CNS*, central nervous system; *EKV*, erythrokeratodermia variabilis; *HPV*, human papillomavirus; *RXLI*, recessive X-linked ichthyosis.



• **Fig. 92.2** Collodion baby born encased in shiny, thickened skin that resembles plastic wrap.



• **Fig. 92.3** Three-week-old male with generalized large scales characteristic of lamellar ichthyosis after shedding of collodion membrane.

TABLE 92.4 Disorders That May Present as a Collodion Baby

Nonsyndromic ichthyoses	Autosomal recessive congenital ichthyosis: lamellar ichthyosis and congenital ichthyosiform erythroderma phenotypes
	Ichthyosis vulgaris
	X-linked recessive ichthyosis epidermolytic ichthyosis
	Bathing suit ichthyosis
	Self-healing collodion baby
	Keratosis linearis with ichthyosis congenital and sclerosing
	Loricrin keratoderma
Syndromic ichthyoses	Ichthyosis follicularis–atrichia–photophobia syndrome
	Neutral lipid storage disease with ichthyosis
	Trichothiodystrophy with ichthyosis
	Conradi-Hünermann-Happle syndrome
	Keratitis-ichthyosis-deafness syndrome
	Loricrin keratoderma
	Arthrogryposis-renal dysfunction-cholestasis syndrome
Metabolic diseases	Holocarboxylase synthetase deficiency
	Gaucher disease type 2
Other diseases	Hypohidrotic ectodermal dysplasia
	Congenital hypothyroidism
	Koraxitrachitic syndrome
	Palmoplantar keratoderma with anogenital leukokeratosis

Autosomal Recessive Congenital Ichthyosis: Harlequin Ichthyosis, Lamellar Ichthyosis, and Nonbullous Congenital Ichthyosiform Erythroderma

ARCI encompasses a spectrum of nonsyndromic autosomal recessive ichthyoses that includes phenotypes ranging from lamellar ichthyosis (LI) to nonbullous congenital ichthyosiform erythroderma (CIE) to harlequin ichthyosis (HI). ARCI is caused by mutations in more than a dozen different genes.[5]

• **Fig. 92.4** Newborn with harlequin ichthyosis born with thick, fissured, platelike scales, severe ectropion and eclabium, and hypoplastic nose and ears.

The classic LI phenotype is characterized by coarse, yellow to brown-black, platelike scales (Fig. 92.3) with varying degrees of underlying erythema, ectropion, and eclabium. Many patients with LI are born with a full or partial collodion membrane, discussed earlier. The classic CIE phenotype is characterized by more prominent erythroderma and finer, white scales that may not be apparent until the collodion membrane sheds. Neonates with CIE often have a less severe collodion membrane or may be born without a membrane.[2] The principles of neonatal management for these phenotypes are the same as those discussed earlier for a collodion baby.

HI is the most severe clinical phenotype of ichthyosis in a newborn and is rarely diagnosed by prenatal ultrasound. These neonates are born with an "armor" of thick, fissured, platelike scales (Fig. 92.4). They have severe ectropion and eclabium and flattened, hypoplastic nose and ears. Constricting bands lead to contractures and digital ischemia. They are at high risk of the complications discussed earlier for collodion babies but have a higher rate of infant mortality. Historically, almost 50% of babies with HI have died in the neonatal period, more than half in the

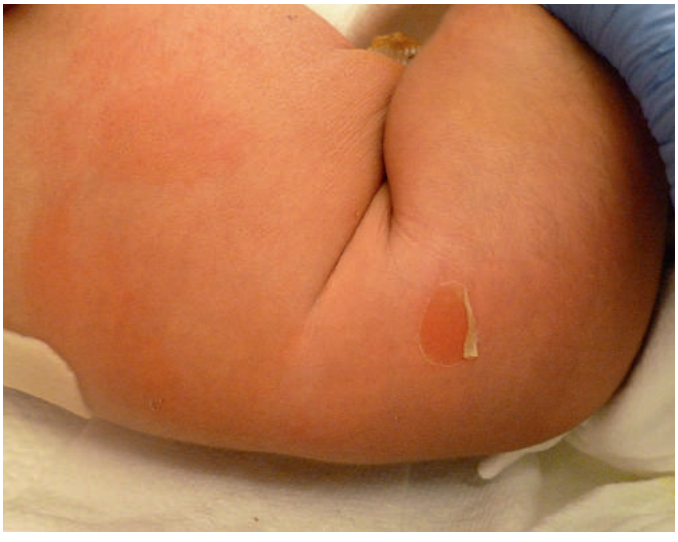
first 3 days of life. The cause of death is most commonly attributed to sepsis and/or respiratory failure.[6] Intensive, multidisciplinary, supportive care is critical for neonates with HI, with detailed recommendations for neonatal care available.[7] Early initiation of therapy with a systemically acting retinoid (such as isotretinoin, 0.5 to 1 mg/kg per day, or acitretin, 0.5 mg/kg per day) before day of life 7 may improve the survival rate. Patients who survive infancy have a lifelong ichthyosis that resembles CIE.

Epidermolytic Ichthyosis

The term *keratinopathic ichthyoses* encompass ichthyoses that are a result of keratin mutations, most of which are autosomal dominant. Epidermolytic ichthyosis (EI), previously referred to as *epidermolytic hyperkeratosis* or *bullous CIE*, is the most common and presents with generalized erythroderma and bullae. The term *epidermolytic hyperkeratosis* describes the histopathologic or ultrastructural features seen on skin biopsy characterized by marked hyperkeratosis (thickened stratum corneum) with clumping and lysis (disintegration) of the epidermal cells above the basal layer.[8] Newborns with EI typically present with widespread erythema and superficial blistering, as a result of the fragility of the epidermis from abnormal keratin production (Fig. 92.5). These neonates may receive a misdiagnosis of epidermolysis bullosa (EB) or staphylococcal scalded skin syndrome. Subtle skin thickening over the elbows, knees, palms, or soles may be diagnostic clues. During the first few months of life, the phenotype gradually evolves into more pronounced skin thickening with verruciform, ridged scales, accentuated in areas of friction, including flexural and intertriginous areas. Malodorous bacterial colonization is common.

Treatment of neonates with EI requires supportive care with attention to gentle handling to minimize blister formation, including use of nonadherent dressings. They are at risk of temperature instability, dehydration, and electrolyte instability and should be closely monitored for bacterial infections.

EI is caused by autosomal dominant mutations in *KRT1* or *KRT10*, either inherited or caused by a spontaneous mutation. It is also possible that the parent of a child with EI may have a congenital epidermolytic epidermal nevus caused by a somatic mosaic keratin mutation in *KRT1* or *KRT10*. Gonadal involvement of the keratin mutation, more common in those with widespread epidermal nevi, results in an offspring affected by generalized EI.[9]



• **Fig. 92.5** Newborn with epidermolytic ichthyosis who exhibited widespread erythema and superficial blisters.

Diagnosis of Ichthyoses

Cutaneous and extracutaneous clinical features are critical in narrowing down the differential diagnosis for a neonate born with scaly or hyperkeratotic skin and can sometimes be sufficient for diagnosis. Family history may be helpful, particularly for ichthyoses with a dominant inheritance pattern.[3] The gold standard for diagnosis is genetic mutation analysis.

Histopathologic findings from a skin biopsy in many patients with ichthyosis are nonspecific, with a few exceptions. EI has characteristic histologic findings of "epidermolytic hyperkeratosis" discussed earlier. Biopsy specimens from an individual with ichthyosis vulgaris, NS, TTD, Refsum syndrome, or Conradi-Hünermann-Happle syndrome (chondrodysplasia punctate X-linked dominant type 2) may show reduced or absent stratum granulosum. Histopathology of loricrin keratoderma is notable for parakeratosis and hypergranulosis.[2] Special immunohistochemical stains or ultrastructural analysis by electron microscopy may be considered in certain cases.

Examination of hair shafts can be helpful for diagnosing NS and TTD. Trichorrhexis invaginata ("bamboo hair" or "ball-and-socket" deformity) is characteristic of NS but is not usually present until after 1 year of age and even then is invariably present. The hair of patients with TTD may show trichoschisis (clean transverse fracture) or trichorrhexis nodosa (nodes from longitudinal splitting of fibers) on light microscopy and characteristic tiger-tail banding on polarized microscopy from low sulfur content.

Further work-up for syndromic ichthyoses is typically based on extracutaneous findings.

Prognosis and Treatment of Ichthyoses

Distinguishing the type of ichthyosis is crucial for offering prognostic information to the family, as prognosis is highly variable. Neonatal care for severe phenotypes, including collodion baby, LI, HI, and EI, was discussed earlier. For all types of ichthyosis, skin care typically involves application of bland emollients to hydrate the stratum corneum.[3] Emollients with keratolytics such as urea, salicylic acid, α-hydroxy acids, and propylene glycol are usually avoided in infancy because of risk of toxicity from absorption. Oral retinoids are primarily considered for patients with HI or rarely in those with severe collodion membrane with delayed shedding.

Epidermolysis Bullosa

Epidermolysis bullosa (EB) encompasses a group of mechanobullous disorders characterized by skin fragility and blister formation. The clinical spectrum is broad, but in the neonatal period most patients have vesicles, bullae, or erosions. Most forms of EB are caused by genetic mutations that result in absent or reduced levels of adhesion proteins normally present at the interface of the epidermis and dermis, which is called the *basement membrane zone* (BMZ). As a result, the epidermis separates from the dermis with less friction or force than usual, resulting in blister formation. In some EB subtypes, the epithelia of the external eye, ear, nose, upper airway, and gastrointestinal and genitourinary tracts are involved. The nature of the mutated protein and the severity of the mutation typically determine the phenotype: whether blistering is

localized or generalized and the extent of extracutaneous involvement. As a general rule, less severe forms of EB tend to be more common and autosomal dominantly inherited, whereas more severe forms are rarer with autosomal recessive inheritance.[10,11]

Classification of Epidermolysis Bullosa

EB classification and nomenclature have evolved over many years and were most recently updated at a 2013 international consensus meeting,[12] although additional subtypes and genes have been identified since.[13] EB is classified into three major types: EB simplex, junctional EB, dystrophic EB, and Kindler syndrome. EB type is determined by the level of the skin at which mechanical fragility and blister formation occur. EB simplex is the most superficial form, with the level of skin cleavage occurring in the epidermis, usually in basal keratinocytes. In junctional EB, blisters form deeper, within the midportion (lamina lucida) of the skin's BMZ. Skin cleavage in dystrophic EB occurs just below the BMZ in the most superficial layer of the dermis (the sublamina densa). In Kindler syndrome, which is very rare, fragility and blister formation are seen at multiple depths within the skin. EB is further subdivided into more than 39 subtypes on the basis of clinical presentation, ultrastructural features, immunohistochemical findings, and the presence of specific genetic mutations.[12] Only the more common or more notable subtypes are discussed in this chapter (Tables 92.5–92.7).

TABLE 92.5 Subtypes of Epidermolysis Bullosa Simplex

Subtype and Previous Eponym	Clinical Features
EBS, generalized severe (EBS, Dowling–Meara)	*Presentation*: birth *Bullae*: herpetiform, generalized *Mucosal involvement*: present or absent *Nails*: dystrophic *Prognosis*: progressive PPK, blistering abates with age
EBS, generalized intermediate (EBS, Koebner)	*Presentation*: birth to early infancy *Bullae*: pressure points *Mucosal involvement*: during infancy *Extracutaneous*: cardiomyopathy (KLHL24) *Nails*: may be lost, but regrow *Prognosis*: abates with age
EBS, localized (EBS, Weber–Cockayne)	*Presentation*: early childhood *Bullae*: acral *Mucosal involvement*: mild to none *Extracutaneous*: nephropathy (CD151) *Prognosis*: abates with age
EBS, intermediate with muscular dystrophy	*Presentation*: newborn–neonatal period *Mucosal involvement*: present *Extracutaneous*: congenital or delayed-onset muscular dystrophy; tooth enamel hypoplasia Prognosis: early death
EBS, severe with pyloric atresia	*Presentation*: birth *Bullae*: generalized *Extracutaneous*: pyloric atresia, GU anomalies *Prognosis*: neonatal death

EBS, Epidermolysis bullosa simplex; *GU*, genitourinary; *PPK*, palmoplantar keratoderma.

TABLE 92.6 Subtypes of Junctional Epidermolysis Bullosa

Subtype and Previous Eponym	Clinical Features
JEB, generalized severe (JEB, Herlitz)	*Presentation*: birth *Bullae*: widespread with nonhealing granulation tissue *Nails*: dystrophic or absent *Extracutaneous*: airway (25%), GI tract, GU systems, eyes, teeth *Prognosis*: death by 2 years of age because of sepsis, failure to thrive, or airway compromise
JEB, generalized intermediate (JEB, non-Herlitz)	*Presentation*: birth *Nails, hair, teeth*: commonly involved *Prognosis*: neonatal death to normal life span
JEB with pyloric atresia	*Presentation*: birth *Bullae*: severe, generalized *Extracutaneous*: pyloric atresia, GU anomalies *Prognosis*: neonatal death

GI, Gastrointestinal; *GU*, genitourinary; *JEB*, junctional epidermolysis bullosa.

TABLE 92.7 Dystrophic Epidermolysis Bullosa

Subtype and Previous Eponym	Clinical Features
RDEB, generalized severe (RDEB, Hallopeau-Siemens)	*Presentation*: birth *Blistering*: widespread, involving skin and mucosa *Nails*: dystrophic or absent *Extracutaneous*: ocular, GI tract, GU system, kidneys, heart *Sequelae*: mutilating scarring, pseudosyndactyly of digits, aggressive SCC *Prognosis*: poor, death most commonly caused by metastatic SCC
RDEB, generalized intermediate (RDEB, non-Hallopeau-Siemens)	*Presentation*: birth *Blistering*: widespread but less severe involvement of skin and mucosa *Nails*: dystrophic or absent *Extracutaneous*: variable to absent *Sequelae*: variable pseudosyndactyly, SCC *Prognosis*: lifelong blistering, risk of early death
DDEB, generalized (DDEB, Cockayne-Touraine, and Pasini)	*Presentation*: birth *Blistering*: most pronounced on hands, feet, knees, and elbows *Nails*: dystrophic or absent *Extracutaneous*: variable GI tract involvement *Prognosis*: lifelong blistering but normal life span
Bullous dermolysis of the newborn	*Presentation*: birth *Blistering*: generalized or localized; spontaneous or induced by trauma *Prognosis*: spontaneous resolution in first year of life

DDEB, Dominant dystrophic epidermolysis bullosa; *GI* gastrointestinal; *GU*, genitourinary; *RDEB*, recessive dystrophic epidermolysis bullosa; *SCC*, squamous cell carcinoma.

Epidermolysis Bullosa Simplex

EB simplex most commonly results from genetic mutations affecting keratins 5 and 14 in the basal layer of the epidermis (see Table 92.5). Blisters typically heal without scarring. Most patients with EB simplex have autosomal dominantly inherited forms and a normal life span. However, some of the recessive forms, including those associated with muscular dystrophy and pyloric atresia, carry a poor prognosis and are associated with early death.[11] Patients with generalized subtypes of EB simplex (intermediate and severe types) present with blistering in the newborn period. Severe generalized EB simplex is characterized by extensive blistering in herpetiform (herpes-like) or arcuate clusters. Mucosal involvement may be seen, nail dystrophy is common, and progressive palmoplantar keratoderma is characteristic. In the intermediate subtype of generalized EB simplex, bullae are most common over pressure points such as the elbows, knees, legs, feet, and hands and may be widespread following the trauma of birth. Mucosal involvement may occur during the newborn period but reduces with age. Nails may be lost but regrow. Localized EB simplex is the most common form of EB. Patients with localized EB simplex are usually asymptomatic during the newborn period, with onset of acral blisters in early childhood.

Junctional Epidermolysis Bullosa

Junctional EB is caused by gene mutations that affect expression of proteins integral to the lamina lucida of the BMZ. All known subtypes of junctional EB are autosomal recessive, and clinical severity is highly variable (see Table 92.6). Tooth enamel hypoplasia is characteristic. Generalized severe junctional EB, previously known as *Herlitz subtype*, is the most severe form and carries the highest risk of early death of all forms of EB.[14] Patients present at birth with widespread mucosal and skin blistering, most pronounced over pressure points (Fig. 92.6). Nails are dystrophic or absent. Exuberant granulation tissue often develops periorally and on the upper back. Airway involvement is typical, and the eyes and gastrointestinal and genitourinary systems are also commonly affected. Prognosis is extremely poor. Most patients die during the first 2 years of life secondary to failure to thrive, sepsis, or respiratory failure.[11] The presentation of the generalized intermediate subtype of junctional EB is variable and less severe than that of the generalized form. Localized subtypes are also seen.

Dystrophic Epidermolysis Bullosa

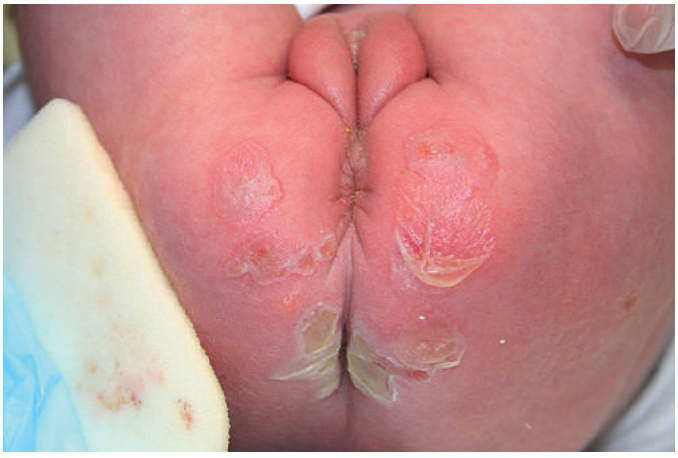
The dystrophic subtypes of EB are all caused by mutations affecting the expression of collagen VII, which forms the anchoring fibrils, critical for adhesion of the basement membrane to the upper dermis. Blistering occurs just below the BMZ, resulting in significant scarring and milia formation (Fig. 92.7). Dystrophic EB can be categorized into autosomal dominant and recessive variants. In general, recessively inherited forms are associated with absence of collagen VII and are more severe than dominant forms, in which collagen VII expression is decreased or abnormal (see Table 92.7).

The generalized severe recessive variant is the most severe subtype of dystrophic EB. Patients present at birth with generalized blistering of the skin and mucosa. Involvement of multiple organ systems, including the eyes, gastrointestinal tract, kidneys, genitourinary tract, and the heart, can be seen. Scarring of the hands and feet can lead to functionally limiting pseudosyndactyly and bone deformation. Anemia and failure to thrive are characteristic.[15] Aggressive squamous cell carcinomas arising in chronic wounds are the most common cause of death in patients with recessive dystrophic EB and develop as early as the teenage years.[14]

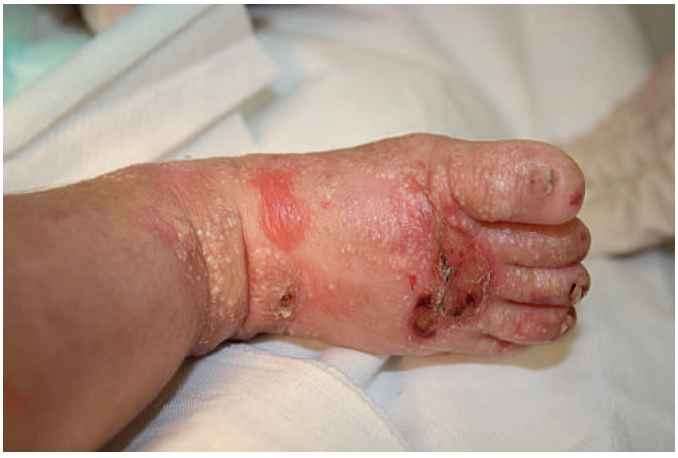
In patients with dominantly inherited forms of dystrophic EB, blistering is typically induced by trauma and limited to the hands, feet, elbows, and knees. Scarring and milia formation occur but do not typically cause functional impairment. Nail dystrophy or loss is common and may be the only manifestation of disease. Life expectancy is usually normal.[11] There is a subtype of dystrophic EB called *bullous dermolysis of the newborn* where skin fragility is usually transient and spontaneously resolves within the first year of life.[16] These patients are often born with absent skin (aplasia cutis), especially on the lower extremities (Fig. 92.8).

Diagnosis of Epidermolysis Bullosa

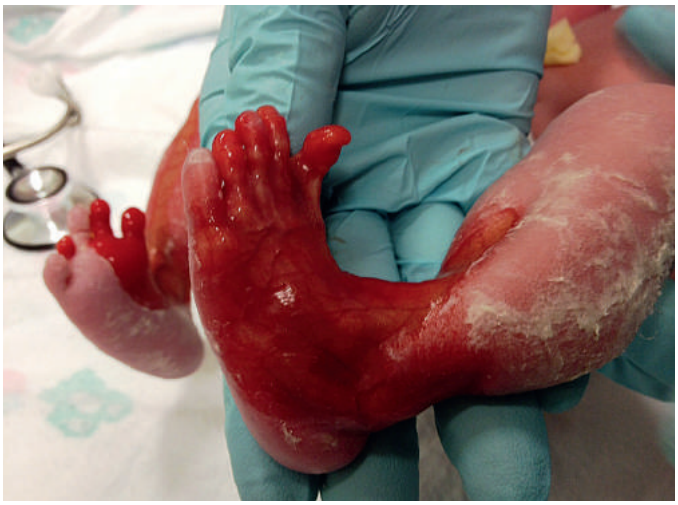
The differential diagnosis for skin blisters or erosions in the neonate is broad but should include EB. Traumatic and infectious causes of blistering should be ruled out. A thorough history, including family history of blistering disorders, should be obtained. Physical examination findings are an unreliable indicator of EB type during the neonatal period, and discussion of long-term prognosis should be deferred until the subtype of EB is determined through testing.[13]



• **Fig. 92.6** Newborn with junctional epidermolysis bullosa who exhibited bullae in the diaper area and other areas of friction.



• **Fig. 92.7** Four-month-old with dystrophic epidermolysis bullosa characterized by bullae, erosions, and milia.



• **Fig. 92.8** Bullous dermolysis of the newborn with absent skin on legs and feet.

Diagnosis of EB type is not straightforward and has traditionally entailed specialized examination of a skin biopsy specimen, which remains essential in many cases. As the accuracy of genetic testing has increased and the cost decreased, current standard of care also includes mutational analysis of DNA obtained from peripheral blood.[17] A list of laboratories that provide testing for EB can be found at http://www.ncbi.nlm.nih.gov/gtr/tests/. In families with a known history of EB, methods of prenatal or preimplantation diagnosis can be considered.

Skin biopsy in a patient with suspected or known EB should be performed at the junction of a fresh blister and normal skin. Ideally, a blister is induced by the rubbing of a pencil eraser against the skin until erythema develops. The level of blister formation (i.e., intraepidermal, lamina lucida, or superficial dermis) can be determined by transmission electron microscopy (TEM) or immunofluorescence antigen mapping (IFM) of newly induced blisters. TEM allows detailed visualization of the epidermal–dermal junction to determine whether blister formation occurs superficial to, within, or deep to the BMZ. IFM uses monoclonal antibodies directed against proteins in the BMZ to help determine the level of split and in some cases will show decreased or absent expression of certain proteins mutated in EB. For optimal accuracy, both should be performed by a laboratory experienced in performing these studies on patients with EB.[17] It is important to note that EB type cannot be determined through routine light microscopy of hematoxylin- and eosin-stained sections.

Management of Epidermolysis Bullosa

Novel and highly effective treatments for EB, including gene replacement therapy and injection of genetically corrected fibroblasts, have shown success in a very small number of patients. The status of ongoing trials can be found at https://clinicaltrials.gov/.[18] For the time being, supportive measures remain the mainstay of therapy. A multidisciplinary approach is important, involving specialists in fields such as dermatology, surgery, gastroenterology, otolaryngology, dentistry, hematology/oncology, wound care, pain management, occupational and physical therapy, nutrition, and psychology.[15,19] EB patients who die in infancy and early childhood most often succumb to sepsis, followed by failure to thrive or respiratory failure. Those with severe subtypes of junctional EB are at markedly higher risk of early death,[14] and in some patients it may be appropriate to emphasize comfort measures over aggressive treatments that prolong life.[20]

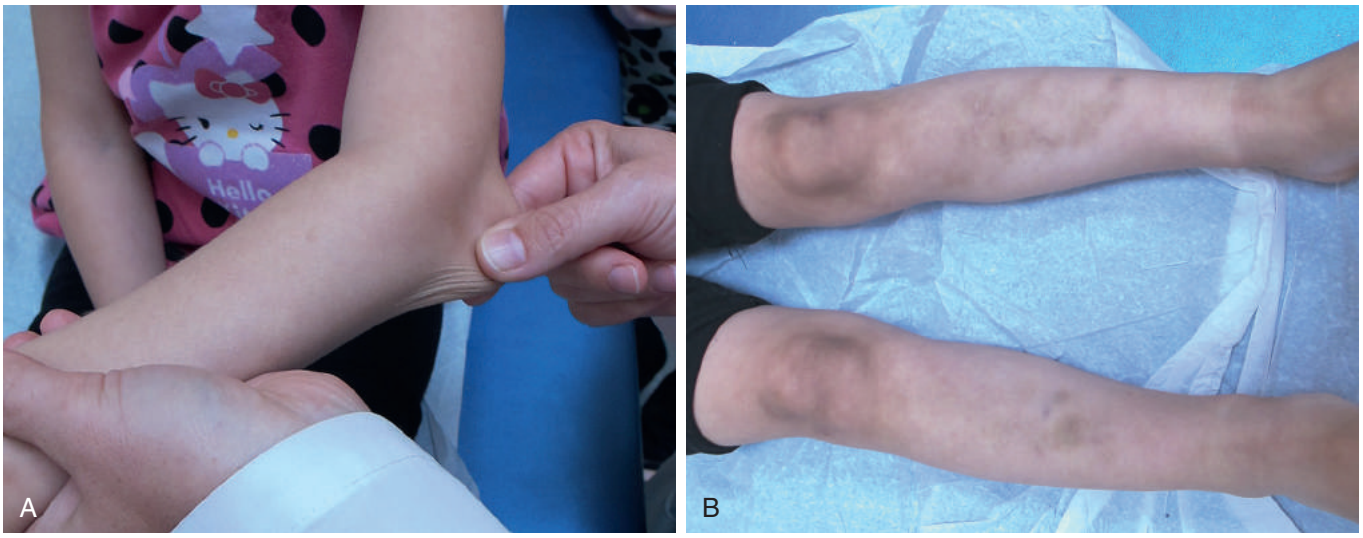
In the neonatal period, prevention of new blisters and prevention of infection are of the utmost importance. Gentle handling and avoiding of frictional trauma should be emphasized. Infants should be rolled or moved with a sheet when possible to avoid friction. When lifting is necessary, one hand should gently support the head and neck, with the other under the buttocks. Ambient temperature should be cool, and routine use of incubators should be avoided, as heat may increase skin fragility. Bedding should be soft, and silicone dressings or padding should be applied to bony prominences and inside diapers. Intact bullae should be gently ruptured and drained with a sterile needle, leaving the roof intact as a biologic dressing. Wounds should be covered with nonadhesive dressings, and these should be changed daily or every other day. Tape and adhesives should never be applied to the skin. Nonadhesive wound dressings can be placed under pulse oximeter probes and electrocardiogram leads. Prevention of infection often involves use of chlorhexidine, dilute bleach, and/or dilute vinegar baths. Topical antibiotics should be used sparingly to avoid resistance. Pain control is essential.[19]

Aggressive nutritional support is essential, especially in patients with feeding difficulties caused by significant mucosal involvement, or pyloric atresia, or those with increased fluid and energy requirements because of widespread blisterings.[19] Soft nipples used for feeding infants with cleft palates should be used in those with oral involvement. EB patients are prone to cutaneous infection, most commonly with *Staphylococcus*, which may disseminate. Indwelling catheters and overuse of topical antibiotics such as mupirocin should be avoided when possible.[10] Tracheostomy may be indicated in patients with airway involvement.[15,20] Surgical repair of pyloric atresia enhances survival in affected patients, particularly those with relatively mild skin involvement.[15] Patients with severe disease should also be monitored for iron-deficiency anemia.

Ehlers–Danlos Syndrome

The Ehlers-Danlos syndromes (EDS) are a heterogeneous group of inherited connective tissue disorders characterized by with variable manifestations of cutaneous hyperextensibility, joint hypermobility, and tissue fragility. The 1997 Villefranche Nosology described six types of EDS. The most recent consensus classification from 2017 describes 13 distinct clinical subtypes with diverse genetic etiology.[21] Premature rupture of membranes is more common in infants with EDS or in pregnancies of affected mothers and may be the presenting sign of the condition.

Classical EDS (cEDS) is the most common subtype and is usually caused by autosomal dominant mutations in the COL5A1 gene, which codes for type V collagen. The skin of affected patients is soft, velvety, doughy and hyperextensible. Importantly, it readily recoils to the normal position after being stretched (in contrast to cutis laxa, discussed later) (Fig. 92.9A). The skin is also fragile with a tendency to form widened, atrophic scars often referred to as *fish-mouth* or *cigarette-paper scars*. Vessel fragility leads to easy bruising (Fig. 92.9B). Hematomas can become fibrotic and may result in soft subcutaneous nodules referred to as *pseudotumors* or hard calcified nodules referred to as *spheroids*. cEDS is also characterized by joint hypermobility and resulting complications.[22] Patients with the autosomal recessive cardiac-valvular form of



• **Fig. 92.9** Three-year-old girl with classic Ehlers-Danlos syndrome characterized by hyperextensible skin (A) and easy bruising (B).

EDS (cvEDS) present with similar skin findings but are also at high risk for severe progressive cardiac-valvular dysfunction.

Vascular EDS (vEDS) is caused by autosomal dominant mutations in the COL3A1 and COL1A1 genes, which code for type III and type I collagen respectively. vEDS carries risk of life-threatening arterial or organ rupture. Cutaneous findings include thin, translucent skin and extensive bruising but not skin hyperextensibility. Patients with more rare and severe forms of EDS may come to attention at birth with multiple anomalies, including severe joint hypermobility resulting in multiple dislocations and subluxations (arthrochalasia EDS), congenital hypotonia with kyphoscoliosis (kyphoscoliotic EDS), ocular abnormalities (brittle cornea syndrome), hypotonia with short stature and bowing of limbs (spondylodysplastic EDS), and congenital contractures (musculocontractural and myopathic EDS).

Dermatosparaxis EDS is a very rare but severe autosomal recessive phenotype which is notable for extremely fragile, lax skin, which may present with tears at birth or with minimal trauma, as well as severe bruisability with risk of subcutaneous hematomas and hemorrhage.[21] It is important to note that infants with EDS have not been shown to be of increased risk for fractures, including those with hypermobile EDS, which does not have a clear genetic basis. This finding should raise suspicion for child abuse in a nonambulatory patient.[23]

Cutis Laxa

The skin of patients with cutis laxa is characterized by loose, redundant, hypoelastic skin that does not recoil after stretching (in contrast with the skin in EDS patients which does recoil and does not form redundant folds). Cutis laxa can be congenital (inherited) or acquired. Mutations in at least 13 different genes which code for proteins involved in dermal elastic fiber production have been reported to cause variants of cutis laxa. The autosomal dominant form is caused by mutations in the *ELN* gene, which encodes elastin. Clinical presentation is highly variable. Cutaneous findings and dysmorphic facial features may be evident at birth. Elastic fibers in extracutaneous sites are often affected, which may result in life-threatening complications, including arterial tortuosity and aneurysms, genitourinary and gastrointestinal diverticulae.[24]

The X-linked form of cutis laxa, once classified as a type of EDS, is also referred to as *occipital horn syndrome*. This disorder is caused by mutations in the *ATP7A* gene leading to impaired copper metabolism and is allelic with Menkes disease. Cutaneous manifestations of these allelic conditions may include soft, doughy skin and hypopigmented, fragile, kinky hair (pili torti).

Ectodermal Dysplasias

The ectodermal dysplasias comprise a heterogeneous group of more than 200 inherited conditions affecting development and/or homeostasis of two or more ectodermal derivatives, including at least one of the following appendages: hair, teeth, nails, and certain glands.[25] Other structures derived from embryonic ectoderm include the central and peripheral nervous systems, mucosal epithelium mammary glands, external ear, melanocytes, and multiple ocular structures. The most recent consensus classification system developed in 2017 groups these conditions based on genotype, molecular pathway and phenotype.[25] Most implicated genes function in one of the following molecular pathways essential to ectodermal development: nuclear factor kappa-B (*NFκB*), *WNT*, and *TP63*.

Characteristic dysmorphic features and hypotrichosis are often the earliest recognized signs of ectodermal dysplasia. Temperature dysregulation is a common complication of hypohidrosis (reduced sweating) and may lead to recurrent fevers of unknown origin. The clinical features of the ectodermal dysplasias are diverse. The most common or notable forms are discussed below and summarized in Table 92.8.

Management of ectodermal dysplasias hinges on establishing the diagnosis, referral to appropriate specialists on the basis of symptoms and known associations, and supportive measures. Early involvement of a geneticist is important, and ideally molecular confirmation of the diagnosis is obtained. For children with hypohidrotic forms of ectodermal dysplasia, avoidance of overheating is paramount. Eye drops and nasal irrigation should be used to lubricate the ocular and respiratory mucosa. Dental assessment should be performed early in life as intervention may be initiated before age 2 years.[26]

TABLE 92.8 Notable Ectodermal Dysplasias[25]

Syndrome	Clinical Features
Hypohidrotic ectodermal dysplasias	Mostly males Hypohidrosis Hypotrichosis Hypodontia Periorbital hyperpigmentation and dermatitis Craniofacial dysmorphology Dry skin
Incontinentia pigmenti	Mostly females Short stature Staged skin involvement Cataracts, microphthalmia Hypodontia Breast aplasia Nail dystrophy Abnormal hair
Ectodermal dysplasia and immunodeficiency 1 (EDAID1)	Mostly males Hypotrichosis Hypohidrosis Immunodeficiency, recurrent infection
Focal dermal hypoplasia (Goltz syndrome)	Mostly females Short stature Facial asymmetry Skin atrophy Narrow auditory canals, hearing loss Hypodontia, oral papillomas Syndactyly Sparse hair
Rapp-Hodgkin syndrome	Short stature Maxillary hypoplasia, cleft lip/palate, hypodontia Hearing loss
Ankyloblepharon-ectodermal defects-cleft lip/palate–clefting (AEC, Hay–Wells syndrome)	Scalp erosions, hypotrichosis Conductive heating loss Maxillary hypoplasia, cleft lip/palate, hypodontia Ankyloblepharon, lacrimal duct atresia
Ectrodactyly, ectodermal dysplasia, and cleft lip/palate syndrome 3 (EEC3)	Blepharophimosis Cleft lip/palate, hypodontia, microdontia Syndactyly, dystrophic nails Hypotrichosis
Clouston syndrome	Nails: dystrophic Hair: normal at birth; becomes sparse, brittle Teeth prone to caries Ocular abnormalities Hyperkeratosis of palms, soles, knees, elbows, knuckles French-Canadian families

Hypohidrotic Ectodermal Dysplasia

HED is the most common type of ectodermal dysplasia and most often results from an X-linked recessive mutation of the ectodysplasin A gene (*EDA*). The classic triad of associated clinical features is hypohidrosis, alopecia, and hypodontia (reduced number of teeth). In X-linked HED, males are hemizygous for the *EDA* mutation and generally display the complete triad, as well as a propensity for respiratory disorders. Most are identified within the first 2 years of life. Females are typically heterozygous for the *EDA* mutation. Their presentation may be subtle and diagnosis delayed.

Infants with X-linked HED may exhibit failure to thrive, nasal congestion, decreased saliva production, and gastroesophageal reflux. Eczema usually presents during the first year of life. Males are more likely to suffer from respiratory problems, including chronic, malodorous nasal congestion, wheezing, and recurrent sinusitis.[27]

Ectodermal Dysplasia and Immunodeficiency 1 (EDAID1) is most commonly caused by mutations in the IKBKG gene on chromosome band Xq28. Affected males present similarly to those with HED, but also with immunodeficiency and multiple infections. Female carriers of more severe mutations in this same gene have IP, as discussed later.

Ankyloblepharon-Ectodermal Defects-Cleft Lip/Palate (AEC, also known as Hay-Wells syndrome) is of particular importance to the neonatologist, as the congenital scalp erosions seen in affected patients can raise concern for neonatal infection or epidermolysis bullosa (discussed above).

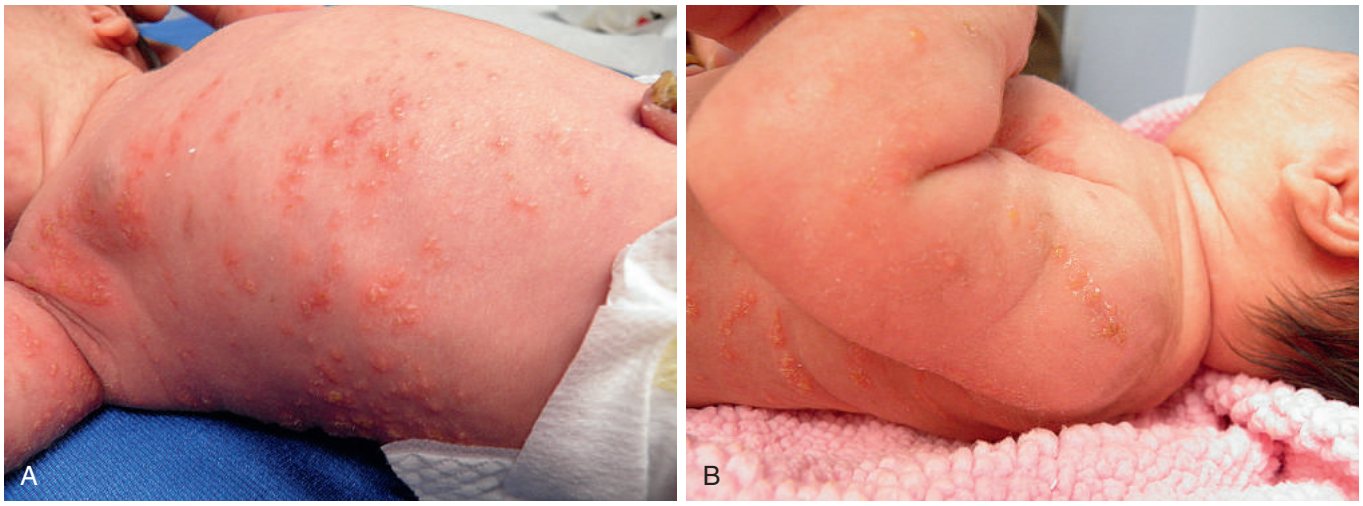
Incontinentia Pigmenti

Incontinentia pigmenti (IP), also referred to as *Bloch-Sulzberger syndrome*, is an X-linked ectodermal dysplasia that variably affects the skin, hair, nails, teeth, eyes, and central nervous system (CNS). The disorder is caused by mutations in the *IKBKG* gene, also called *NEMO* (which encodes nuclear factor κB essential modulator), localized to chromosome band Xq28. The vast majority of patients with IP are female, suggesting causative mutations in *IKBKG* are lethal in males, but there are rare reports of affected males with Klinefelter syndrome (XXY genotype), somatic mosaicism, or hypomorphic alleles.[28,29] Females with a missense mutation in *IKBKG* may have a son with X-linked ectodermal dysplasia with immunodeficiency, discussed above. Females with IP are functionally mosaic because of random X chromosome inactivation early in embryologic development (lyonization), which explains the variability in clinical phenotype. Cutaneous lesions of IP demonstrate mosaicism and typically follow Blaschko lines.

Cutaneous Findings

Almost all patients with IP will exhibit cutaneous features at birth or within the first few weeks of life. Four classic stages of skin lesions have been described, but not all patients will exhibit each stage, and there can be overlapping of stages. Stage 1 is characterized by erythema and vesiculobullous or pustular lesions in a linear and whorled pattern following Blaschko lines (Fig. 92.10). This stage can last from weeks to months and then resolves spontaneously but can recur even years later, often precipitated by infection. The distribution along Blaschko lines can help distinguish cutaneous lesions of IP from herpes or bacterial infection.

In stage 2 there are linear, hyperkeratotic, verrucous papules and plaques that may or may not have evolved from previous vesiculobullous lesions. The verrucous stage is usually distributed on the extremities and is most pronounced on the hands or feet.



• **Fig. 92.10** Newborn with incontinentia pigmenti who exhibited extensive vesiculobullous lesions on the trunk (A) and extremities (B), some following Blaschko lines.

These lesions often resolve by about 6 months of age but rarely persist or recur.

Stage 3 is characterized by linear and whorled brown to slate-gray hyperpigmentation along Blaschko lines and typically involves both the trunk and extremities or just the trunk. The distribution does not usually correspond to prior inflammatory or verrucous lesions. This stage begins within the first few weeks to months of life and may last for years, often resolving spontaneously by adolescence or adulthood.

Less than half of patients exhibit stage 4, with atrophic, hypopigmented, or hypovascular streaks lacking adnexa (hair follicles or sweat glands). These are often located on the posterior lower extremities and may be subtle and thus underreported. The atrophic lesions are persistent and may be the only cutaneous finding in adulthood.[30] Patients with IP may also have abnormal hair and nails.

Extracutaneous Findings

Ocular abnormalities (both retinal and nonretinal) are one of the most common and manifestations and the most important to identify early. Retinal vasculopathy leading to retinal ischemia and subsequent detachment is an important cause of blindness. Vasculopathy can be detected within the first days of life, and blindness prevented with laser therapy. CNS involvement typically presents within the first year of life may include seizures, motor impairment, or learning difficulties and intellectual disability. Brain MRI and EEG are recommended.[31] Dental anomalies are common and may affect both deciduous and permanent teeth. Mammary gland, and skeletal abnormalities as well as pulmonary hypertension have been reported.

Diagnosis

Major criteria for the diagnosis of IP include characteristic skin findings and mutation in the *IKBKG* gene (as well as dental anomalies). Minor diagnostic criteria include eosinophilia, hair, nail, or mammary abnormalities, peripheral neovascularization of the retina and characteristic skin histology.[31] A biopsy of cutaneous lesions in stage 1 will reveal characteristic infiltration of the epidermis by eosinophils with intraepidermal vesicles and dyskeratosis. Stage 1 may also be associated with a striking peripheral leukocytosis and eosinophilia. These findings can help differentiate vesiculobullous or pustular lesions of IP from infectious causes (such as herpes, varicella, or bullous impetigo), Langerhans cell histiocytosis, EB, autoimmune bullous diseases, bullous mastocytosis, or child abuse. Histopathology of stage 2 lesions shows papillomatosis, hyperkeratosis, and dyskeratosis; however, these same findings can be seen in epidermal nevi. Skin biopsy findings from stage 3 or stage 4 lesions are nonspecific.

Prognosis and Treatment

The cutaneous findings in IP, other than stage 4 atrophic or hypopigmented lesions, resolve spontaneously. Vesiculobullous lesions may require dressings or wound care, with monitoring for superinfection. All patients with suspected IP should have a thorough ophthalmology evaluation as soon as possible after birth. Careful neurologic examination is essential, and neuroimaging and electroencephalographic studies should be performed in patients with signs of CNS involvement. Dental evaluations should begin by age 2 years.[30]

Focal Dermal Hypoplasia (Goltz Syndrome)

Focal dermal hypoplasia (FDH), also known as *Goltz syndrome*, is a rare ectodermal dysplasia with skin, skeletal, ocular, and other manifestations caused by mutations in the *PORCN* gene. Like IP, FDH is an X-linked dominant disorder and predominantly affects females (90%), who are functional mosaics, as mutations are often lethal in males. Cutaneous lesions are usually present at birth, including linear streaks of dermal hypoplasia associated with telangiectasias or pigmentary changes that follow Blaschko lines, yellow to red-brown nodules or outpouching caused by herniation of fat through hypoplastic dermis, and ulcers caused by congenital absence of skin (aplasia cutis). Erythematous papillomas affecting the skin or mucosa may appear later. Nail hypoplasia or ridging, alopecia, and dental abnormalities are common. Skeletal manifestations include craniofacial findings and limb malformations such as ectrodactyly or syndactyly. X-ray of long bones reveals longitudinal striations of the metaphyses

(osteopathia striata). A number of developmental abnormalities of the eye have been associated with FDH. Other developmental anomalies, including congenital diaphragmatic hernia, have been described. Most individuals have normal development, but developmental delay has been reported. Treatment of affected individuals requires a multidisciplinary approach.[32,33]

Disorders With Generalized Hypopigmentation

Oculocutaneous albinism (OCA) is characterized by generalized hypopigmentation of the skin, hair, and eyes, with additional ocular abnormalities. OCAs are classified into "classic" nonsyndromic types and syndromic forms with additional systemic manifestations. There are eight identified forms of nonsyndromic OCA, each associated with a specific gene mutation.[34] The implicated gene mutations affect proteins integral to melanin production or distribution. Severity of pigment loss and ocular manifestations differ depending on the OCA type and mutation severity. For example, the most severe type of OCA, OCA type 1A, is caused by a nonsense mutation in the tyrosinase gene, resulting in complete absence of melanin production. OCA type 1B is caused by a missense mutation in the same gene, allowing various amounts of melanin production. OCA types 1 to 4 account for most cases, and their pathogenesis and clinical features are summarized in Table 92.9.[35]

Hermansky-Pudlak, Chédiak-Higashi, and Griscelli syndromes are syndromic forms of OCA. The Hermansky-Pudlak syndromes are most commonly observed in families of Puerto Rican descent. In addition to OCA, patients have abnormal platelet function and are prone to bleeding. The intestines, lungs, kidneys, and heart may also be affected. Patients with Chédiak-Higashi syndrome have impaired phagocytosis and are prone to pyogenic infections. Griscelli syndrome is associated with neurologic or immunologic abnormalities. Patients with metabolic disease may also present with generalized hypopigmentation. For example, those with Menkes disease are unable to metabolize copper and therefore lack this essential cofactor for tyrosinase activity and melanin production.[36]

Diagnosis of Oculocutaneous Albinism

In patients with severe forms of OCA the diagnosis is often clinically apparent during the neonatal period. However, presentation may be subtle in patients with some melanin production, such as those with OCA types 2 and 3 born to darker-skinned parents. Comparison of skin, hair, and eye color with that of other family members may be helpful. Genetic testing performed at specialized centers currently identifies causative mutations in approximately 80% of OCA patients.[36]

Treatment of Oculocutaneous Albinism

Treatment of OCA is geared toward addressing visual impairment and preventing sun-induced carcinogenesis. Early referral to and regular monitoring by an ophthalmologist is critical. Vigilant sun avoidance and protection, with use of sunscreen, UV-protective clothing, and eyewear, is essential. Sunscreens with physical blockers—namely, zinc oxide and titanium dioxide—are generally considered to be safest in infants and children. Clinical trials of

TABLE 92.9 Congenital Disorders With Generalized Loss of Skin Pigmentation[35,36,49]

Syndrome	Clinical Features
OCA types 1A and 1B	Most common OCA type in white Europeans Complete loss of (OCA type 1A) or significantly reduced (OCA type 1B) pigmentation: white or very light skin and hair Ocular: pink irides at birth become blue gray in adulthood, reduced visual acuity, strabismus, photophobia, nystagmus, misrouting of the optic nerves at the chiasm, foveal hypoplasia
OCA type 2 (brown OCA)	Most common OCA type in equatorial Africans Minimal to moderate skin pigmentation Hair white, golden, or red Irides translucent blue to light brown Ocular: similar to OCA type 1
OCA type 3 (rufous OCA)	Most common OCA type in southern Africans Skin red, freckled, or hypopigmented Reddish hair Light eyes Ocular: nystagmus, strabismus, positive red reflex Can be subtle: comparison with parents can aid in diagnosis
OCA type 4	Most common in Japanese descent Light skin and hair Translucent irides Ocular: mild nystagmus and photophobia, decreased visual acuity, absent macula
Hermansky-Pudlak syndrome	OCA, similar to OCA type 1 Abnormal platelets Easy bruising and bleeding Pulmonary fibrosis Cardiomyopathy Intestinal inflammation and hemorrhage Renal failure Most common in Puerto Rican descent
Chédiak-Higashi syndrome	Variable skin hypopigmentation Ocular: similar to OCA type 3 Gingivitis Hepatosplenomegaly Recurrent pyogenic infections Progressive neurologic decline
Griscelli syndrome	Partial albinism, with hypopigmented skin, silver-gray hair Visual problems Neurologic impairment Immunodeficiency, hemophagocytic syndrome
Menkes disease	"Doughy" inelastic, hypopigmented skin Hairs short, sparse, twisted (pili torti) Microcephaly, brachycephaly, pudgy cheeks Small stature Neurodegeneration: hypotonia, seizures, hypothermia Failure to thrive Impaired vision

AD, Autosomal dominant; *AR*, autosomal recessive; *OCA*, oculocutaneous albinism; *XLR*, X-linked recessive.

medications designed to enhance melanin production in patients with albinism and related disorders are currently under way.[35]

Disorders With Localized Hypopigmentation

Piebaldism

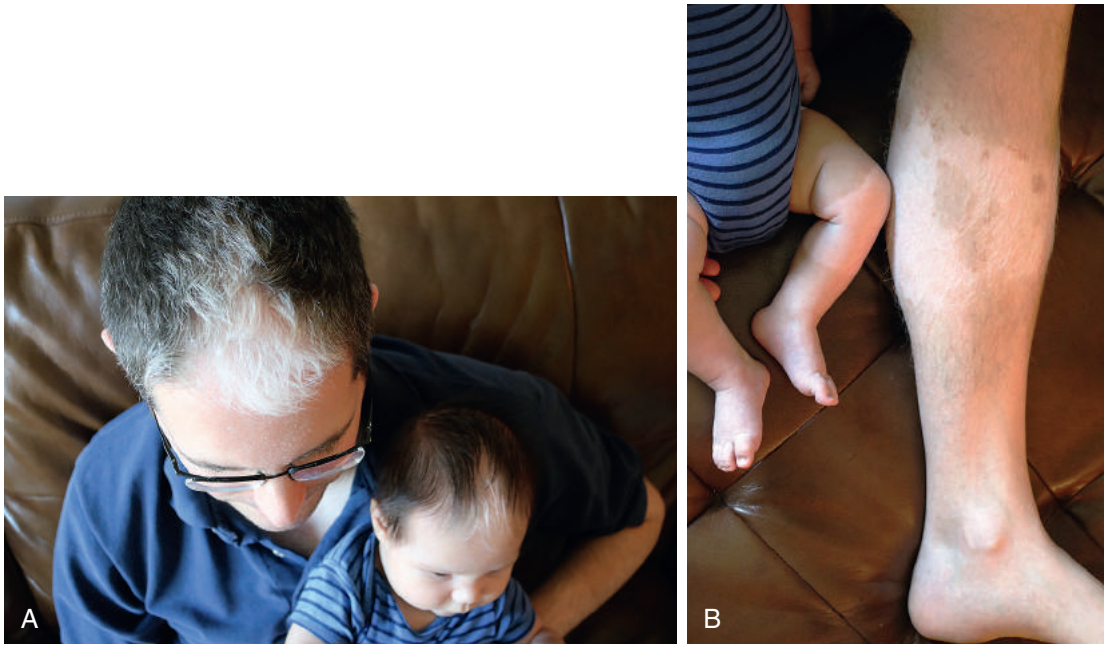
Piebaldism is an autosomal dominant disorder characterized by circumscribed areas of leukoderma (absence of skin pigment). The anterior hairline is affected in 80% to 90% of cases, resulting in a white forelock (poliosis circumscripta) (Fig. 92.11A). This is frequently the sole manifestation.[37] Well-demarcated depigmented patches with islands of normal pigmentation and hyperpigmentation are typically present at birth and remain stable through adulthood (Fig. 92.11B), although a few cases of spontaneous repigmentation have been reported.[38] Characteristically, the ventral body is more affected, particularly the central forehead, anterior trunk, arms, and legs. The dorsal midline, hands, feet, and periorificial regions are typically spared. Diagnosis is clinical and can be confirmed with molecular analysis of the *KIT* gene. Skin biopsy of depigmented areas is not necessary but when performed reveals absent or markedly reduced numbers of melanocytes. In the neonatal period, management of piebaldism hinges on vigilant photoprotection of amelanotic skin. Camouflaging makeup may be used for cosmetic purposes. Surgical and laser treatments are evolving and may be considered later in life.

The differential diagnosis for an infant with localized loss of skin or hair pigmentation includes disorders with systemic complications such as Waardenburg syndrome and tuberous sclerosis (Table 92.10) as well as nonsyndromic pigmentary anomalies. *Hypomelanosis of Ito* is a historical term used to describe linear and whorled hypopigmentation along Blaschko lines that may be an isolated cutaneous finding or associated with extracutaneous abnormalities, including neurologic or musculoskeletal conditions. Hypomelanosis of Ito is no longer considered a distinct entity but rather encompasses a group of disorders associated

TABLE 92.10 Syndromes With Congenital Localized Loss of Skin Pigmentation[37,49]

Syndrome	Clinical Features
Piebaldism	White forelock (poliosis circumscripta) Depigmented macules and patches (leukoderma) Heterochromic irides Rare: Hirschsprung disease, deafness
Waardenburg syndrome	White forelock Early graying (by age 35 years) Heterochromic, bichromic, or bright blue irises Depigmented macules or patches Congenital sensorineural deafness Bony anomalies Facies: dystopia canthorum, synophrys, broad nasal root, nose hypoplasia, smooth or shortened philtrum Hirschsprung disease (type 4)
Tuberous sclerosis	Hypomelanotic ash leaf spots, "confetti-like" hypomelanotic macules Facial angiofibromas Collagenomas/shagreen patches Forehead fibrous plaques Periungual fibromas Neurologic: cortical tubers, subependymal nodules or astrocytomas, developmental delay, seizures, infantile spasms, behavioral disorders Other: retinal achromic patch or hamartomas, renal angiomyolipomas or cysts, cardiac rhabdomyomas, lymphangioleiomyomatosis, etc.
Pigmentary mosaicism	Hypopigmented patches, whorls, or streaks along Blaschko lines Associated with various neurologic, ocular, dental, or musculoskeletal abnormalities or isolated cutaneous finding

AD, Autosomal dominant.



• **Fig. 92.11** Father and son with piebaldism manifesting itself as white forelock (A) and well-demarcated depigmented patches with islands of normal pigmentation and hyperpigmentation (B).

with various mosaic defects, and many prefer the term *pigmentary mosaicism* to describe the associated cutaneous findings. Other terms have been used to describe congenital hypopigmentation or depigmentation, including *patterned dyspigmentation, segmental pigmentation disorder*, and *nevus depigmentosus*. Congenital vitiligo has also been rarely reported. In any patient born with localized hypopigmentation or depigmentation, a thorough history, family history, and physical examination should be conducted, with further work-up directed by any abnormalities found.[39,40]

Porphyrias

The porphyrias are a group of disorders caused by mutations in enzymes involved in heme biosynthesis that result in accumulation of porphyrin or porphyrin precursors. Most porphyrias are autosomal dominant, but some are autosomal recessive, X-linked, or have more complex inheritance patterns. Cutaneous manifestations are due to the photosensitizing effects of porphyrins. Phototoxicity can be immediate with burning pain, erythema, and edema and/or delayed with blisters, dyspigmentation, and scarring. Traditionally, the porphyrias have been classified on the basis of the organ in which porphyrins or porphyrin precursors accumulate as erythropoietic (accumulation in bone marrow erythroid cells), hepatic (accumulation in liver), or mixed (i.e., hepatoerythropoietic porphyria). The porphyrias may be also categorized on the basis of primary symptoms, with "acute porphyrias" predominated by neurovisceral attacks and "cutaneous porphyrias" predominated by skin photosensitivity.[41]

The erythropoietic porphyrias (congenital erythropoietic porphyria [CEP], erythropoietic protoporphyria [EPP], X-linked EPP [XLP]) and hepatoerythropoietic porphyria (HEP) can present in early infancy with severe photosensitivity and are reviewed in the following sections. Box 92.1 lists disorders that can present with photosensitivity in infancy. Phototherapy-induced eruptions because of transient porphyrinemia may be seen in neonates with hemolytic disease of the newborn.[42,43]

The hepatic porphyrias are categorized by neurovisceral attacks, with variable cutaneous photosensitivity, and usually present later in childhood or adulthood. Exceptions include homozygous variants of hereditary coproporphyria, variegate porphyria, acute intermittent porphyria, and aminolevulinic acid dehydratase porphyria, which are exceedingly rare but can present in infancy.

Congenital Erythropoietic Porphyria

CEP, previously known as *Günther disease*, is an autosomal recessive erythropoietic cutaneous porphyria caused by mutations in the gene *UROS* encoding uroporphyrinogen III synthase. The disease is characterized by severe photosensitivity and hemolytic anemia with splenomegaly. CEP may present in utero as nonimmune hydrops fetalis. Severe photosensitivity usually begins in infancy and can sometimes present in the neonatal period with blistering after phototherapy for hyperbilirubinemia. Scarring, hyperpigmentation, hypertrichosis, and disfigurement develop in photoexposed areas over time (Fig. 92.12). There are variable ocular manifestations. Urine may be faint pink to dark red from accumulation of uroporphyrin. The teeth also develop a reddish-brown color (erythrodontia). Teeth, urine, and stool may fluoresce under Wood lamp examination.

The diagnosis of CEP can be made on the basis of elevated uroporphyrin and coproporphyrin levels in erythrocytes, urine, and stool and can be confirmed by measurement of uroporphyrinogen III synthase activity or gene mutation analysis. Historically, CEP has been managed primarily with photoprotection and long-term erythrocyte cell transfusions. More recently, CEP has been cured with hematopoietic stem cell transplant, but there are significant risks of transplant complications, and novel therapeutics are being studied.[44]

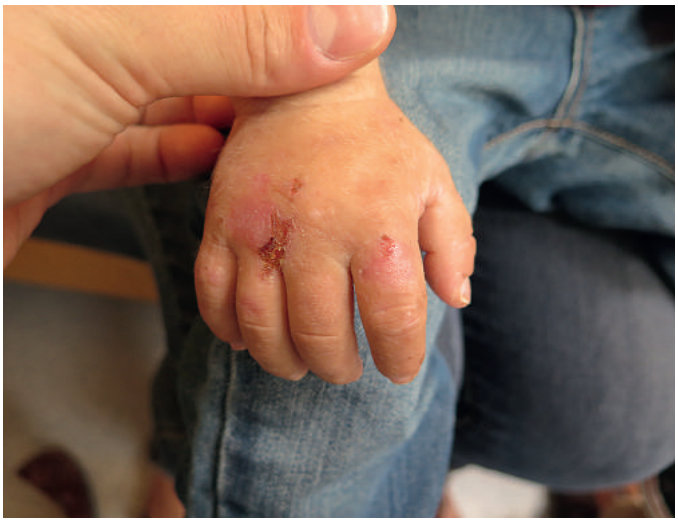
Erythropoietic Protoporphyria and X-Linked Erythropoietic Protoporphyria

EPP is the most common form of porphyria in childhood and is caused by autosomal recessive mutations in the gene *FECH* encoding ferrochelatase. XLP, also referred to as *X-linked dominant protoporphyria*, is an X-linked form that results from mutations in the *ALAS2* gene, the product of which catalyzes the first committed step of heme biosynthesis. XLP has a phenotype very similar to that of EPP but with higher concentrations of erythrocyte protoporphyrin and a higher incidence of liver disease.[45]

Clinical manifestations of EPP include immediate painful photosensitivity to sunlight and sometimes fluorescent lighting. In infancy, this may manifest itself as episodes of crying within minutes of UV exposure, and older children may complain of stinging or a burning sensation in exposed areas. Prolonged exposure may

• BOX 92.1 Disorders That Present With Photosensitivity in Infancy

Porphyrias
Transient porphyrinemia
Xeroderma pigmentosum
Cockayne syndrome
Bloom syndrome
Rothmund-Thomson syndrome
Smith-Lemli-Opitz syndrome
Hartnup disease
Neonatal lupus erythematosus
Phototoxic or photoallergic reaction



• Fig. 92.12 Two-year-old male with congenital erythropoietic porphyria who has bullae, crusted erosions, hyperpigmentation, and hypertrichosis in sun-exposed areas. He initially exhibited blistering after phototherapy for hyperbilirubinemia as a neonate.

lead to erythema, edema, and petechiae and vary rarely vesicles or bullae. Long-term UV exposure results in shallow atrophic scars and thickened, leathery skin around the mouth (pseudorhagades) and overlying knuckles.

Hepatic involvement can range from mild liver dysfunction to rare liver failure. Cholelithiasis may cause severe abdominal pain. Anemia is usually mild or absent. Diagnosis of EPP and XLP can be made on the basis of elevated erythrocyte protoporphyrin concentration, and red blood cells will fluoresce under Wood lamp examination. Increased stool protoporphyrin concentration may also be detected. The diagnosis is confirmed by genetic testing. Management in infancy includes sun avoidance and protection, with symptomatic treatment of photosensitivity reactions. Patients should be monitored for liver disease and microcytic anemia.[46] Afamelanotide is a targeted treatment option for adults with EPP.[47]

Hepatoerythropoietic Porphyria

HEP is an exceedingly rare disorder caused by homozygous or compound heterozygous mutations in the gene encoding uroporphyrinogen decarboxylase. Heterozygous mutations or sporadic mutations in the same gene result in porphyria cutanea tarda, the most common porphyria in adults. HEP usually presents with severe bullous photosensitivity before age 2 years, with subsequent hypertrichosis and scarring, resembling CEP. The diagnosis can be made on the basis of highly elevated erythrocyte zinc protoporphyrin concentration, along with elevated urine and stool porphyrin concentrations, and can be confirmed by genetic testing.[48]

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93

Infections of the Skin

MARKUS D. BOOS AND ROBERT SIDBURY

KEY POINTS

- Owing to their cutaneous, immunologic, and renal immaturity, newborns (especially premature neonates) are at increased risk of skin infection.
- As a group of potentially life-threatening but often treatable diseases, infections must always be considered in a newborn with skin lesions.
- Prompt diagnosis and initiation of therapy are crucial to prevent devastating long-term sequelae, particularly in instances of disseminated disease.
- *Staphylococcus aureus*, *Streptococcus* species, *Candida albicans*, and herpes simplex virus are the most common causes of skin infection in the neonate.

Staphylococcus aureus Infections

Staphylococcus aureus is ubiquitous and harbored as a commensal organism in about one-third of the population, with a predilection for the nares (especially in children), perineum, and other moist cutaneous surfaces.[1] It is more likely to be present early if the child is born via cesarean delivery rather than vaginally, reflecting colonization via the bacterial microbiota of adult skin.[2] However, by 1 week of age, most newborns will exhibit nasopharyngeal or umbilical colonization by *S. aureus*.

Invasive infection of infants by *S. aureus* remains a healthcare challenge. A retrospective study of staphylococcal infections in 348 neonatal intensive care units (NICUs) demonstrated that the incidence of invasive *S. aureus* infection (defined as a positive culture result from blood, cerebrospinal fluid (CSF), sterile fluid, or an abscess) was 44.8 per 10,000 infants. Most of these infections were caused by methicillin-sensitive strains.[3] Cutaneous signs of invasive *S. aureus* infection are mediated by local or circulating bacterial toxins; *S. aureus* is therefore responsible for skin lesions of protean morphology.[4]

Impetigo

Impetigo is a group of superficial skin infections caused by *S. aureus*, group A streptococcus (GAS), or both. Neonatal bullous impetigo is caused by *S. aureus* and can occur in nursery-based, epidemic patterns, often attributed to nasal carriage of *S. aureus*.

Clinical Findings

Impetigo is one of the most common neonatal skin infections. Bullous impetigo manifests itself as flaccid vesicles/bullae or pustules on an erythematous base, most often seen in the diaper area, periumbilically, or in skin folds (Fig. 93.1). Ruptured lesions leave behind a moist red base with a characteristic collarette of scale.[5,6] Lesions of bullous impetigo are usually not closely grouped, which distinguishes this infection from herpes simplex virus (HSV) infection. Aggressive cases of *S. aureus* bullous impetigo may also present as widespread desquamation in a degloving pattern.[7]

In contrast, nonbullous impetigo is characterized by thin-walled vesicles or pustules, often at sites of broken skin on the extremities or face. These lesions rapidly burst and assume their characteristic appearance of variably pruritic, eroded red patches with a superimposed honey-colored crust. In both forms of impetigo, affected areas heal without scarring.

Etiology

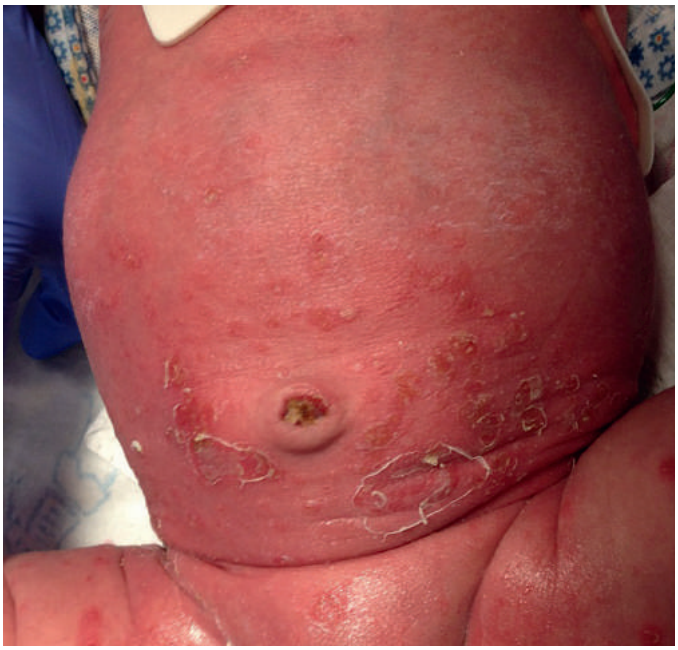
S. aureus is the exclusive cause of bullous impetigo and the primary cause of nonbullous impetigo. GAS may also be associated with the nonbullous form. Bullous impetigo is mediated by local production of exfoliative toxin A (ETA) or B (ETB); these toxins cleave the desmosomal protein desmoglein 1 in the superficial layers of the epidermis. This loss of adhesion between keratinocytes results in characteristic flaccid blisters.

Diagnosis

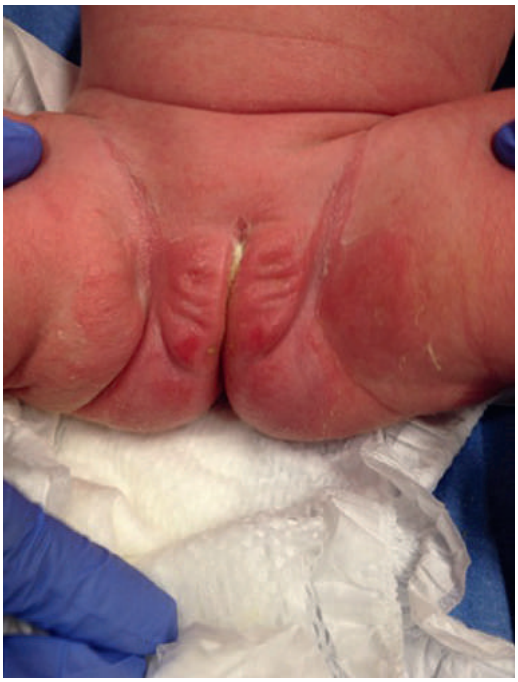
Diagnosis is supported by the presence of gram-positive cocci in clusters on Gram stain of a swab from a pustule, vesicle, or crusted plaque of impetigo. Confirmation is made by bacterial culture taken from blood, skin, and soft tissues.

Treatment

Bullous impetigo is benign if treated early, although extensive local proliferation with exotoxin production or dissemination can be life threatening. Treatment should be instituted promptly and isolation maintained until lesions have ceased to spread, no longer have associated crusting, and have begun to reepithelialize. Infants should be closely monitored, and a high index of suspicion should be maintained for evidence of systemic disease. Infants with periumbilical lesions are at risk of bacterial omphalitis. Extremely limited infections may be treated with topical mupirocin, but this form of therapy should be used with caution in neonates. More extensive lesions require a systemically (most recommend parenterally) administered penicillinase-resistant antibiotic for 7 to 10 days. Antibiotic choice should ultimately be guided by sensitivities of cultured organisms, especially with the rising incidence of



• **Fig. 93.1** Bullous Impetigo. Widespread superficial erosion with characteristic collarettes of scale in a periumbilical distribution.



• **Fig. 93.2** Staphylococcal Scalded Skin Syndrome. Widespread erosions centered around the inguinal folds in a newborn. Note the diffuse background erythema.

methicillin-resistant strains. In those cases, clindamycin, vancomycin, or linezolid would be considered.

Importantly, *S. aureus* outbreaks in nurseries continue to be documented, and it has been suggested that these epidemics may be underreported.[4,8,9] These outbreaks may present as multiple cases of staphylococcal scalded skin syndrome (SSSS), pustulosis, bullous impetigo, abscesses, or other varied presentations of staphylococcal infection.[8] Factors that have been proposed to increase the likelihood of staphylococcal outbreaks include crowded nurseries, poor adherence to hand washing by medical staff, inadequate umbilical cord care, nursery staff carriage of the organism, and lack of isolation early in the course of an outbreak. As such, universal hand-washing protocols, a reduction in overcrowding, increased nurse-to-patient ratio, and staff monitoring for carriage have all been proposed to mitigate the risk of nursery outbreaks. Aggressive monitoring of patients via active surveillance cultures and subsequent treatment of *S. aureus*–colonized neonates with topical mupirocin therapy and chlorhexidine baths have also been successful in limiting staphylococcal infections.[10]

Staphylococcal Scalded Skin Syndrome

In contrast to the localized effects of the exfoliative toxins that mediate bullous impetigo, SSSS is a generalized manifestation of circulating toxin produced by *S. aureus*. Early diagnosis and treatment of SSSS can be lifesaving.

Clinical Findings

SSSS is most common in full-term infants and young children, although cases of affected premature infants have also been reported.[11,12] Affected infants demonstrate abrupt onset of temperature instability, lethargy, and irritability, with subsequent generalized skin tenderness and erythema. Initial erythema tends to occur on the face but subsequently becomes more marked in intertriginous areas (Fig. 93.2).[13] Facial swelling, conjunctivitis, and significant periorificial crusting is common; crusting accompanied by radial fissuring about the mouth gives afflicted children a characteristic appearance. Notably, mucosal surfaces are spared in SSSS. Following these initial signs and symptoms, focal or widespread flaccid bullae may develop within hours to days with subsequent desquamation. This is easily elicited by light stroking of intact skin (Nikolsky sign). Following desquamation, affected areas appear as painful shiny denuded patches. At this stage, diffuse skin involvement may cause problems with thermoregulation, fluid and electrolyte balance, and superinfection.[4]

SSSS must be distinguished from toxic epidermal necrolysis (TEN), a life-threatening condition involving full-thickness epidermal necrosis, most commonly secondary to medication administration.[14] A distinguishing feature of these two conditions is the presence of mucosal blistering and hemorrhagic crusting in TEN.

Etiology

The signs and symptoms of SSSS are the result of circulating exfoliative toxins produced from an often subclinical focus of *S. aureus* infection. Infants are thought to be more at risk of developing SSSS because of immunologic and renal immaturity, which allows excess accumulation of exfoliative toxin within the circulation. Specifically, significantly lower anti-exfoliative toxin antibodies have been noted in patients with SSSS compared with healthy controls.[15] In contrast to bullous impetigo, which is more commonly associated with ETA, SSSS is more frequently caused by ETB. Additionally, while *S. aureus* is identifiable in the blisters of bullous impetigo, in SSSS, *S. aureus* is present at a primary distant site such as the nose, mouth, conjunctiva, umbilicus, or circumcision site. Fresh skin lesions are therefore sterile and blister fluid is culture negative.

Diagnosis

If the diagnosis is in question, a skin biopsy sample prepared for frozen section can be examined emergently. The presence of an

intraepidermal rather than a full-thickness blister with separation at the dermal-epidermal junction distinguishes SSSS from TEN, allowing rapid initiation of appropriate therapy. If the clinical impression is strong, swabs from potential niduses of infection (nasopharynx, conjunctiva, umbilicus, etc.) may identify the primary focus. Gram staining may be performed emergently, while bacterial culture will confirm the diagnosis. Bullous lesions do not contain organisms. Blood culture specimens should be obtained because sepsis, although uncommon, may occur.

Treatment

Empiric therapy with a cephalosporin- or penicillinase-resistant penicillin, such as oxacillin, is the treatment of choice for SSSS; additional coverage to include methicillin-resistant *S. aureus* (MRSA) should be considered in areas with higher prevalence of MRSA and patients with severe systemic disease.[16] Clindamycin may also be considered as a first-line agent for treatment of SSSS because of its superior cutaneous penetration, as well as its ability to inhibit bacterial toxin production. However, reports assessing antibiotic resistance in SSSS have shown that there are certain geographic areas in which clindamycin-resistant strains predominate.[16] As such, empiric treatment with both oxacillin and clindamycin may be useful until antibiotic sensitivities return.[17] In cases of widespread disease, fluid and nutritional support and maintenance of normal body temperature may be required. Close monitoring for secondary superinfection (particularly with gram-negative organisms) is also warranted. Approximately 2 to 3 days after initiation of therapy, denuded areas become dry and desquamation ensues. Crusted, flaky areas may be treated with normal saline compresses. Application of a bland ointment emollient may promote skin healing and resolution. Additional treatment of family members may be considered in instances of recurrent staphylococcal skin infections. As in bullous impetigo, the intraepidermal cleavage plane is superficial and therefore does not result in scarring in the absence of secondary infection or other complications.

Streptococcus Species Infections

Cutaneous streptococcal infections occur in the newborn but are less common than staphylococcal infections. Nevertheless, outbreaks of GAS in nurseries have been reported[18,19] following introduction of the organism via maternal carriers or nursery personnel. Omphalitis is a common manifestation, although cellulitis, pustular eruptions, and paronychia may also be seen. GAS dissemination may occur and often presents as respiratory distress, a toxic shock-like syndrome, lethargy, abdominal distension, and poor oral intake.[20] However, certain classic signs of invasive disease, such as fever, may not be present, particularly in neonates less than 5 days old. Meningitis is more commonly seen in affected individuals 5 days of age or older. Since sepsis and meningitis can result, infected infants should be identified and treated promptly with strict isolation. Disinfection of the umbilical stump reservoir and penicillin prophylaxis for carriers and exposed infants have been the most effective measures in preventing spread. Active GAS infections respond readily to penicillin.

Group B streptococcus (GBS) is one of the most frequently encountered pathogens in the newborn nursery and remains a primary cause of neonatal sepsis. Early-onset disease (during the first week of life), probably acquired during labor and delivery, most commonly becomes manifest as septicemia with respiratory distress and shock. Late-onset disease (7 days to 3 months) is acquired after birth and more commonly associated with meningitis and adverse neurodevelopmental outcomes.[21]

Skin infections caused by GBS are uncommon but have been documented. The most common skin manifestation of GBS is cellulitis, often of the face and neck.[22,23] This so-called GBS cellulitis-adenitis syndrome typically occurs from 1 to 11 weeks of age and is characterized by progressive erythema and swelling. Extracutaneous symptoms include fever, irritability, and poor oral intake. Erosions, abscesses, and necrotizing fasciitis have also been noted with GBS. A diagnosis of GBS can be made via blood culture, which is often positive; CSF cultures are also recommended. Penicillin G or ampicillin is effective as first-line therapy.

Omphalitis

Omphalitis is a bacterial infection of the umbilical stump that presents around day 3 of life. It is commonly caused by *S. aureus*, *Staphylococcus epidermidis*, *Streptococcus* species (spp.), *Escherichia coli*, *Clostridium difficile*, *Klebsiella*, and *Pseudomonas*; these pathogenic bacteria may be contracted from the birth canal or other nonsterile exposures, including the hands of individuals assisting in delivery.[24] Outbreaks in nurseries have been described and are often attributable to staphylococcal or streptococcal infections. Risk factors for the development of omphalitis include unplanned home birth, septic delivery, prematurity, low birth weight, umbilical cord catheterization, prolonged rupture of membranes, and perinatal maternal infection.[25]

Clinical Findings

Omphalitis is characterized by edema, erythema, and tenderness around the umbilicus. Purulent, malodorous discharge may be present. Infection can evolve to cellulitis or lymphangitis or extend deeper into the subcutis or along the abdominal wall, leading to systemic signs of infection or necrotizing fasciitis. Other potential complications include sepsis, peritonitis, bowel ischemia, abscesses, and hepatic vein thrombosis.[24]

Diagnosis

Gram stain and bacterial culture from moist umbilical stump fluid can be performed, but because this area can be contaminated easily, clinical correlation is needed. Infection must be differentiated from embryonic duct remnants.

Treatment

Systemically acting ampicillin and gentamicin can be used empirically to treat both gram-positive and gram-negative organisms until culture and sensitivities are obtained. Intravenous antibiotics can be switched to enteral antibiotics once the skin improves clinically. Appropriate umbilical cord care is an important component of preventing omphalitis. Typically, dry cord care (keeping the cord clean and open to air or covered with a clean cloth) is recommended for neonates born in the hospital and/or in high-resource countries. In contrast, application of topical chlorhexidine is recommended for children born outside of the hospital setting, in areas with high neonatal mortality rates, or in situations where hygiene is poor and infection rates are increased.[24]

Candida Species Infections

Candida albicans is a yeast species that typically exists with humans as a commensal organism. Colonization of the gastrointestinal,

respiratory, and cutaneous surfaces occurs rapidly after birth and is thought to occur primarily via the maternal genitourinary tract, although skin contact may contribute. Nevertheless, decreased host immunity, altered surface microbiota, and epidermal immaturity all contribute to *Candida* pathogenesis and invasive disease.[26,27] Development of active infection may occur in utero, during delivery, or postnatally.

Localized *Candida* Infection (Primary Cutaneous)

Oral Candidiasis (Thrush)

Oropharyngeal candidiasis is characterized by adherent white patches on a normal or erythematous base, most typically on the buccal mucosa or the tongue. These patches are characteristically recalcitrant to physical removal and may be asymptomatic or cause discomfort, resulting in decreased oral intake.[28] Although the peak incidence of *C. albicans* colonization of the oropharynx is estimated to occur at 4 weeks of age,[29] most infants affected do not subsequently develop oral thrush. In a study of 650 infants in a NICU in India, however, approximately 3% developed symptomatic oral candidiasis at a mean age of 10.5 days, with birth asphyxia noted to be the only significant risk factor for this occurrence.[30] A more recent prospective study evaluating oral lesions of Turkish children in an outpatient setting found a 13.6% incidence of oral candidiasis in neonates less than 1 month of age.[31] Notably, thrush is more common in neonates born to mothers with symptomatic *Candida* vulvovaginitis rather than those who are simply colonized. However, there does not appear to be an association between the mode of delivery (vaginal vs. cesarean section) and the development of oral thrush in the first year of life.[32]

Candida *Diaper Dermatitis*

Candida diaper dermatitis is exceedingly common. Cutaneous candidiasis is characterized by widespread, confluent erythema with scalloped edges and characteristic involvement of moist, intertriginous areas. Peripheral white scale and satellite papules or pustules may be evident.[28] Alternatively, candidiasis may present as multiple pink papules with overlying scale that merge into broader plaques. In addition to the diaper area, intertriginous sites, including the neck folds and axillae, may also be infected with *Candida*, as may the nails.

Diagnosis of Localized Cutaneous Candida *Infection*

Presumptive diagnosis can be made by physical examination and history, but microscopic examination of scrapings suspended in 10% potassium hydroxide for yeast and pseudohyphae is useful. The diagnosis may be confirmed by identification of the organism in culture.

Treatment

Nystatin is a polyene antifungal with activity against *Candida* but not dermatophytes. Oral lesions usually respond promptly to a course of nystatin suspension: 100,000 to 200,000 units, administered by mouth four times daily for 7 to 14 days. In refractory cases, an increased dosage of nystatin or systemic therapy may be instituted.[33] Orally or intravenously administered fluconazole has also been used for the treatment of recalcitrant oral thrush or in infants at high risk of dissemination. Nevertheless, its higher relative cost makes it a less suitable first-line treatment.

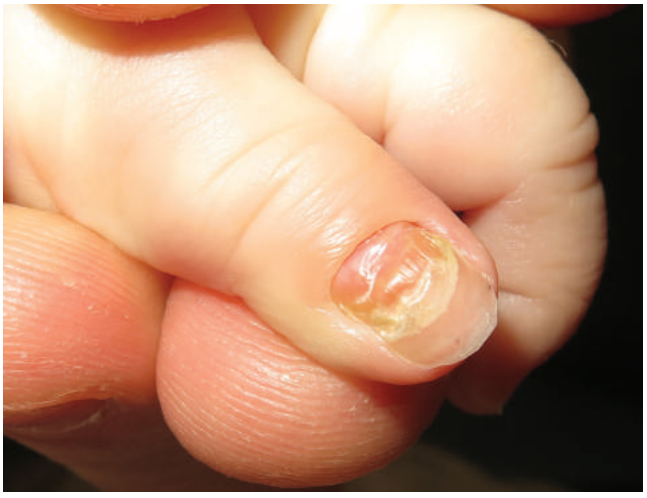
Localized cutaneous candidiasis in an otherwise healthy infant is most easily treated with topical agents, including nystatin, an imidazole antifungal (e.g., miconazole, ketoconazole), or ciclopirox olamine.[34] A recent study examining the efficacy of a 7-day course of 0.25% miconazole nitrate ointment in the treatment of diaper candidiasis showed a clinical cure rate of approximately 50%; cure rates for recurrent episodes were even higher and did not result in antifungal resistance.[35] Importantly, if a breastfeeding mother is also affected, treatment of the mother with nystatin cream or orally administered fluconazole may be indicated. Gentian (crystal) violet is an antiseptic dye effective against *Candida* species. In a 0.5% or 1% aqueous solution, it has proven to be a safe and effective treatment for thrush but is less commonly used because of side effects, including transient purple staining of the skin and mucosal ulceration. Carcinogenicity in mice has been reported.

Congenital (Intrauterine) Candidiasis

Congenital candidiasis refers to widespread candidiasis (rarely with extracutaneous involvement) that results from ascending intrauterine infection where *Candida* spp. cross the fetal membrane and infect surfaces that contact amniotic fluid. Congenital candidiasis occurs within 6 days after birth, although it is most commonly evident on the first day of life.

Clinical Findings

Lesions of congenital candidiasis may at times be seen on the placenta and fetal membranes, including characteristic miliary granulomas of the umbilical cord.[33] These umbilical cord lesions are discrete yellow-white flat-topped papules measuring 0.5 to 4 mm in diameter. Congenital cutaneous candidiasis (CCC) in affected infants is characterized by erythematous macules, papules, and vesicopustules with intense background erythema, often in various stages of development. The back, extensor extremities, and intertriginous areas are most commonly affected, while the face, oral mucosa, and diaper area are comparatively spared. Palmar and plantar pustules, paronychia, and nail involvement characterized by onychomadesis (nail shedding) and yellow hyperkeratotic bands (Fig. 93.3) help distinguish this condition from more common, benign neonatal dermatoses.[36] In rare instances, nail changes are the sole manifestation of CCC,



• **Fig. 93.3** Onychodystrophy Secondary to Congenital Candidiasis. Note the yellow, hyperkeratotic bands characteristic of the condition.

and a high index of suspicion must be maintained to make the correct diagnosis.[37] A scald-like erythema reminiscent of disseminated neonatal candidiasis (see later) has also been reported in full-term infants but is more characteristic of CCC presenting in premature neonates with a birth weight below 1000 g.[36,38] Skin lesions usually resolve with desquamation within 1 to 2 weeks, and nail changes improve spontaneously with time. The prognosis is good in full-term infants, but that of affected low-birth-weight infants is guarded.[34]

Diagnosis

The differential diagnosis of vesicopustular eruptions in neonates is broad, and includes CCC (Box 93.1). Rapid bedside diagnosis can be made via a potassium hydroxide preparation that reveals budding yeasts and pseudohyphae. Positive results on cultures from an intact pustule or skin scrapings, as well as evidence of fungal elements present in the stratum corneum on histologic analysis of skin biopsy tissue, are also diagnostic. Cultures of skin, blood, urine, and CSF are usually negative but should always be considered when CCC is included in the clinical differential diagnosis; they are required when systemic disease is clinically suspected, as well as in all preterm infants.

Treatment

In well full-term infants with an uncomplicated course, active nonintervention with close monitoring may be sufficient as CCC resolves spontaneously within 1 to 2 weeks.[38] Alternatively, topical antifungals may be considered. In at-risk neonates, particularly low birth weight or preterm infants, empiric systemic treatment should be initiated while awaiting culture results. Recommended treatment of CCC includes systemic amphotericin or fluconazole for 14 or more days, which has been shown to prevent dissemination and mortality secondary to *Candida* spp.[39] Other risk factors that may warrant systemic treatment of at-risk neonates include antibiotic or corticosteroid therapy, indwelling catheters, and parenteral nutrition. Clinical findings, including respiratory distress, widespread burn-like dermatitis, or other signs or symptoms of systemic infection, also warrant more aggressive therapy.

Disseminated/Invasive Candidiasis

In contrast to congenital candidiasis, invasive candidiasis (IC) is a disseminated form of neonatal candidiasis characterized by *Candida* infection in otherwise sterile body fluid, such as blood, urine, or CSF. It is more commonly seen in premature, very low birth weight (VLBW) infants, and is typically acquired via the vaginal canal or handling in the nursery, although congenital onset may also occur. In contrast to congenital candidiasis, invasive neonatal candidiasis usually presents after the first week of life. While IC is uncommon in infants weighing more than 2500 g, its incidence increases to 5% to 10% in neonates weighing less than 1000 g. The most common causes of IC are *C. albicans* and *C. parapsilosis* species.[40]

The risk factors for systemic infection include VLBW (< 1500 g), indwelling catheters, endotracheal tubes, broad-spectrum antibiotic therapy (most specifically with third-generation cephalosporins), steroid administration, and parenteral nutrition with intravenous lipid emulsions.[41] Infection rates differ across medical centers owing to differences in the aforementioned modifiable risk factors. Mortality is estimated at approximately 20% to 30% across all affected neonates, with even greater rates of accompanying morbidity in survivors.[41,42]

Clinical Findings

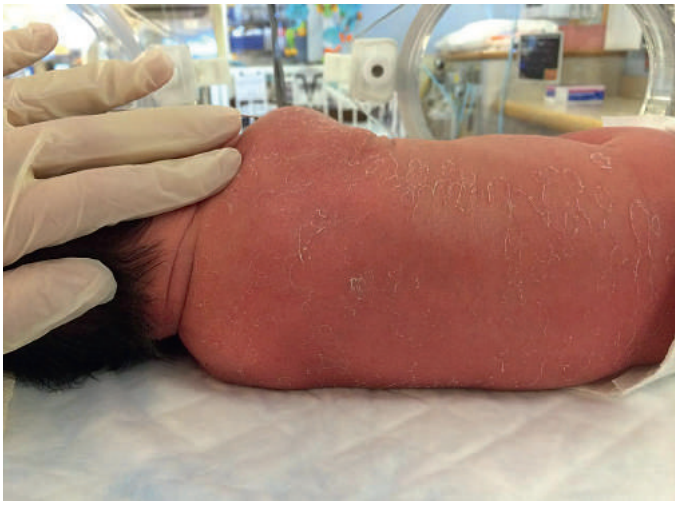
Skin manifestations include erosive, burn-like dermatitis followed by desquamation, scaly and erythematous patches, papules, pustules, and abscesses (Fig. 93.4). Intertriginous accentuation and involvement of the oral mucosa is often appreciated.[38] Reports of perioral or diffuse hyperpigmentation in the context of disseminated *Candida* spp. infections have also been reported.[43] Systemic involvement occurs via hematogenous spread from gastrointestinal and cutaneous reservoirs, most frequently involving the kidney, central nervous system (CNS), and eyes. The spleen, liver, heart, bones, and joints may also be affected. Respiratory distress, temperature instability, apnea, bradycardia, abdominal distension, and hypotension are suggestive of IC and should prompt a thorough evaluation. Supportive laboratory features may include an elevated white blood cell count and thrombocytopenia, as well as persistent hyperglycemia and glycosuria.[40]

Diagnosis

The differential diagnosis of IC with cutaneous involvement is similar to that for CCC and includes neonatal vesiculopustular eruptions that range from benign, self-limited cutaneous processes to rapidly progressive, life-threatening diseases (see Box 93.1). Early and correct diagnosis is essential. Organisms from skin are usually demonstrable on potassium hydroxide or calcofluor white preparations and cultures of scrapings from involved skin.

• BOX 93.1 Causes of Vesicopustular Eruptions in the Newborn

- Transient neonatal pustular melanosis
- Erythema toxicum neonatorum
- Staphylococcal impetigo
- Congenital candidiasis
- Herpes virus infections
- *Listeria*
- Syphilis
- Langerhans cell histiocytosis



• Fig. 93.4 Congenital Candidiasis. "Sunburn-like" erythema with accompanying superficial desquamation on the back of a neonate.

The diagnosis of disseminated candidiasis can be expedited by a positive touch preparation of a punch biopsy specimen of skin. Using this technique, the practitioner firmly imprints the dermal side of the specimen on a microscope slide and then assesses it for yeast after potassium hydroxide preparation or Gram staining. Histologic examination of specimens from the placenta and umbilical cord prepared with the appropriate stains may also be supportive if fungal elements are demonstrated.

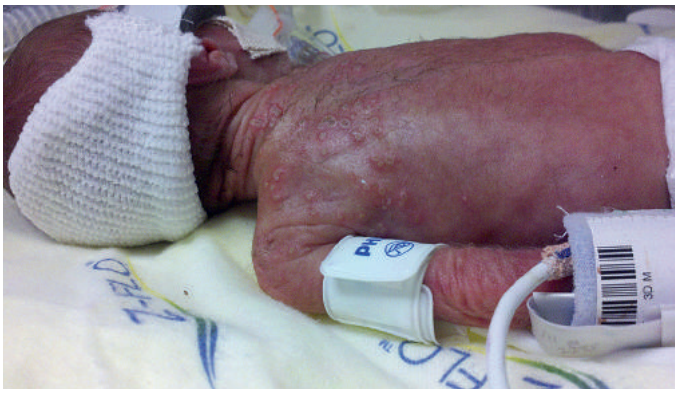
Disseminated disease can be difficult to diagnose, as culture of the organism from blood or CSF is estimated to have a sensitivity of 50% or less, and fungal antigen detection systems appear to have low sensitivity (and have also not been tested in neonates).[40,42] Although urine culture often leads to false-positive results, such findings are strongly associated with systemic disease in infants at risk and should be interpreted in the clinical context. In addition to blood, urine, and CSF cultures, ophthalmologic examination, chest x-ray for pulmonary involvement, echocardiogram, and head and abdominal ultrasonography should be considered.

Prognosis and Treatment

Systemic infection with *C. albicans* in premature infants is a serious infection with high morbidity and mortality. Sequelae of IC include neurodevelopmental impairment, deafness, retinopathy of prematurity, chronic lung disease, and renal failure.[40,44] Minimizing the aforementioned risk factors for IC and instituting meticulous hand hygiene are considered effective means of prevention. Probiotic supplementation has also been posited to provide protection against IC.[45] Empiric first-line treatment should be initiated while diagnostic results are awaited in patients for whom suspicion of IC is high, as this results in improved clinical outcomes. Among the various antifungals used to treat IC, fluconazole and amphotericin B are the agents of choice (including liposomal amphotericin B if urinary tract involvement is excluded).[42,46] Echinocandins such as caspofungin are less favored but may also be considered, although they are inappropriate for cases with ophthalmologic involvement as they are unable to penetrate the vitreous. Fluconazole prophylaxis has been recommended for neonates weighing less than 1000 g cared for in NICUs with high rates of IC (≥ 15%). However, a randomized controlled trial evaluating the effects of fluconazole prophylaxis in patients weighing less than 750 g in NICUs with lower rates of IC (including most of those in the United States and European Union) showed no reduction in the rates of IC or death, suggesting that universal administration of fluconazole prophylaxis in this population may not be appropriate.[47] Therapy is recommended for 21 days after microbiologic clearance as documented by culture and imaging.

Primary Cutaneous Aspergillosis

Primary cutaneous aspergillosis (PCA) is an increasingly common cutaneous infection in neonates, particularly the premature. This increased incidence is due in part to improved survival of extremely premature infants, as well as increased exposure to fungal spores made airborne by construction in and around hospitals.[48] PCA is thought to occur primarily via traumatic insult, with subsequent inoculation through intravenous line placement or use of adhesive tape, elastic dressings, armboards, cyanoacrylate adhesives or contaminated gauze.[49] Contributing factors also include an immature skin barrier and immune system, frequent use of systemic corticosteroids in NICU patients, and administration of broad-spectrum antibiotics.[50,51]



• **Fig. 93.5** Primary Cutaneous Aspergillosis. Erythematous plaques and broad pustules, many with dusky centers, on the back of a premature neonate.

Clinical Findings

PCA typically presents as an erythematous papule, patch, or plaque that with time can become pustular and may eventuate in cutaneous ulceration with crusting and, at times, purpura (Fig. 93.5). The differential diagnosis for PCA includes other deep fungal infections, as well as cutaneous *Pseudomonas* infection or inflammatory conditions, including cutaneous polyarteritis nodosa or pyoderma gangrenosum.[50]

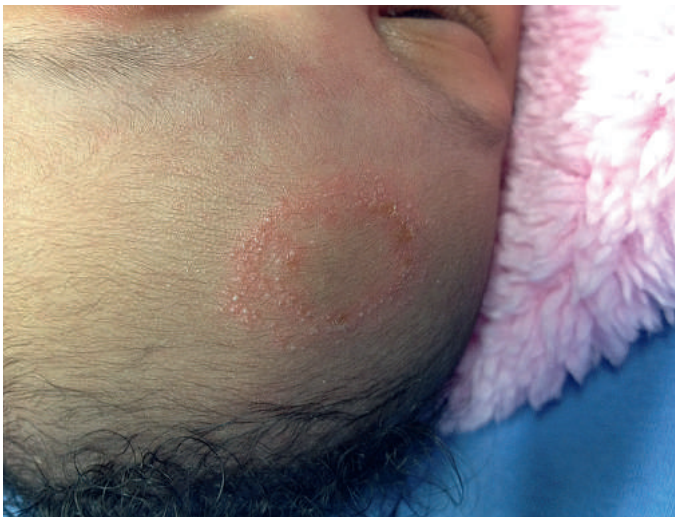
Diagnosis and Treatment

A diagnosis of PCA is made via skin biopsy for histologic analysis and tissue culture. Systemic involvement must also be excluded, particularly when lesions are multifocal. Specific evaluation, including blood culture, chest x-ray, echocardiogram, funduscopic examination, lumbar puncture, and abdominal ultrasonography, should be considered when one is evaluating a patient with known or suspected PCA.

The treatment of choice for PCA is amphotericin B, with consideration of use of its liposomal formulation. Voriconazole may be used as an alternative. For single lesions, surgical debridement should be considered to prevent systemic dissemination.[50,51]

Tinea Capitis and Tinea Corporis

Although uncommon in neonates, dermatophytoses, such as tinea corporis (involving the body) and tinea capitis (involving the scalp), are important infections to recognize as they can be clinically mistaken for more serious disorders. Most commonly caused by superficial invasion of the epidermis by *Trichophyton* and *Microsporum* species, dermatophytoses are thought to occur in infants in part because of epidermal and immune immaturity. Other predisposing factors in hospitalized infants (particularly in the NICU setting) include moist, humid environments, administration of broad-spectrum antibiotics, and frequent application of tapes and instrumentation.[52]



• **Fig. 93.6** Tinea Corporis. Annular erythema with central clearing on the forehead of an infant. Note the presence of erythematous papules and superficial scale on the periphery of the lesion.

Clinical Findings

Clinically, neonatal tinea capitis presents with a variable combination of alopecia, erythema, and scale. An annular morphology may be appreciated, and lymphadenopathy may also be present. It may or may not be accompanied by tinea corporis (dermatophytosis of the body), which classically appears as an annular patch or thin plaque with central clearing and peripheral erythema and scale (Fig. 93.6).[53] Other more atypical presentations reported include pustules, nodules, and vesicles.[54] As such, the differential diagnosis for neonatal tinea includes neonatal lupus, Langerhans cell histiocytosis, impetigo, HSV infection, and benign diagnoses, including seborrheic dermatitis.

Diagnosis and Treatment

Consideration of dermatophyte infection in the neonate is important as recognition of this infection can prevent costly unnecessary procedures and evaluations to exclude more serious diagnoses, including neonatal lupus. Potassium hydroxide preparations are a useful bedside diagnostic tool to quickly diagnose tinea corporis or tinea capitis, while dermatophyte culture and skin biopsy may also be considered in more atypical presentations.

Treatment consists of either topical or oral antifungal therapy. Topical therapy two or three times daily with agents, including azole antifungals or terbinafine, can help clear even scalp infections in neonates and is an appropriate first treatment choice as the risk of systemic absorption and potential side effects is low.

For more information regarding the diagnosis, prevention, and management of fungal infections in neonates, see Chapter 36, Fungal Infections in the NICU.

Herpes Simplex Virus Infection

HSV exists as two viral types: HSV-1 and HSV-2. Historically, HSV-2 was associated with genital eruptions, while HSV-1 was associated with orolabial infections, although the incidence of HSV-1-associated genital herpes appears to be increasing.[55] Neonatal infection can be associated with either serotype. Despite a high prevalence of HSV infections in adults, neonatal herpes is relatively uncommon. Nevertheless, early recognition and prompt initiation of therapy for neonatal herpes are critical as the consequences of delaying therapy can be devastating. While approximately 5% of cases of HSV infection in the neonatal period are due to in utero exposure, with another 10% occurring after birth, most occur in the perinatal period.

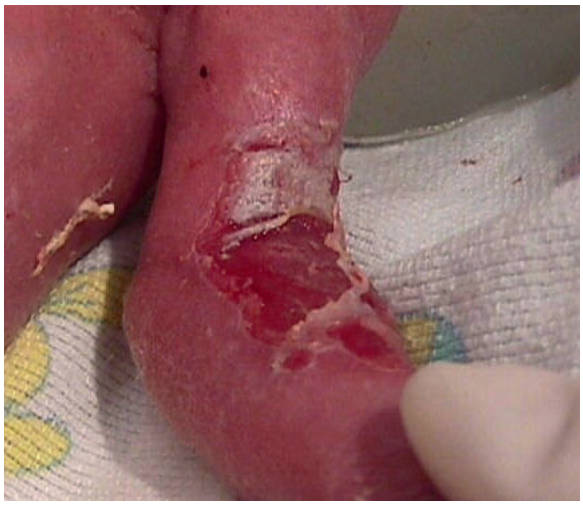
Clinical Findings

Neonatal HSV infection is classified on the basis of the extent of involvement: skin, eye, and/or mouth (SEM) disease without visceral or CNS involvement; CNS disease; or disseminated disease. Infection typically presents within the first week to two weeks after birth, with a slightly later onset of CNS disease at approximately 16 to 17 days. Neonatal HSV infection may present with temperature instability, lethargy, irritability, or poor oral intake or a sepsis-like syndrome that may include new-onset seizures, pneumonia, and disseminated intravascular coagulation. Approximately 60% to 80% of afflicted neonates exhibit characteristic skin lesions or develop them during the course of the disease. Nevertheless, there remains a significant proportion of patients who never develop skin vesicles throughout their disease course.[55,56]

Approximately 45% of infected infants exhibit SEM disease, often presenting at about day 10 to 12 of life.[57] Characteristic cutaneous lesions begin as erythema that quickly evolves into isolated or grouped vesicles on an erythematous base. Continued evolution may result in pustules, crusts, or erosions. Vesiculation typically occurs at sites of trauma, including the presenting part and sites of fetal electrode placement.[58] Conjunctivitis is relatively common in SEM disease, although oral involvement remains rare. With early institution of therapy, infants with SEM disease have an excellent prognosis, with low rates of morbidity and a mortality rate of zero. Nevertheless, if infection is left untreated, it will progress to disseminated or CNS disease in three-quarters of all cases.[58] Additionally, infants with SEM disease and three or more recurrences of cutaneous vesicles in the first 6 months of life appear to be at greater risk of neurologic impairment.[59]

In contrast, approximately 30% of infected infants have CNS disease, with an increased associated mortality and risk of neurodevelopmental abnormalities. Up to 70% of infants with CNS disease will have cutaneous involvement during the course of their infection.[57] Prematurity and the presence of seizures at onset of therapy appear to portend a worse prognosis in infants with CNS disease, even with therapy (acyclovir era). The remaining 25% of infants have evidence of disseminated disease (sepsis, liver failure, encephalitis, disseminated intravascular coagulation, respiratory distress). Forty percent of patients in this group do not manifest any cutaneous findings.[60] The prognosis of disseminated disease is worse than for other forms of neonatal HSV infection; treatment with high-dose acyclovir has improved outcomes compared with historical measures, but mortality associated with disseminated disease is still estimated at approximately 30%. Almost 20% of survivors have neurodevelopmental delays.

Infants infected in utero (congenital HSV infection) have a distinct clinical presentation (Fig. 93.7). Skin lesions are almost always present at birth and include widespread erosions and bullae, scars, hyperpigmentation, hypopigmentation, and aplasia cutis. Other frequent findings include microcephaly, hydranencephaly, intracranial calcification, chorioretinitis, optic atrophy, and microphthalmia.[55,58]



• **Fig. 93.7** Congenital Herpes Simplex Virus Infection. Broad ulceration with peripheral vesiculation and desquamation on the left arm of a neonate. (Photo courtesy of Albert Yan, MD, The Children's Hospital of Philadelphia.)

Etiology

Most cases of neonatal herpes simplex are the result of vertical transmission in the perinatal period. Most cases of neonatal HSV infection in developed countries are secondary to HSV-1 infection, reflecting the increased prevalence of HSV-1 associated with genital herpes. The usual route of infection is via intrapartum contact with genital mucosa during a phase of active viral shedding, which may be symptomatic or asymptomatic. Nevertheless, physicians should also be aware of uncommon means of HSV transmission associated with certain ethnic or religious groups. For instance, postnatal transmission of HSV has been reported in ultra-Orthodox Jewish populations in which orogenital suctioning of blood is performed following ritual circumcision.[61]

Epidemiology

In developed countries, the incidence of neonatal HSV infection ranges from 1.6 to 33 per 100,000 live births.[60] When compared with the risk of genital herpes reactivation, the risk of neonatal herpes is greater with maternal first episode primary or first episode nonprimary infection (the latter category reflects acquisition of an HSV serotype in individuals with preexisting protective antibodies versus the other serotype).[62] Unfortunately, it has been estimated that up to 80% of mothers who transmit HSV to their infants have no known history of genital herpes.[55] Vaginal delivery, prolonged rupture of membranes, maternal infection with HSV-1, and use of instrumentation during delivery (i.e., fetal scalp electrodes) are additional risk factors for acquisition of HSV in the neonate.

Diagnosis

A high index of suspicion is required, particularly in cases without cutaneous manifestations. When characteristic herpetic lesions are present, the differential diagnosis includes other causes of vesicopustular eruptions (see Box 93.1). A Tzanck smear test or direct fluorescent antibody detection using skin scrapings from the base of a fresh vesicle is a relatively rapid means of detecting cutaneous HSV infection, but the diagnostic yield of both is variable, largely influenced by the age, quality, and handling of the specimen. Viral culture and polymerase chain reaction (PCR) for HSV DNA are the preferred methods for diagnosing neonatal HSV infection; the sensitivity of PCR in identifying HSV DNA in CSF samples is 75% to 100%.[60] Obtaining specimens from the CSF, oropharynx, anus, conjunctivae, and nasopharynx is recommended; evaluation of peripheral blood via PCR is also useful in establishing a diagnosis of neonatal herpes, although it does not assist in identifying the extent of disease. Notably, HSV serologic tests are not a useful diagnostic adjunct. If they are elevated, serum alanine aminotransferase levels may indicate disseminated disease.

Treatment

Strategies to decrease the risk of vertical transmission of HSV include cesarean delivery, maternal prophylactic antiviral therapy, and limiting use of invasive monitoring techniques in mothers shedding HSV at the time of delivery.[62] Specifically, birth via cesarean delivery for women with active lesions or prodromal symptoms has been shown to reduce the risk of neonatal HSV infection.[63] Maternal suppressive therapy with acyclovir is known to reduce the frequency of both genital lesions near term and cesarean delivery, but whether this reduces acquisition of HSV by the neonate remains unclear.

Early treatment with antiviral agents is critical to decrease the risk of serious complications and death. All cases of presumptive neonatal HSV infection should be treated with intravenous acyclovir therapy, with a recommended dosage of 60 mg/kg/day administered every 8 hours at 20 mg/kg/dose.[64] A notable side effect of this therapy, however, is transient neutropenia that warrants ongoing monitoring. Nephrotoxicity has also been documented in patients treated with acyclovir, although this appears less common in patients with normal renal function whose fluid status is closely monitored. Treatment is recommended for 14 days for SEM disease and 21 days for disseminated and CNS disease. In cases of CNS involvement, repeated evaluation of CSF for HSV infection via PCR should be performed at the conclusion of therapy, and, if the findings are positive, it should be continued until PCR evaluations return negative.[57]

For more information on diagnosing and managing viral infections in neonates, please refer to Chapter 34.

Acknowledgments

We would like to thank Dakara Rucker Wright and Bernard A. Cohen for their contributions to previous editions of this chapter.

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94

Common Newborn Dermatoses

KATE KHORSAND AND ROBERT SIDBURY

KEY POINTS

- Papulopustular lesions on the palms and/or soles in the first month of life likely represent a self-limited inflammatory process such as eosinophilic pustular folliculitis or infantile acropustulosis—but scabies should be ruled out.
- Neonatal acne (a.k.a. *cephalic pustulosis*) is typically multifactorial and self-limited. Treatment is not necessary, but topical agents aimed at reduction of the commensal yeast *Malassezia* can be beneficial.
- Evidence suggests that early use of emollients in infants at risk of developing atopic dermatitis may prevent later disease.
- Subcutaneous fat necrosis of the newborn is generally benign and self-limited but when extensive can cause hypercalcemia.

This chapter describes a group of cutaneous disorders commonly found in neonates. Most of these disorders are benign and are either self-limited or treated with ease. Recognition of these common neonatal dermatoses is important so as to distinguish them from more serious conditions, sparing patients unnecessary work-up and treatment. Each section includes common clinical features and presentation, notes on establishing the diagnosis (with a short differential diagnosis), the cause if known, and a brief discussion of basic treatment and prognosis.

Erythema Toxicum Neonatorum

Clinical Findings

Erythema toxicum neonatorum (ETN) is a benign inflammatory condition affecting newborns. Estimates of incidence range from 7% to 41%, and it is seen more commonly in white newborns with a higher birth weight and greater gestational age.[1] The condition presents in the first 1 to 3 days after birth as irregularly shaped erythematous macules that can develop overlying vesicles or pustules (Fig. 94.1). ETN most commonly affects the trunk but can involve the face and extremities as well. The palms and soles are typically spared. Affected infants are otherwise healthy. The lesions persist for 1 to 2 weeks and then spontaneously resolve without sequelae.

Diagnosis

The diagnosis is often clinical but can be further supported by the scraping of a pustule, smearing the contents onto a slide, and staining with Wright or Giemsa stain.[2] This will reveal a large number of eosinophils. The differential diagnosis of ETN includes other benign entities such as transient neonatal pustular melanosis, miliaria, infantile acropustulosis, and eosinophilic pustular folliculitis. Additionally, infections such as folliculitis, candidiasis, impetigo, and herpes should be considered and ruled out. If a biopsy sample is obtained, histopathologic features include intraepidermal vesicles filled with eosinophils and a mixed intradermal inflammatory infiltrate localizing around the superficial pilosebaceous follicle.

Etiology

The cause of ETN remains unclear. Studies have shown the presence of inflammatory mediators, as well as increased eosinophil and macrophage activity[3,4] in affected skin compared with unaffected skin. This has led to the theory that ETN could represent a cutaneous immunologic response to microbial colonization of the hair follicles after birth.[4]

Treatment and Prognosis

No treatment is required for this benign condition. It is usually asymptomatic. Parents can be reassured and informed that the condition will spontaneously resolve in a matter of weeks. Prolonged courses or recurrence is rare.

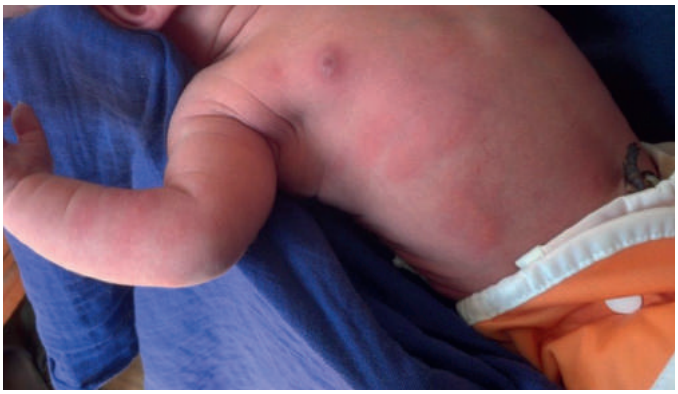
Transient Neonatal Pustular Melanosis

Clinical Findings

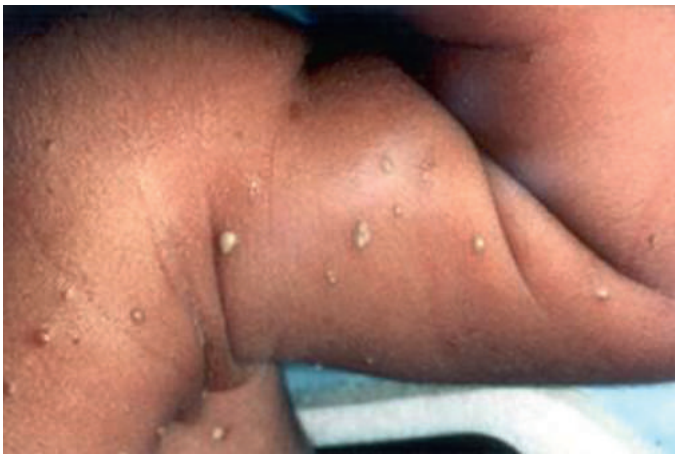
Transient neonatal pustular melanosis is a benign, self-limited condition that presents at birth in affected infants. It is more common in African-American infants.[5] There are three clinical phases of transient neonatal pustular melanosis. At birth, the neonate has very fragile and superficial 2- to 10-mm pustules located most commonly on the forehead, under the chin, on the lower back, and on the shins. These pustules are often wiped off during the initial cleaning after birth. Next, a fine collarette of scale is noted around the resolving pustule. Last, hyperpigmented brown macules develop at the site of the previous pustules (Fig. 94.2).[2] The first two phases usually last 1 to 2 weeks, but hyperpigmentation can persist for several months.[6]

Diagnosis

Diagnosis is clinical, but it can be supported by a scraping from the pustule, with Wright staining of the contents. This will reveal a predominance of neutrophils. The differential diagnosis



• **Fig. 94.1** Erythema Toxicum Neonatorum. Pinpoint pustules overlying ill-defined erythema on the trunk and extremity of a healthy newborn consistent with erythema toxicum neonatorum.



• **Fig. 94.2** Transient Neonatal Pustular Melanosis. Intact discrete pustules on non-inflamed base, some ruptured, with residual hyperpigmentation.

includes ETN, and these conditions can overlap. Additional considerations include miliaria, acropustulosis of infancy, and infectious diseases such as candidiasis, impetigo, and herpes, which should be ruled out.

Etiology

The underlying cause of transient neonatal pustular melanosis is unknown. Studies linking it with ETN suggest a similar cause.

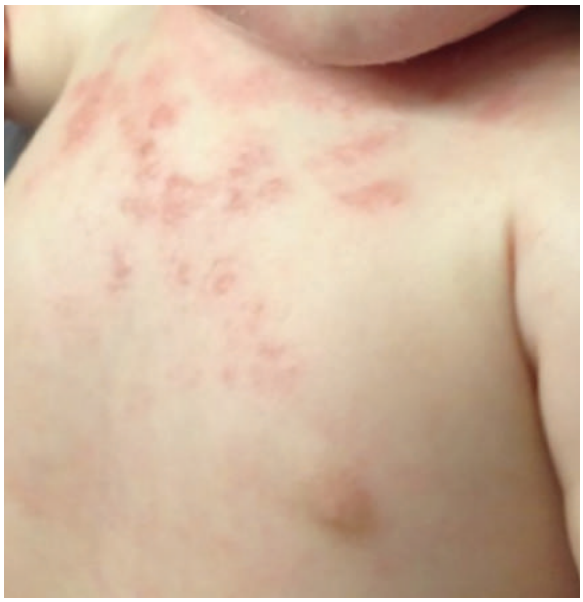
Treatment and Prognosis

No treatment is required for this benign and self-limited condition. Parents should be informed that while the initial skin findings resolve quickly, the residual hyperpigmentation may take months to completely resolve.

Eosinophilic Pustular Folliculitis

Clinical Findings

Eosinophilic pustular folliculitis (EPF) is a vesiculopustular eruption that can occur in several distinct settings. The infantile form



• **Fig. 94.3** Eosinophilic Pustular Folliculitis. Grouped folliculocentric eosinophil-rich erythematous papules and pustules on the chest.

is relatively rare and presents with pruritic follicular lesions typically on the extremities or scalp, although truncal involvement is described (Fig. 94.3). Crops of lesions will occur discretely, not in an annular array as in adults, and persist for days to weeks before resolving without sequelae. EPF tends to present somewhat later than erythema toxicum but has been described on the first day after birth.[7]

Diagnosis

Diagnosis is clinical, and persistence past several weeks generally distinguishes EPF from erythema toxicum, although peripheral eosinophilia may be supportive. Histologic examination demonstrates an eosinophilic follicular infiltrate.

Etiology

The cause is unknown.

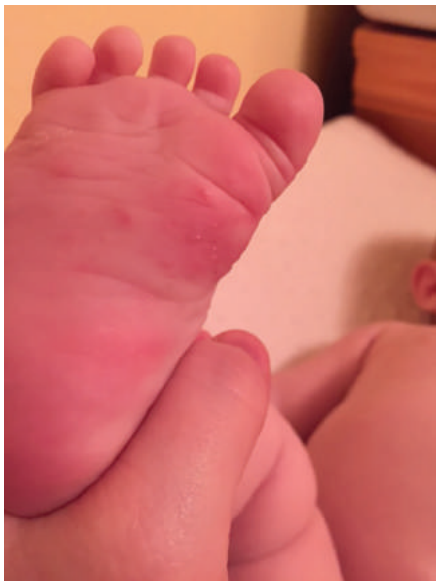
Treatment and Prognosis

Treatment is symptomatic and can include emollients, topical corticosteroids, and antihistamines. Topical indomethacin therapy has been used in refractory cases.[8]

Acropustulosis of Infancy

Clinical Findings

Acropustulosis of infancy is a relatively uncommon pruritic, vesiculopustular rash that occurs on the hands and feet with spread to the wrists and calves. First described in 1979, this rash is closely related to preceding infections and infestations, with scabies preceding approximately 50% of cases.[9,10] The eruption is most common in African-American, Hispanic, and Asian children and presents at less than 1 year of age. Crops of intensely pruritic vesicles and pustules appear on the hands and feet, persist for around 2 weeks, and then resolve (Fig. 94.4). Frequent recurrences are the



• **Fig. 94.4** Infantile Acropustulosis. Discrete, grouped erythematous papules and pustules on the sole of the foot.

norm, with each eruption decreasing in intensity until eventual complete resolution around 2 to 3 years of age.

Diagnosis

Diagnosis is primarily clinical, and a history of preceding scabies can be quite helpful in making the diagnosis. If a biopsy sample is obtained, it shows necrolysis of the keratinocytes with intraepidermal pustules filled with neutrophils and eosinophils.[11] The differential diagnosis includes scabies (concurrent infestation must be ruled out and treated if present), pustular psoriasis, coxsackievirus infection, eosinophilic folliculitis, and less likely, an allergic contact dermatitis.

Etiology

The cause of acropustulosis is unknown, but the condition is known to frequently follow prior scabies infestation or coxsackievirus infection.

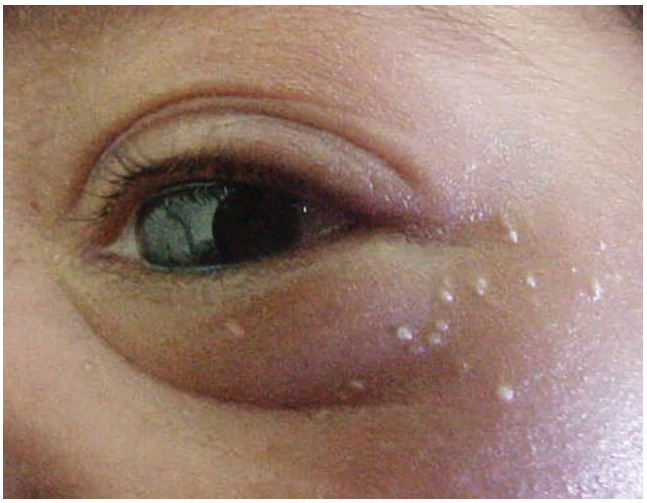
Treatment and Prognosis

The recurrent episodes of acropustulosis of infancy will eventually resolve around 2 to 3 years of age, but episodes can be managed. Ruling out scabies and treating it if it is present should be a first step.[12] Topical treatment with mid-potency to high-potency corticosteroids is effective for discrete episodes but will not help to prevent relapses.[10] Historically, acropustulosis of infancy was treated with orally administered dapsone, but this is no longer frequently used because of the risk of hemolysis and methemoglobinemia in patients with glucose 6-phosphate dehydrogenase deficiency.

Milia

Clinical Findings

Milia are an extremely common benign entity, seen at birth in 40% to 50% of newborns. They are more common with longer gestations and in infants born to multigravidas.[13] Milia (singular, *milium*) are tiny, white monomorphic papules with a smooth surface. They can number from several to dozens. They are an entity distinct from miliaria, which is discussed next. Milia can be present anywhere on the body but occur most commonly on the face, predominantly on the forehead, cheeks, and chin (Fig. 94.5).



• **Fig. 94.5** Milia. Numerous, discrete, pinpoint monomorphic white papules around the eye.

Diagnosis

A useful diagnostic tool is expression of the milia contents, which will resemble a tiny, white pearl. This is the keratinocyte debris that forms the interior of the cyst. The differential diagnosis includes sebaceous hyperplasia, which would also present on the face but tend to be more yellow, and miliaria, which presents primarily on the body and in greater numbers with distinct clinical variants.

Etiology

Milia are tiny inclusion cysts that form in the epidermis. The epidermal tissue invaginates, often around a vellus hair, and forms a cyst with a wall of keratin-producing cells.

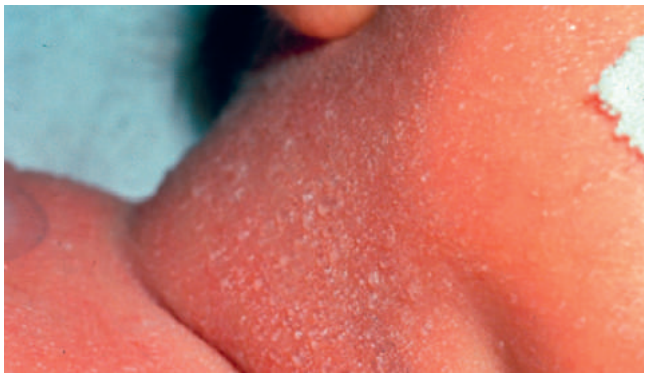
Treatment and Prognosis

Treatment of milia is not required. They tend to resolve spontaneously over several months. Of note, the presence of multiple large or persistent milia can rarely be associated with syndromes such as epidermolysis bullosa or orofaciodigital syndrome.

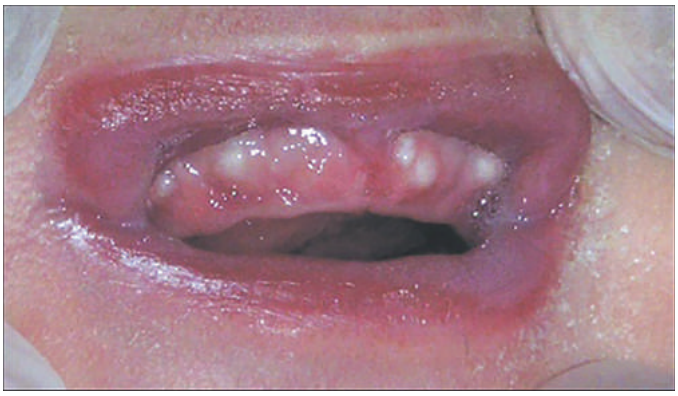
Miliaria

Clinical Findings

Miliaria is a benign rash due to obstruction of the eccrine duct occurring in the first 1 to 2 weeks of life, with rapid resolution within days. There are multiple variants depending on the location of the eccrine duct obstruction, with differing clinical presentations. Miliaria crystallina occurs when eccrine ducts within or below the stratum corneum are obstructed and causes small clear



• **Fig. 94.6** Miliaria Crystallina. Numerous, discrete, easily ruptured superficial vesicles. (From Antaya RJ, Robinson DM. Blisters and pustules in the newborn. *Pediatr Ann*. 2010;39:635-645.).



• **Fig. 94.7** Epstein's Pearls. Discrete, opaque yellow keratin-rich papulonodules along the alveolar ridge of the mouth.

vesicles that can be easily wiped away (Fig. 94.6). Miliaria rubra is caused by obstruction of the eccrine duct at the level of the epidermis and is thought to be related to overheating. This causes erythematous papules or papulopustules primarily on the head, neck, face, and trunk. Dermal inflammation contributes to the clinical presentation. Miliaria profunda is caused by occlusion of the eccrine duct at or below the dermal-epidermal junction and causes a mildly inflammatory papular eruption that is rare in newborns.

Diagnosis

The diagnosis of miliaria is made clinically, although scraping of a vesicle or pustule with subsequent Wright staining will reveal few to no cells in miliaria crystallina and lymphocytes in miliaria rubra. The differential diagnosis includes ETN, transient neonatal pustulosis, milia, eosinophilic folliculitis, and infectious entities. Recurrent episodes of pustular miliaria rubra could be a sign of the rare and potentially fatal condition type I pseudohypoaldosteronism, which is an inherited disorder of mineralocorticoid resistance leading to a salt-wasting crisis.

Etiology

Miliaria is caused by obstruction of the eccrine duct, possibly by sweat accumulation. Neonates can have incompletely canalized eccrine ducts, predisposing them to developing obstruction and subsequent miliaria.[14]

Treatment and Prognosis

No treatment is required for this benign condition, which will self-resolve. Prevention of overheating can reduce the incidence of miliaria rubra.

Epstein Pearls and Bohn Nodules

Clinical Findings

Epstein pearls and Bohn nodules are very common and benign cysts that form in the mouths of neonates. They present as single or clustered 1- to 2-mm smooth white to yellow papules. Epstein pearls are more often seen in infants born to multigravidas, after longer gestations, and with higher birth weights.[15] Bohn nodules are often mistaken for natal teeth, prompting referral.

Diagnosis

Epstein pearls are found on the palate, typically at the midline of the junction between the hard and soft palate. Bohn nodules are found on the alveolar ridge, most commonly in the maxillary region (Fig. 94.7). They are distinguishable from neonatal teeth in their location in addition to their appearance; neonatal teeth are usually located in the region of the lower incisors. Radiographic examination would distinguish between these diagnoses.[15]

Etiology

Both Epstein pearls and Bohn nodules are inclusion cysts. They are thought to originate from remnants of odontogenic epithelium or to potentially be remnants of salivary glands.[16]

Treatment and Prognosis

Spontaneous resolution will occur with time, typically within the first several weeks to months after birth. No treatment is required.

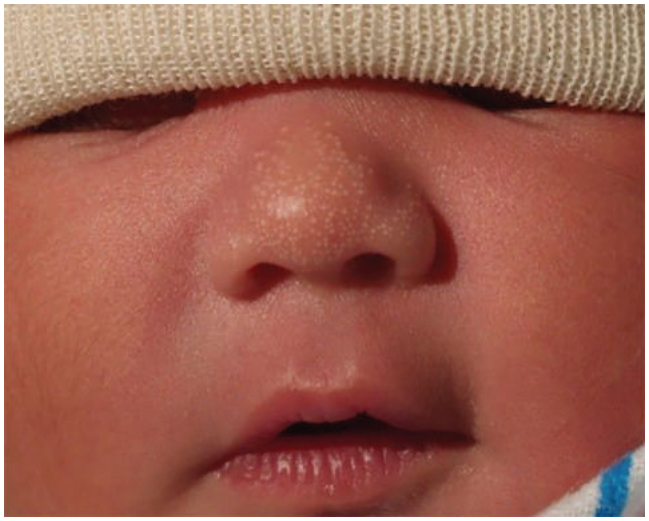
Sebaceous Hyperplasia

Clinical Findings

Sebaceous hyperplasia is a common finding, seen in approximately half of newborns,[17] and is typically most noticeable surrounding the nose and upper lip (Fig. 94.8). It presents as regular, smooth white to yellow papules that can be grouped around follicles.

Diagnosis

The differential diagnosis includes milia and neonatal acne (neonatal cephalic pustulosis).



• **Fig. 94.8** Sebaceous Hyperplasia. Pinpoint yellow macules and papules along the sebaceous triangle on the nose.

Etiology

Sebaceous hyperplasia in newborns is thought to occur secondary to maternal androgens, which stimulate the sebaceous glands in utero.[4,17]

Treatment and Prognosis

No treatment is required, and the findings will spontaneously resolve in the first few weeks of life.

Neonatal Cephalic Pustulosis (Neonatal Acne)

Clinical Findings

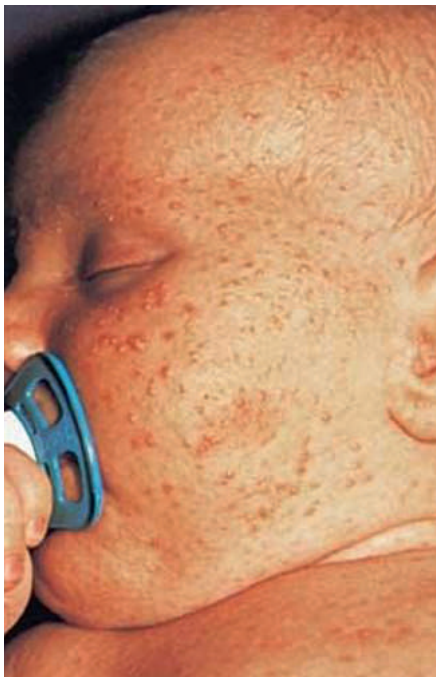
Neonatal cephalic pustulosis, which is also known by the more common term of *neonatal acne*, consists of erythematous papules and pustules on the cheeks, chin, and forehead of infants (Fig. 94.9). It occurs within the first 30 days after birth and can last up to several months. Comedones are absent. It is an entity distinct from infantile acne, which occurs at 3 to 6 months of age and can last for years.

Diagnosis

Diagnosis is made primarily on the clinical appearance, although Wright stain of a smear will reveal neutrophil predominance. The differential diagnosis includes miliaria, milia, ETN, or rarely, severe infectious processes such as candidiasis or folliculitis.

Etiology

The cause of neonatal cephalic pustulosis is debated but is thought to be related to skin colonization with *Malassezia* species. *Malassezia* colonization begins at birth and increases during the first few weeks after birth from approximately 5% of newborns colonized during the first week to 30% colonized by the second to fourth week.[18] This increased *Malassezia* colonization is thought to trigger an inflammatory reaction that some believe becomes neonatal cephalic pustulosis.[2,19] However, others suggest that the degree of skin colonization by *Malassezia* does not differ between affected and unaffected infants and that there is no correlation between the severity of disease and *Malassezia* isolation.[18] If, and why, *Malassezia* colonization causes an inflammatory reaction in some neonates but not in others is undetermined.



• **Fig. 94.9** Neonatal Cephalic Pustulosis (Neonatal Acne). Small monomorphic papules and pustules on the head and neck of a newborn consistent with neonatal cephalic pustulosis or neonatal acne. (From Sidbury R, Paller AS. The diagnosis and management of acne. *Pediatr Ann*. 2000;29:17–24.)

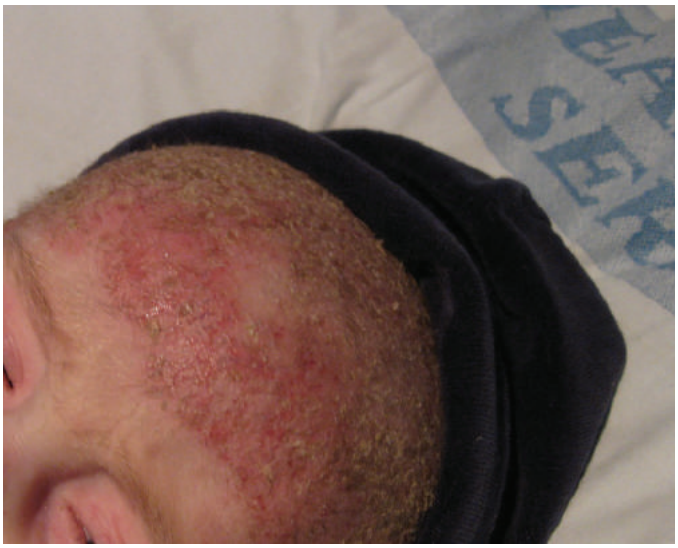
Treatment and Prognosis

No treatment is required for this benign self-limited condition. However, the lesions can take months to resolve. Topical treatment with imidazole antifungals, such as clotrimazole, econazole, or ketoconazole, may be effective at shortening the duration of the eruption.

Seborrheic Dermatitis

Clinical Findings

In newborns, seborrheic dermatitis (SD) is an extremely common finding and presents as irregular salmon pink patches with waxy scaling on the scalp, forehead, and nasolabial folds (Fig. 94.10). It can also involve the diaper area. Appearing around 2 to 4 weeks of age, this condition usually resolves or abates by 1 year of age. Severe cases can become secondarily infected. A strong association between atopic dermatitis (AD) and infantile SD has been postulated, suggesting that infantile SD may precede the development of AD or that these conditions may be on the same spectrum of disease.[20]



• **Fig. 94.10** Seborrheic Dermatitis. Waxy yellow loosely adherent scaly plaques on an erythematous base on the forehead and scalp.

Diagnosis

Diagnosis is clinical, and the differential diagnosis includes AD, psoriasis, Langerhans cell histiocytosis, or another superficial fungal infection. AD may overlap with SD, as discussed previously, and psoriasis does not typically present this early. Langerhans cell histiocytosis is a more serious, but fortunately rare, condition.

Etiology

In adults, SD is related to *Malassezia* overgrowth, but it is unclear if this is the pathogenesis behind the disorder in infants, although that has been suggested by several historical studies.[21,22]

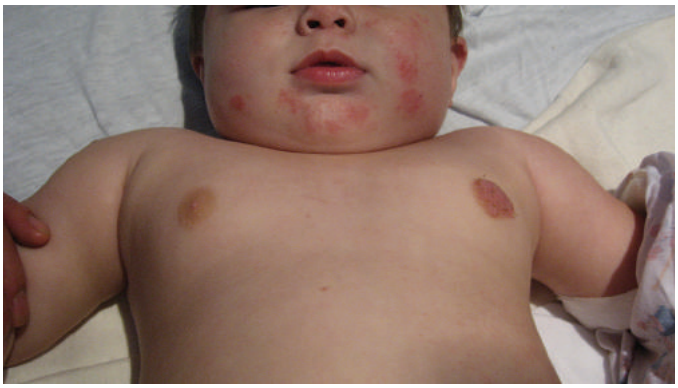
Treatment and Prognosis

This condition often resolves spontaneously or abates by 1 year of age. Both topical antifungals and topical steroids have been used to treat this condition. Ketoconazole was shown to be equally as efficacious at treating SD as steroid creams such as hydrocortisone,[23] and therefore this could be a viable treatment option. Treatment of the scalp with olive oil should be avoided, as this medium can encourage yeast growth.[24]

Atopic Dermatitis

Clinical Findings

AD is an extensive topic, spanning all ages of childhood into adulthood. This section will focus on infantile AD, as there are multiple informational sources for this common and important condition as it pertains to later childhood. AD, commonly known as *eczema*, is a chronic, relapsing condition defined by distinctive cutaneous manifestations and associations with additional atopic conditions such as asthma and allergic rhinitis. It is extremely common, affecting 15% to 20% of the population, particularly in developed countries. In more than half of cases, the onset of AD is in infancy.[25] In infants the most common areas of involvement include the cheeks (often seen concurrently with an irritant dermatitis secondary to saliva), neck, flexural folds, wrists, ankles,



• **Fig. 94.11** Atopic Dermatitis. Ill defined erythematous papules coalescing into plaques on the cheeks with characteristic nipple inflammation.

and notably the nipples (Fig. 94.11). In infantile eczema, involvement of the extensor surfaces (skin on the opposite side of a joint) and trunk is seen more commonly than flexural involvement. Erythema, scaling, lichenification, and excoriation are common clinical findings. Itching is the predominant symptom. The diaper area and nasal tip are notably spared. Superinfection can be common. There is significant overlap with SD, as discussed previously. As infants age, the "typical" presentation of AD often evolves with prominent involvement of the antecubital and popliteal fossae with associated diffusely dry skin.

Diagnosis

Diagnosis is made clinically, and biopsy is rarely performed. The differential diagnosis of AD is broad and includes common entities such as SD, contact dermatitis, and irritant dermatitis. More rarely, severe AD can be associated with recurrent infections of various forms in multiple rare immunologic disorders, including Wiskott-Aldrich syndrome, chronic granulomatous disease, hyper-immunoglobulin E (IgE) syndrome, and severe combined immunodeficiency. Severe recalcitrant eczema with other unusual infections should raise awareness of these serious conditions particularly in the setting of failure to thrive. If lesions appear superinfected with crusting, bacterial culture should be performed.

Etiology

The exact cause of AD remains elusive. At its core, AD appears to be a condition of defective skin barrier function and immune alteration. In AD, there is a prominence of the T-helper2–T-cell subsets, which produce interleukin-4 and promote production of IgE, eosinophilia, mast cell proliferation, and release of histamine. Loss of filaggrin mutations has been linked to individuals with early-onset and persistent AD.[26,27] Filaggrin acts in the top layer of the skin and maintains skin barrier function. When the skin's barrier function is impaired, it is increasingly susceptible to exogenous triggers. Nearly all patients with AD carry *Staphylococcus aureus* on their skin, and staphylococcal exotoxins can act as superantigens that stimulate T-cell responses.[28] The impact of food and food allergy, a common question from parents, is only relevant in a minority of patients. Food allergies are more common in atopic individuals, and in some cases of AD recalcitrant to treatment, food allergies may play a role in the persistence of the disease. A Cochrane review stated that maternal dietary antigen avoidance during pregnancy had no impact on the development of AD in

the first 18 months of life and could increase the risk of preterm birth or decreased birth weight.[29] Breastfeeding during the first 4 months of life has been shown to reduce the incidence and severity of AD in high-risk infants,[30] although prolonged breastfeeding has not impacted its incidence.[31]

Treatment and Prognosis

There is no cure for eczema; therefore, the goal of treatment is to manage signs and symptoms and reduce recurrences. Spontaneous resolution often occurs as children age. Maintenance therapies include gentle bathing techniques such as limiting a gentle soap to soiled areas and avoidance of long, hot bathing. Diligent moisturization at least twice daily and always after bathing with a thick, bland emollient is important to restore cutaneous moisturization and maintain barrier function. Treatment of active rash with appropriate strength topical corticosteroids, rarely stronger than class 6 or 7 (e.g., hydrocortisone 2.5% or 1%, respectively) is first-line treatment for symptomatic AD refractory to emollients and good skin care. Second-line agents include topical calcineurin inhibitors such as tacrolimus or pimecrolimus, but these agents are not approved for use in children younger than 2 years. Adjunctive topical or systemic antibiotics address concurrent skin infection when present, and dilute bleach baths may be preventive.[32] Treating inflammation as described earlier is the best way to reduce itch, but when there is sleep disruption, a sedating antihistamine such as diphenhydramine or hydroxyzine may help, if it is age appropriate.

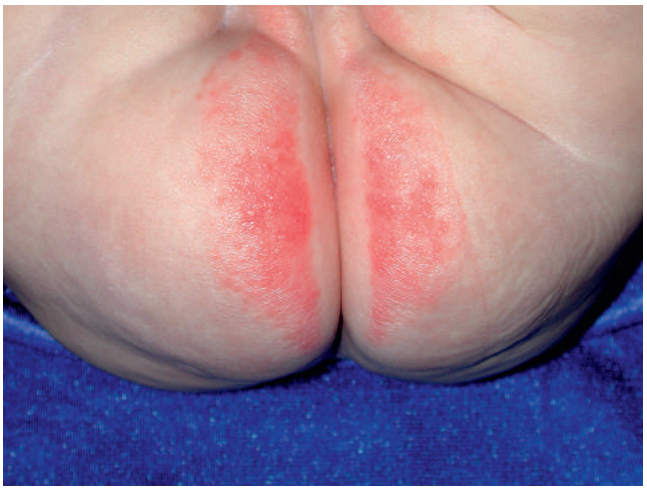
Diaper Dermatitis

Clinical Findings

Diaper dermatitis (DD) is likely the most common skin condition of infants. The vast majority of affected infants are otherwise healthy, but occasionally DD can suggest an underlying systemic disorder. DD is primarily an irritant process, occurring when the skin in contact with the diaper becomes inflamed because of friction and contact with urine, feces, cleansing materials, and other irritants. The clinical presentation includes erythema, with mild scaling of the gluteal crease, buttocks, and convex surfaces of the pubic area and perianal rim (Fig. 94.12). There is often relative sparing of the skinfolds, which are protected from direct contact with the diaper. Erosions or plaques can be present in more severe cases, and discrete ulcerations are seen in a severe form of DD known as *Jacquet dermatitis*. This distinctive noduloerosive presentation is seen more frequently in association with cloth diaper use.

Diagnosis

DD is a clinical diagnosis, and as such, histopathology is usually not helpful. Other infectious and noninfectious causes of rashes in the diaper area should be excluded. Infectious causes of diaper rashes include congenital syphilis and infections caused by *Candida* or other fungi, *Streptococcus*, *Staphylococcus* (including staphylococcal scalded skin syndrome), herpes simplex virus, and human immunodeficiency virus. These infectious diaper area eruptions are usually clinically distinct. *Candida* infections present with bright red, relatively well-demarcated patches and plaques with satellite lesions in the groin region and perianal rim.



• **Fig. 94.12** Irritant Contact Dermatitis. Ill-defined confluent erythematous plaques symmetrically along the convexity of the buttocks.

Staphylococcal infection presents most commonly in the diaper area as bullous impetigo with scattered bullae and vesicles. Streptococcal infections present as a brightly erythematous perianal patch. Skin findings caused by infections are often found in areas additional to the diaper region. Other considerations include infantile SD, allergic contact dermatitis, nutritional deficiencies (such as acrodermatitis enteropathica in zinc deficiency), Langerhans cell histiocytosis, and rare metabolic disorders. Similar findings will often be identified in other nondiapered skin areas, which can be a distinguishing feature.

Etiology

DD is exceedingly common, with most infants experiencing one or more episodes. Multiple factors contribute, including occlusion friction, and maceration. When the barrier has been compromised by these factors, chemical or biologic irritants in urine and feces continue to drive the dermatitis. Further irritants such as soaps, diaper wipes, and numerous topical products perpetuate the problem.

Treatment and Prognosis

Prevention of DD starts with maintenance of skin barrier function and prevention of irritation. The use of disposable diapers, particularly the superabsorbent variety, has been shown to be better at preventing DD than the use of cloth diapers.[33] Avoiding prolonged skin contact with soiled diapers is an important aspect of basic infant care. Potential irritants or sensitizers should be identified and removed. Commercial diaper wipes are a common culprit. Barrier pastes such as zinc oxide ointment and zinc paste as well as petroleum jelly are bland skin protectants. Thick layers of these protective agents should be applied, and with diaper changes, only a partial layer should be removed to protect the underlying skin. Mineral oil provides a way to remove zinc preparations without rubbing the skin excessively. In the absence of *Candida* infection, a low-potency topical steroid can be helpful. If *Candida* infection is suspected, an anti-*Candida* agent can be added. Daily bathing is acceptable, but harsh soaps and scrubbing should be avoided. DD is self-limited and episodic.

Harlequin Color Change

Clinical Findings

Harlequin color change is a rare but benign event. It was first reported in 1952. It represents an abrupt deep vascular red color change to one side of the body, with contralateral blanching and sharp midline demarcation (Fig. 94.13). It can involve the entire body or can be more localized to a single body area. It occurs most commonly in the newborn and is more often seen in premature infants.

Diagnosis

Diagnosis is by clinical appearance. Rapid resolution is the norm.

Etiology

Harlequin color change is thought to be due to the immaturity of the hypothalamic control center in newborns, which causes asymmetric control of the sympathetic peripheral vascular tone, resulting in skin color change with a sharp midline demarcation.

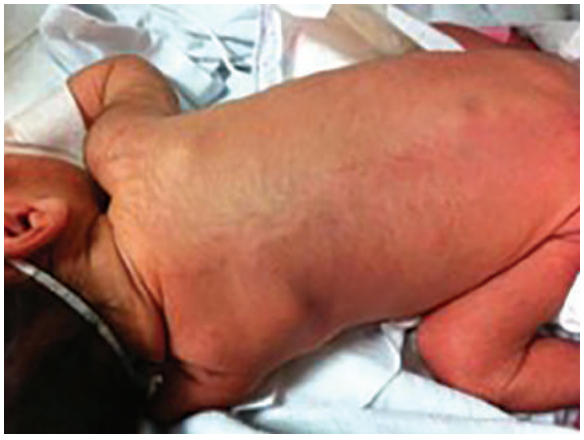
Treatment and Prognosis

No treatment is required for this entity. It is benign and rapidly resolves on its own. Recognition and education are important, as harlequin color change is a surprising and alarming occurrence for parents. Harlequin color change is unrelated to harlequin ichthyosis.

Subcutaneous Fat Necrosis

Clinical Findings

Subcutaneous fat necrosis is a relatively uncommon condition affecting full-term infants within the first several weeks after birth. It presents as single or multiple, poorly demarcated, tender nodules and plaques that are firm and have an underlying dusky reddish-purple hue (Fig. 94.14). The lesions are located in areas with fat pads, including the buttocks, thighs, arms, face, and shoulders. Calcification of the lesions can occur and can be associated with hypercalcemia.



• **Fig. 94.13** Harlequin Color Change. Unilateral macular erythema along the trunk.

Diagnosis

Skin biopsy will reveal subcutaneous granulomatous infiltration with multinucleated giant cells and damaged lipocytes containing characteristic needle-shaped clefts. Soft tissue necrosis and inflammation may cause local production of vitamin D, leading to hypercalcemia. Differential diagnosis includes sclerema neonatorum, which is a rare life-threatening panniculitis characterized by diffuse, progressive skin hardening.

Etiology

Subcutaneous fat necrosis is thought to be caused by some form of intrauterine or perinatal trauma. It has been associated with maternal cocaine use, hypothermia, meconium aspiration, and perinatal asphyxia.[34]

Treatment and Prognosis

This condition is often self-limited, but care must be taken to monitor calcium levels because of the risk of severe hypercalcemia and to assess the newborn for evidence of renal injury because of nephrocalcinosis.[35] Appropriate immediate treatment should be instituted if there is evidence of either of these complications. Resolution of subcutaneous fat necrosis occurs over a period of weeks to months. Bisphosphonates have been suggested for use in subcutaneous fat necrosis, but appropriate controlled clinical trials are still lacking.

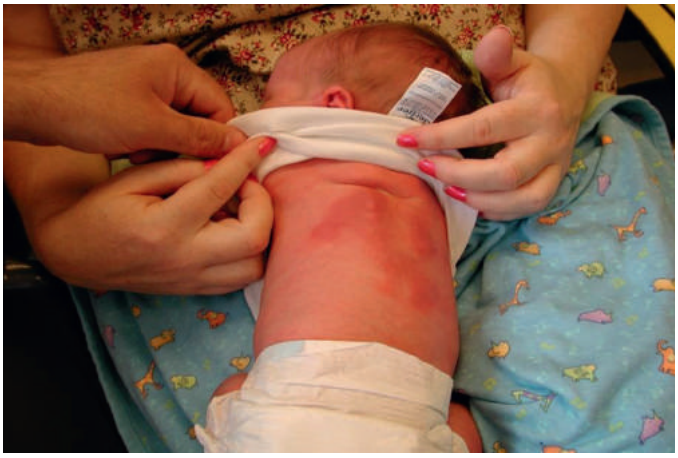
Cutis Marmorata

Clinical Findings

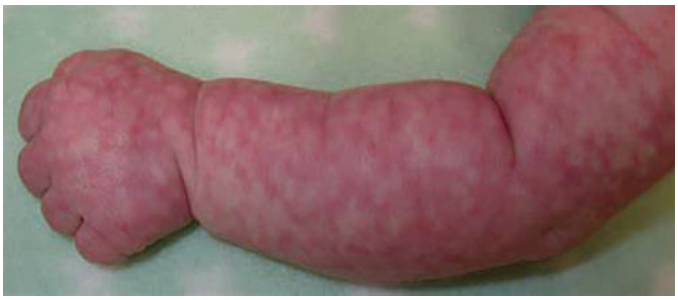
Cutis marmorata is a common finding, particularly in premature infants. It presents with a netlike violaceous reticular blanching pattern affecting the extremities more than the trunk (Fig. 94.15). It is exaggerated with cooling and resolves with warming.

Diagnosis

Diagnosis is made by clinical observation. Findings should resolve with warming, and the condition itself should resolve by



• **Fig. 94.14** Subcutaneous Fat Necrosis. Brawny, indurated, erythematous plaque on the back of an otherwise healthy infant with associated hypercalcemia.



• **Fig. 94.15** Cutis Marmorata. Reticulate violaceous erythematous patches on the upper extremity.

approximately 1 month of age. Persistent cutis marmorata past 1 month of age can be associated with genetic abnormalities such as Down syndrome, trisomy 18, and Cornelia de Lange syndrome. Cutis marmorata that persists after warming, and is localized and asymmetric, could represent cutis marmorata telangiectatica congenita, a distinctive vascular anomaly that can be associated with limb atrophy. Another diagnostic possibility is livedo reticularis, which presents with a clear vascular pattern that can be related to underlying vascular inflammation/vasculitis; however, this condition is quite rare in infants.

Etiology

Cutis marmorata occurs because of transient shifts in skin blood flow.

Treatment and Prognosis

Warming should resolve the cutaneous findings. If cutis marmorata is unresponsive to warming or persists past several months of age, further work-up for underlying causes may be required.

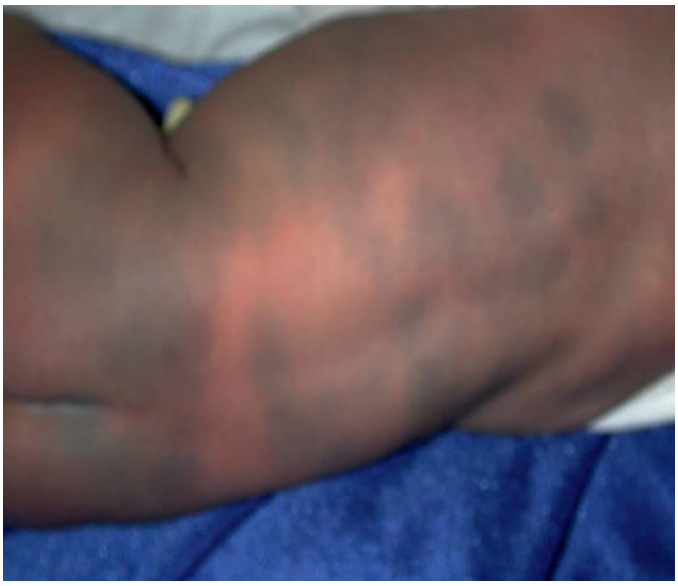
Dermal Melanocytosis

Clinical Findings

Dermal melanocytosis (DM), previously known as *Mongolian spots*, is an exceedingly common congenital cutaneous finding in children of African-American, Asian, and Hispanic descent. It presents as well-demarcated, deep blue to gray pigmented macules and patches that are most commonly seen on the buttocks and low back (Fig. 94.16). DM is usually an isolated finding and is not typically associated with tissue malformations or systemic disorders. There are very rare case reports of DM associated with inborn errors of metabolism (e.g., GM1 gangliosidosis) and other potential associations with a vascular nevus called *phakomatosis pigmentovascularis*, which is a form of twin spotting that occurs when two genetically distinct patches involve nearby or corresponding body areas.[36]

Diagnosis

Diagnosis is by clinical appearance. The differential diagnosis includes postinflammatory hyperpigmentation, nevus of Ito, vascular malformation, and child abuse. Bruising concerning for child abuse is suggested when the color of the lesion changes over time from blue green to brown, and if the lesion is tender to palpation. Biopsy of DM is rarely performed but would show increased numbers of dermal melanocytes.



• **Fig. 94.16** Dermal Melanocytosis. Gunmetal gray macules and patches on the back of a healthy infant consistent with dermal melanocytosis (a.k.a. *Mongolian spots*).

Etiology

DM is caused by an excessive number of dermal melanocytes. The bluish-gray coloration is due to the Tyndall effect (light reflection off the dermal-based melanocytes).

Treatment and Prognosis

No treatment is required for this benign and self-limited condition. DM tends to fade over time, with studies showing that 42% of cases disappear by 1 year of age.[37] Features that portend persistence past 1 year of age are multiple dark and/or large patches and presence in locations other than the sacrum. Most of these lesions fade by several years of age. Laser treatment of the lesions is not recommended. Because of possible confusion with bruising as mentioned earlier, it may be prudent to document DM when it is first encountered.

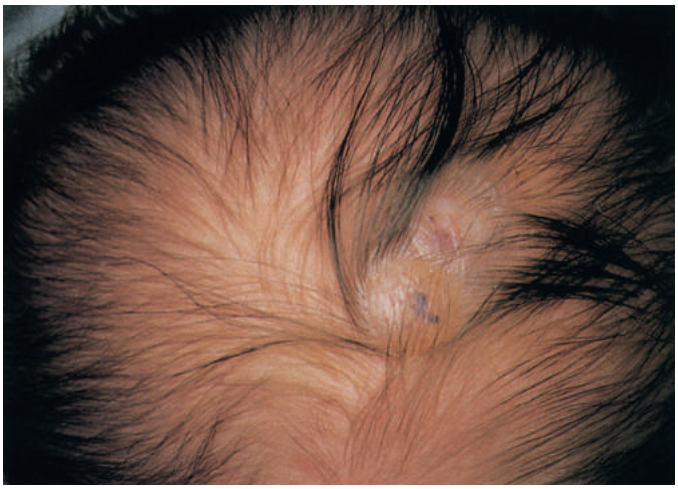
Aplasia Cutis Congenita

Clinical Findings

Aplasia cutis congenita (ACC) is a rare congenital skin defect that is localized and is most often seen on the scalp as an isolated lesion.[38] It presents with a localized loss of skin of variable thickness and is rarely associated with underlying skeletal abnormalities. Findings can include an open ulceration or a healed atrophic scar (Fig. 94.17). The clinical finding of a "hair collar sign" that presents as a ring of darker, longer hair surrounding the lesion represents an increased risk of an underlying neurodevelopmental abnormality.

Diagnosis

Diagnosis is clinical. Imaging of the central nervous system should be performed if a hair collar sign is present. Variants of ACC include focal dermal hypoplasia, or Goltz syndrome. Scalp ulcerations can be seen in infectious processes such as herpes simplex virus infection or can be due to perinatal trauma such as scalp



• **Fig. 94.17** Aplasia Cutis Congenita. Atrophic alopecia plaques at the vertex scalp.

electrode placement. Extensive ACC may be associated with limb hypoplasia (e.g., Adams-Oliver syndrome) or a variant of epidermolysis bullosa (e.g., congenital localized absence of skin, aka *Bart syndrome*).

Etiology

There are numerous theories regarding the cause of ACC. These include primarily intrauterine events such as intrauterine trauma, vascular abnormalities, intrauterine infection, and maternal medications.[39] No firm cause has been elucidated to date.

Treatment and Prognosis

The lesions of ACC will heal without intervention, but scarring will be present. Identification and management of the rare underlying neurodevelopmental abnormality are important aspects of care for these patients. In especially large lesions, surgical intervention may be required to close the defect. Scar revision can be performed later in life if desired.

Suggested Readings

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Vascular Anomalies and Other Cutaneous Congenital Defects

DEEPTI GUPTA AND ROBERT SIDBURY

KEY POINTS

- Infantile hemangiomas (IHs) are the most common soft tissue tumor of infancy, with a reported incidence of 4% to 5% in mature newborns and up to 23% in extremely preterm infants.
- There is often parental concern regarding the risk of hemorrhage in IHs, but serious bleeding rarely occurs, and there is not a risk of coagulopathy or Kasabach-Merritt phenomenon.
- Propranolol is a nonselective beta blocker that was serendipitously discovered to effectively treat IHs.
- Port-wine stains can occur in isolation but also can be related to an underlying genetic disorder, of which *Sturge–Weber syndrome* is the most common. Laser therapy can yield remarkable improvement for many port-wine stains, minimizing the emotional pain that accompanies facial disfigurement.
- Localized areas of hypopigmentation on the skin of the newborn may be isolated phenomena, or they may be markers of extracutaneous abnormalities.
- Congenital melanocytic nevi are hamartomas derived from neural crest cells that form in utero and are often classified by the largest diameter of their adult projected size. The risk of melanoma in small and intermediate-sized nevi is low, but for large and giant congenital melanocytic nevi, the risk of melanoma is higher, ranging from 4.5% to 10%.

The spectrum of congenital cutaneous defects can be organized by the type of tissue or cell of origin or on the basis of their location within the skin. This chapter presents information on the most common and clinically significant vascular anomalies and other congenital cutaneous defects.

Vascular Anomalies

There has been long-standing confusion regarding the nomenclature and pathogenesis surrounding cutaneous vascular anomalies, which has led to delays in diagnosis and improper treatment and management. Terms such as *cavernous hemangioma* were used to describe both vascular tumors and vascular malformations, and terms such as *hemangioma* have been used indiscriminately to encompass various vascular tumors of differing behavior, prognosis, morphology, and treatment. A classification system for vascular anomalies was originally proposed by[1] and was most recently updated in 2015[2] by the International Society for the Study of Vascular Anomalies to allow more precise diagnosis, categorization, and management. Vascular anomalies can be largely divided into two groups—vascular tumors and vascular malformations—with a smaller third group of provisionally unclassified vascular anomalies (Table 95.1). Vascular tumors demonstrate cellular hyperplasia, while vascular malformations comprise a single malformed and dysplastic vessel or a combination of such vessels.[3,4] Vascular tumors can be further subdivided into benign, locally aggressive, and malignant tumors. Vascular malformations are subdivided into categories based on the predominant type of anomalous channel, arising either from a single abnormal channel type or a mixed malformation comprising a combination of capillary, venous, lymphatic, or arterial vessel involvement. Simple and combined vascular malformations can be further divided according to their flow characteristics. Vascular malformations can also be classified by their association with other clinical features or associated syndromes (Table 95.2). A complete list of the various vascular tumors and malformations is given in Table 95.1, with highlighting of the most common vascular anomalies described within the text.

Vascular Tumors

Infantile Hemangiomas

Infantile hemangiomas (IHs) are the most common soft tissue tumor of infancy. They have a reported incidence of 4% to 5%[5–7] in mature newborns, and incidence rates of up to 23% have been reported in preterm infants weighing less than 1000 g.[8] Infantile hemangiomas have a female (2.3 to 2.9 times higher) and white predominance.[9] The most significant risk factor for the development of IHs is low birth weight, with the risk increasing by 40% for every 500-g decrease in birth weight.[10] Additional risk factors include prematurity, multiple gestation, preeclampsia, placental abnormalities, advanced maternal age, and in vitro fertilization.[6,7]

Clinical Features

IHs have a characteristic and unique growth pattern that is similar in both full-term and premature infants. At birth, IHs are either absent or barely evident. Within the first few weeks after birth, a precursor lesion is present, which can appear as either a pale area of vasoconstriction or a telangiectatic red macule or a "bruise-like" area (Fig. 95.1). There is a short latency period of 1 to 3 weeks before initiation of a rapid proliferation phase in most hemangiomas. This rapid growth phase occurs within the first 3 months after birth, with most of the growth occurring between 5 and 8 weeks of

TABLE 95.1 Classification of Vascular Anomalies

Vascular Tumors	Vascular Malformations

Benign
• Infantile hemangioma/Hemangioma of infancy
• Congenital hemangioma
  • Rapidly involuting (RICH)[a]
  • Noninvoluting (NICH)
  • Partially involuting (PICH)
• Tufted angioma[a,b]
• Spindle-cell hemangioma
• Epithelioid hemangioma
• Pyogenic granuloma (aka lobular capillary hemangioma)
Others
• Hobnail hemangioma
• Microvenular hemangioma
• Anastomosing hemangioma
• Glomeruloid hemangioma
• Papillary hemangioma
• Intravascular papillary endothelial hyperplasia
• Cutaneous epithelioid angiomatous nodule
• Acquired elastotic hemangioma
• Littoral cell hemangioma of the spleen
Related lesions
• Eccrine angiomatous hamartoma
• Reactive angioendotheliomatosis
• Bacillary angiomatosis
Locally aggressive or borderline vascular tumors
• Kaposiform hemangioendothelioma[a,b]
• Retiform hemangioendothelioma
• Papillary intralymphatic angioendothelioma (PILA), Dabska tumor
• Composite hemangioendothelioma
• Pseudomyogenic hemangioendothelioma
• Polymorphous hemangioendothelioma
• Hemangioendothelioma not otherwise specified
• Kaposi sarcoma
• Others
Locally aggressive
• Kaposiform hemangioendothelioma (with or without Kasabach-Merritt phenomenon)[a,b]
• Retiform hemangioendothelioma
• Papillary intralymphatic angioendothelioma
• Kaposi sarcoma
Malignant vascular tumors
• Angiosarcoma
• Epithelioid hemangioendothelioma
• Others
	Simple
	• Capillary malformation
	• Venous malformation
	• Lymphatic malformation
	• Arteriovenous malformations
	• Arteriovenous fistula
	Mixed/combined malformation
	• Capillary-venous malformation
	• Capillary-lymphatic malformation
	• Capillary-arteriovenous malformation
	• Lymphatic-venous malformation
	• Capillary-lymphatic-venous malformation
	• Capillary-lymphatic-arteriovenous malformation
	• Capillary-venous-arteriovenous malformation
	• Capillary-lymphatic-venous-arteriovenous malformation

[a]May be associated with thrombocytopenia or consumptive coagulopathy.
[b]Expert consensus is that these lesions lie along a spectrum, rather than being distinct entities.
ISSVA Classification of Vascular Anomalies ©2018 International Society for the Study of Vascular Anomalies Available at https://www.issva.org/UserFiles/file/ISSVA-Classification-2018.pdf Accessed October 2022.

age.[11] It is important to note that up to 80% of IHs have completed their growth by 3 months of age. Their growth is also limited to increasing volume within a predefined area rather than exhibiting a true radial growth phase, which is seen more characteristically in other neoplasms.[12] After a more rapid growth phase, IHs can continue to grow more slowly up until 9 to 12 months of age. There is then an observed phase of relative stabilization followed by spontaneous regression. Regression of a hemangioma occurs slowly over many years, with approximately 90% regression noted by 4 years of age.[13] Clinical signs of regression include dulling of the bright red color to a more purple color and a central gray-white discoloration that spreads centrifugally. If the white discoloration occurs in infants younger than 3 months, it can sometimes be a marker of impending ulceration. Regression does not indicate complete resolution and normalization of the underlying skin in all cases. There can be remaining telangiectasias, anatomical distortion most notable in facial lesions, and permanent textural changes characterized by residual fibrofatty tissue and skin laxity because of loss of elastic

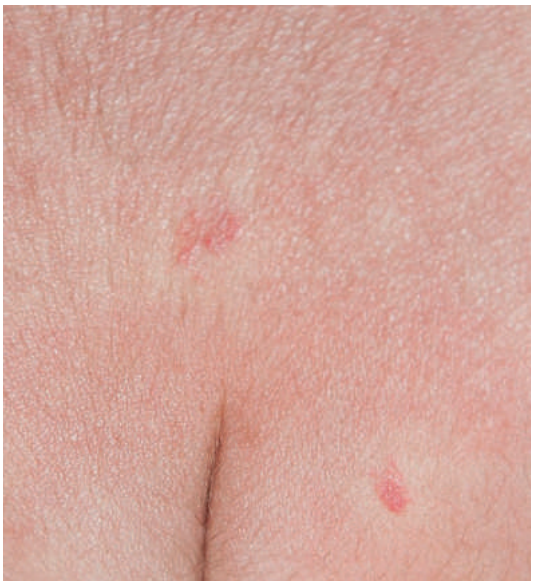
TABLE 95.2 Syndromes Associated With Vascular Malformations

Vascular Malformation Type	Associated Syndrome	Clinical Features	Other Features
Capillary malformation/ port-wine stain	Sturge-Weber syndrome	• Facial port-wine stain (often involving forehead or V1 distribution) • Ipsilateral eye abnormalities (choroidal vascular anomalies, increased ocular pressure, buphthalmos, glaucoma) • Leptomeningeal and brain abnormalities	Mutations in *GNAQ* in syndromic and nonsyndromic port-wine stains identified
	Nova syndrome	• Capillary malformation of the glabella • Neurologic malformations (Dandy–Walker malformation, hydrocephalus, cerebellar agenesis, mega cisterna magna)	
	Phakomatosis pigmentovascularis	Combination of cutaneous vascular and pigmentary abnormalities Classification into five subtypes: 1. Capillary malformation, epidermal nevus 2. Capillary malformation, dermal melanosis, with or without nevus anemicus 3. Capillary malformation, nevus spilus, with or without nevus anemicus 4. Capillary malformation, dermal melanosis, nevus spilus, with or without nevus anemicus 5. Cutis marmorata telangiectasia congenita, dermal melanosis	Phakomatosis pigmentovascularis has been associated with other vascular anomaly syndromes, including Sturge–Weber syndrome and Klippel-Trenaunay syndrome
	Beckwith–Wiedemann syndrome	• Prominent nevus simplex/capillary malformation of the philtrum and glabella • Hemihypertrophy • Visceromegaly • Macroglossia • Omphalocele	Intelligence is often not impaired. Overgrowth syndrome, therefore increased risk of Wilms tumor—screening is recommended.
	Macrocephaly–capillary malformation syndrome/ megalencephaly–capillary malformation–polymicrogyria syndrome	• Macrocephaly • Capillary malformation most prominent of the central face (philtrum and glabella) but can occur anywhere • Overgrowth of the body and brain (megalencephaly, hemihypertrophy) • Brain malformations (polymicrogyria, hydrocephalus) • Digital abnormalities (syndactyly, polydactyly) • Joint laxity	Overgrowth syndrome, therefore increased risk of Wilms tumor—screening is recommended. *AKT3, PIK3CA, PIK3R2* mutations detected. Developmental delay and seizures often occur.
Venous malformations	Blue rubber bleb nevus syndrome (Bean syndrome)	Multifocal venous malformations of the skin, mucosa, and gastrointestinal tract. Present as small black-blue papules and skin-colored nodules. Involvement of the palms and soles is common. Gastrointestinal bleeding is common.	Gastrointestinal bleeding is common and is a unique feature of blue rubber bleb nevus syndrome. Bleeding can result in chronic anemia and can require transfusions.
	Glomuvenous malformation syndrome	Small to large segmental venous malformations with cobblestoned appearance and bluish-purple color. Often painful to palpation.	Autosomal dominant inheritance or sporadic, mutations in glomulin gene (*GLMN*)
	Familial venous malformation cutaneous and mucosal	Small venous malformations mainly involving skin and mucosa but can also involve gastrointestinal tract, brain, and skeletal muscle. Usually asymptomatic.	Autosomal dominant inheritance. Often associated with *Tie2* mutation.
	Maffucci syndrome	• Venous malformation like skin nodules, rare presentation in infancy • Enchondromas, benign cartilage-forming tumors within the medullary cavity of the bone leading to bony distortion, fragility, and shortening of the affected limb. Hands and feet are involved 90% of the time.	Malignant transformation of the enchondromas can occur over time. Somatic mosaic mutations in *IDH1* and *IDH2* have been identified in cases.
	Cutis marmorata telangiectatica congenita	• Capillary malformation, reticulated, can have focal areas of atrophy and ulceration (see Fig. 95.34) • Limb asymmetry of affected limb	Other reported abnormalities: glaucoma and other ocular anomalies, cardiac defects, syndactyly, brain and spinal cord abnormalities
Lymphatic malformation	Gorham syndrome	• Multiple lymphatic malformations present in the bone, skin, and soft tissue • Progressive destruction of the bone associated with lymphatic malformation	

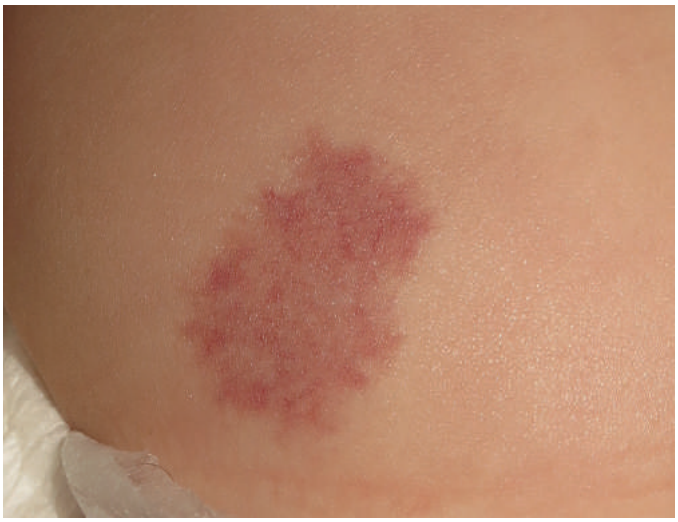
Continued

TABLE 95.2 Syndromes Associated With Vascular Malformations—cont'd

Vascular Malformation Type	Associated Syndrome	Clinical Features	Other Features
Arteriovenous malformation	Capillary malformation–arteriovenous malformation syndrome	• Multiple capillary malformations, congenital and acquired • Arteriovenous malformations of the central nervous system and soft tissues	Autosomal dominant Mutation in *RASA1*
	Cobb syndrome	• Spinal arteriovenous malformation • Vascular malformation of the skin (extremities or trunk)	
	Hereditary hemorrhagic telangiectasia	• Telangiectasias of the skin and mucosa • Epistaxis • Arteriovenous malformation of the lungs, brain, and liver	Autosomal dominant Infants with hereditary hemorrhagic telangiectasia can present with intracranial hemorrhage in the neonatal period
Vascular malformation and overgrowth syndromes	Beckwith-Wiedemann syndrome	• Prominent nevus simplex/capillary malformation of the philtrum and glabella • Hemihypertrophy • Visceromegaly • Macroglossia • Omphalocele	Intelligence is often not impaired. Overgrowth syndrome, therefore increased risk of Wilms tumor—screening is recommended.
	Macrocephaly–capillary malformation syndrome/ megalencephaly–capillary malformation–polymicrogyria syndrome	• Macrocephaly • Capillary malformation most prominent of the central face (philtrum and glabella) but can occur anywhere • Overgrowth of the body and brain (megalencephaly, hemihypertrophy) • Brain malformations (polymicrogyria, hydrocephalus) • Digital abnormalities (syndactyly, polydactyly) • Joint laxity	Overgrowth syndrome, therefore increased risk of Wilms tumor—screening is recommended. *AKT3, PIK3CA*, and *PIK3R2* mutations detected. Developmental delay and seizures often occur.
	Klippel-Trenaunay syndrome	• Combined slow-flow vascular malformation (capillary malformation, capillary malformation–venous malformation, capillary malformation–lymphatic malformation–venous malformation) in a characteristic geographic morphology • Venous varicosities • Overgrowth or undergrowth of the affected limb • Leg length discrepancy	At risk of coagulopathy, pulmonary embolism, contractures
	Parkes Weber syndrome	• Fast-flow vascular malformation (arteriovenous malformation) and arteriovenous shunts • Vascular stain • Overgrowth of the affected limb • Leg length discrepancy	Can occur as part of capillary malformation–arteriovenous malformation syndrome due to *RASA1* mutation, therefore family history should be obtained
	CLOVES syndrome	• Combined vascular malformation (capillary malformation, capillary malformation–lymphatic malformation–venous malformation, lymphatic malformation, arteriovenous malformation) • Epidermal nevus • Lipomas/truncal lipomatosis • Skeletal anomalies/scoliosis • Sandal gap deformity of toes	*PIK3CA* mutation. Risk of Wilms tumor.
	Proteus syndrome	• Slow-flow vascular malformation (capillary malformation, capillary malformation–lymphatic malformation–venous malformation, lymphatic malformation) • Connective tissue nevi	Growth is progressive
	PTEN hamartoma tumor syndrome (Cowden syndrome, Bannayan-Riley-Ruvalcaba syndrome)	• Vascular malformation (arteriovenous malformation, lymphatic malformation) • Epidermal nevus • Collagenomas • Lipomatosis	Segmental overgrowth. At risk of internal malignancies such as breast, thyroid, and endometrial cancers. Syndrome has characteristics of penile lentigines, developmental delay, and macrocephaly.



• **Fig. 95.1** A hemangioma precursor sign presenting as a pale vasoconstrictive halo surrounding two faint erythematous macules on the lumbosacral region of an infant.

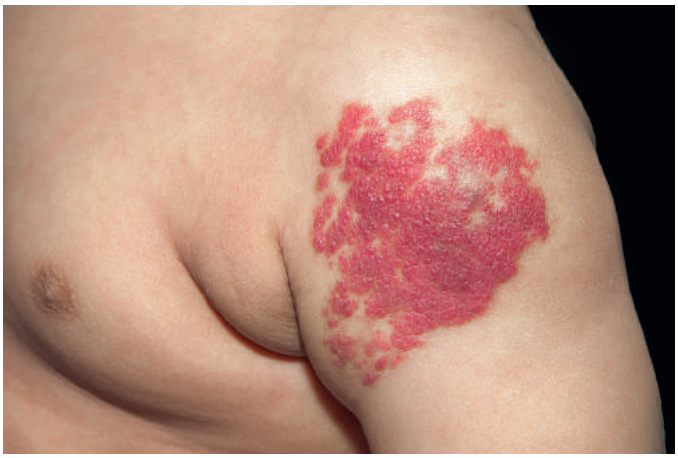


• **Fig. 95.2** Infantile hemangioma with minimal or absent growth. A 4-month-old male with a reticulated vascular patch on the lower back.

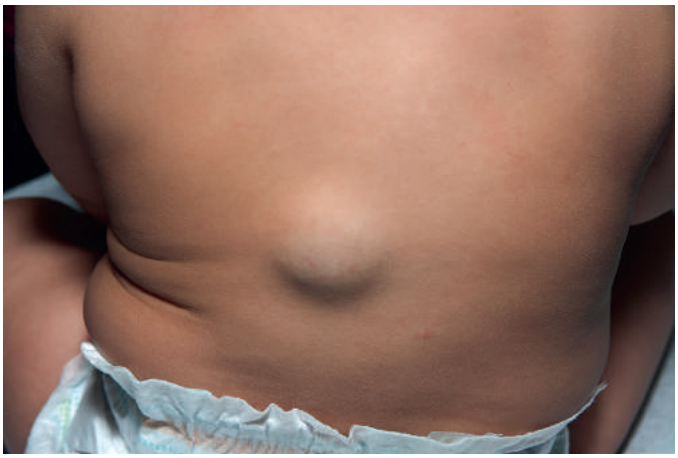
fibers causing fine wrinkling of tissue. Permanent scarring can also result from hemangiomas that were previously ulcerated.

While proliferation is often a key characteristic of IHs, it should not be considered an absolute defining feature. There is a unique subset of IHs known as *abortive hemangiomas* or *infantile hemangiomas with minimal or absent growth* in which the proliferation phase is absent (Fig. 95.2).[14] These hemangiomas present preferentially on the lower extremities and sometimes can be confused with capillary malformations (CMs). Although they lack the proliferation phase, they do spontaneously regress.

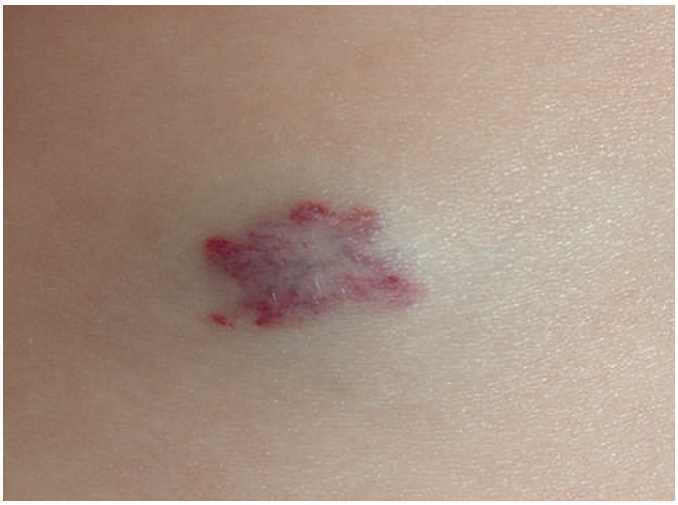
The clinical appearance of an infantile hemangioma is dictated by the depth of involvement, which can also lend itself to differences in the timing of growth. Superficial hemangiomas (Fig. 95.3) are located in the upper dermis and present as elevated bright-red, well-demarcated papules or plaques, which are sometimes in lay language referred to as *strawberry hemangiomas*. In their early proliferation phase, small bright-red papules can be seen arising from fine telangiectatic vessels, which can be a distinguishing feature between IHs and CMs that have a similar appearance in early



• **Fig. 95.3** Infantile hemangioma, superficial type, located on the upper arm of an infant.



• **Fig. 95.4** Infantile hemangioma, deep type, located on the lower back of an infant.



• **Fig. 95.5** Infantile hemangioma, mixed type, with both superficial and deep components.

infancy. Deep infantile hemangiomas (Fig. 95.4) are confined to the deep dermis and subcutis and present as bluish, dome-shaped tumors or nodules, with ill-defined borders. They can present in isolation or more commonly as a mixed infantile hemangioma (Fig. 95.5) with both superficial and deep components. The deep

component of IHs often has a distinct growth pattern in which they tend to appear later, around 2 to 3 months of age, and grow for a longer period, sometimes even over years, as compared with their superficial counterparts.[12,15] Hemangiomas are further characterized by their shape, pattern, and extent of involvement—whether focal or segmental. Focal hemangiomas seem to grow from a single point, whereas segmental hemangiomas are thought to arise from an embryonic developmental unit or placode and comprise a pattern on the skin correlating with these developmental units. Segmental hemangiomas are at risk of associated anomalies and syndromes. Large facial or scalp hemangiomas should be evaluated for the possibility of PHACE (*p*osterior fossa abnormalities, infantile *h*emangioma, *a*rterial abnormalities, *c*oarctation of the aorta, *e*ye abnormalities) syndrome, which can also have associated midline defects such as supraumbilical raphe and sternal cleft abnormalities and therefore are sometimes referred to as *PHACES*. Perineal hemangiomas or segmental hemangiomas in the lumbosacral area should be evaluated for the possibility of underlying spinal and genitourinary abnormalities with possible LUMBAR (*l*ower body hemangioma, *u*rogenital abnormalities, *u*lceration, *m*yelopathy, *b*ony deformities, *a*norectal malformations, and *r*enal abnormalities) syndrome, also known as *SACRAL* (*s*pinal dysraphism, *a*nogenital abnormalities, *c*utaneous, *r*enal and urologic abnormalities, associated with *a*ngioma of the *l*umbosacral localization) or *PELVIS* (*p*erineal hemangioma, *e*xternal genitalia malformations, *l*ipomyelomeningocele, *v*esicorenal abnormalities, *i*mperforate anus, and *s*kin tag) syndrome. Segmental hemangiomas, like deep IHs, can sometimes have a prolonged growth phase, which in rare cases may last for years.

There is often parental concern regarding the risk of hemorrhage in IHs, and parents should be reassured that serious bleeding rarely occurs. Coagulopathy and risk of Kasabach-Merritt phenomenon (KMP) was originally thought to be associated with large infantile hemangioma, but it has been shown that KMP does not occur with IHs and is seen only with other vascular tumors, such as tufted angiomas and kaposiform hemangioendotheliomas.[16]

Pathogenesis

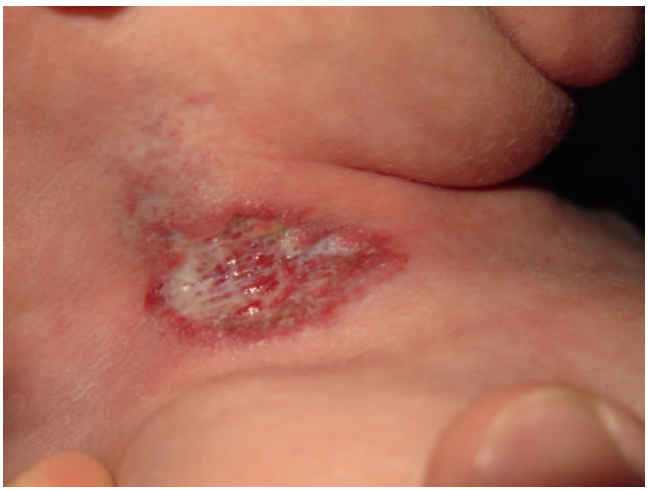
IHs form as a result of dysregulation of both vasculogenesis and angiogenesis. There are many proposed theories as to the trigger that initiates this dysregulation, without any one unifying cause, but it is likely that hypoxia plays an important role. There is strong evidence in support of IHs being derived from endothelial stem and progenitor cells.[17] Isolation of $CD133^+$ endothelial progenitor cells from infantile hemangioma tissue and injection of these progenitor cells into nude mice lead to formation of tumors with the unique immunohistochemical and growth characteristics of IHs.[17] The endothelial cells that were isolated from IHs were also found to be clonal in nature, suggesting that these tumors were caused by a somatic mutation in one or more genes regulating endothelial cell proliferation.[18] The endothelial cells in infantile hemangioma express a unique phenotype of cell surface markers with positive staining for glucose transporter 1 (GLUT1), merosin, and antigen Lewis Y, which is also expressed by placental endothelial cells, but are not present in any other tumor or vascular malformation.[19] Histologically, IHs have increased cellularity and form small sinusoidal vascular channels. It has been suggested that IHs may result from ectopic placental tissue given the similarity in their endothelial cell profiles and the fact that the placenta and IHs share a similar life cycle.[20] Further evidence suggestive of this link relates to the increased risk of development of IHs in low birth weight infants—particularly those whose mothers had preeclampsia or placenta previa, both of which are associated with placental hypoxia. Hypoxia likely plays a large role in the development of IHs and is thought to be an important triggering signal for their development,[21,22] with in utero hypoxia as a risk factor for localized hemangiomas and regional hypoxia from arterial abnormalities as a risk factor for segmental hemangiomas. It is likely that this hypoxic stress acts as a triggering signal and leads to overexpression of vascular endothelial growth factor via the hypoxia-inducible factor α pathway, which then leads to proliferation of $CD133^+$ endothelial progenitor cells that are present in fetal tissue and causes them to differentiate into immature endothelial cells, along with pericytes, dendritic cells, and mesenchymal cells with adipogenetic potential. The adipogenetic potential may play a role during regression—when blood vessels are replaced with fibrofatty tissue. During the involution phase, endothelial cells are also noted to express caspases, which are known markers of apoptosis.

Diagnosis

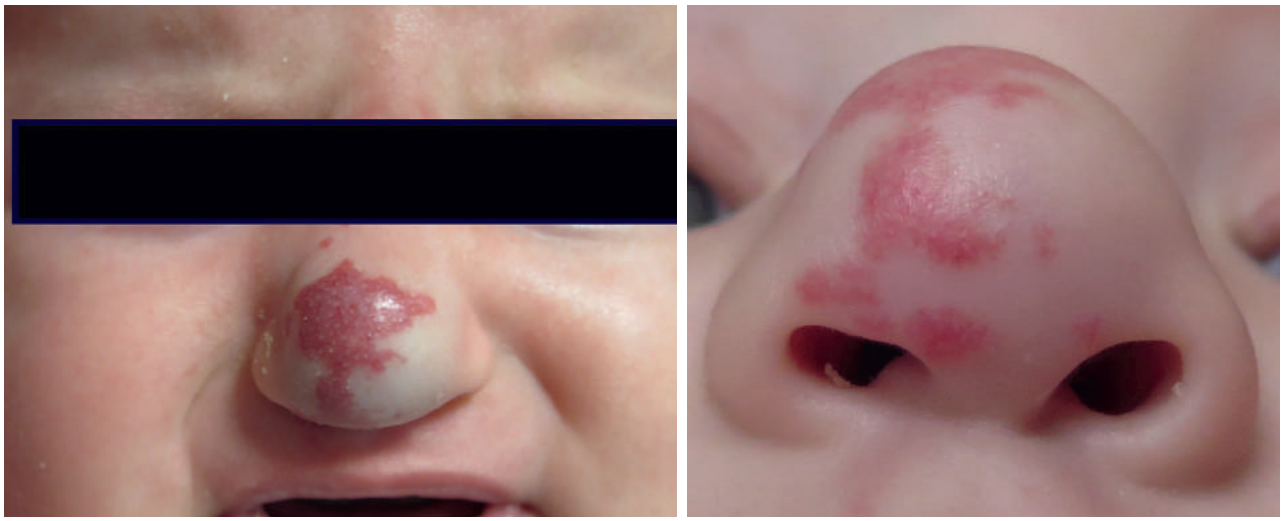
In most cases, a hemangioma can be diagnosed by its clinical appearance and characteristic pattern of evolution. A lesion that deviates from this typical picture presents a diagnostic dilemma. Doppler ultrasound examination can be easily performed and may be helpful in distinguishing between an infantile hemangioma and low-flow malformation or nonvascular tumor. Other imaging modalities—magnetic resonance imaging (MRI) or angiography—may be indicated for large or obstructive lesions (e.g., ocular, upper airway) to help define the extent of involvement or associated abnormalities (e.g., PHACE or LUMBAR syndrome).[9,23] Skin biopsy is diagnostic for nonvascular tumors, which can mimic vascular birthmarks (e.g., pilomatricoma, juvenile xanthogranuloma [JXG], Langerhans cell histiocytosis, infantile myofibromatosis, rhabdomyosarcoma). IHs can also be differentiated from other vascular tumors by staining with GLUT1, an immunohistochemical marker that is highly selective and specific for IHs. GLUT1 is also expressed at the blood–brain barrier and in placental tissue but has not been found in any other vascular tumors, including congenital hemangiomas. Its discovery has helped in making the correct diagnosis of these vascular tumors, especially in cases with atypical presentations.[20] It should be noted that congenital hemangiomas (NICH, RICH, PICH) are Glut-1 negative.

Complications

While the majority of IHs are uncomplicated and spontaneously regress without any need for intervention, in approximately 10% to 15% of cases, complications can arise requiring treatment.



• **Fig. 95.6** Painful ulcerated infantile hemangioma on the neck.

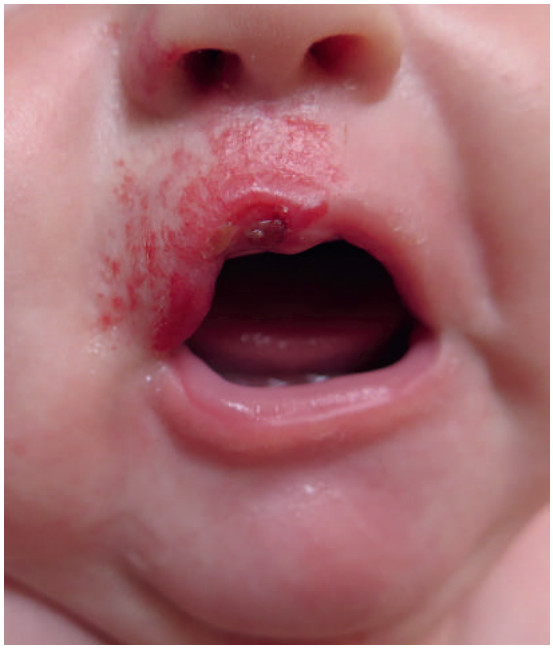


• **Fig. 95.7** Nasal tip infantile hemangioma with both superficial and deep components. Nasal tip hemangiomas are at high risk of causing disfigurement.

Local Complications

Ulceration. Ulceration is the most common complication observed in IHs and can be seen in up to 30% of cases (Fig. 95.6).[24] It can cause significant pain and discomfort and permanent scarring in the area of ulceration. Ulceration has been described to be more likely to occur during two points during the life cycle of the hemangioma: either just before the rapid proliferation phase, which can be the presenting sign of the hemangioma, or at the end of the growth phase (usually around 4 months of age). The exact mechanism causing ulceration is unknown but is thought to be related to tissue hypoxia, with the tumor outgrowing its blood supply.[25] Ulceration is more commonly seen in large hemangiomas, those with segmental distribution, and those with mixed morphology with both superficial and deep components.[25–27] Areas of friction or those exposed to moisture for a long time, such as the lower lip, neck, intertriginous areas, and anogenital and diaper region, are at high risk of ulceration. Nearly one-third of all ulcerated hemangiomas are found in the diaper region.[28] Various treatment modalities have been shown to be effective in treating ulcerated hemangiomas, but all ulcerated hemangiomas benefit from local wound care, and combination therapy is more effective than monotherapy.[29] Treatment options included topically administered brimonidine, 0.2% or timolol, 0.5%, topical antibiotics with mupirocin or metronidazole, pulsed dye laser treatment, and systemic therapy with propranolol. Topical analgesics can also be useful to minimize pain and discomfort.[30]

Disfigurement. In addition to the transient disfigurement (hemangiomas located on the central face), large hemangiomas, and those with a significant superficial component, can predispose the affected child to permanent scarring. IHs involving the nasal tip are at risk of a bulbous nasal tip or a "Cyrano" deformity (Fig. 95.7). This is caused by splaying of the alar cartilage during the proliferative growth phase. Early intervention with initiation of propranolol therapy can preserve the contour of the nose.[31] If a persistent deformity develops, surgical debulking may be needed. Similarly, IHs located on the lip are at risk of deformity and disruption of the natural contours of the lips, in addition to being at high risk of ulceration and permanent scarring (Fig. 95.8).[32] Large hemangiomas of the central chest or hemangiomas involving the breast tissue in females can also be quite disfiguring, leading to permanent breast hypoplasia in some cases (Fig. 95.9).[33]



• **Fig. 95.8** Partial segmental infantile hemangioma of the lip and nose with ulceration of the lip.

Disfigurement alone, regardless of threat to function, is a reasonable indication for medical therapy in certain cases, and should be considered. Regression of hemangiomas does not always ensure complete normalization of the underlying skin and therefore can lead to lifelong psychosocial and emotional sequelae experienced by both the family and the patient.[34]

Functional Complications

Periocular Hemangiomas. Hemangiomas located on the lid or around the orbit are at risk of causing visual impairment and can lead to amblyopia in severe cases. Amblyopia can be caused by direct pressure on the globe, causing astigmatism or myopia, or because of the size of the hemangioma there can be visual axis obstruction or strabismus.[35] Larger and segmental hemangiomas in the periorbital area pose the greatest risk of ocular

complications. Deep retrobulbar IHs may present with proptosis and can also cause strabismus and visual acuity changes. Deep and mixed infant hemangiomas can also cause tear duct obstruction and exposure keratopathy. Aggressive and early initiation of treatment along with evaluation by a pediatric ophthalmologist can help prevent some of these complications.[36,37] Further imaging, such as MRI, may be needed to assess the extent of the hemangioma or the presence of a deeper component.

Auricular Hemangiomas. With infantile hemangiomas of the ear there is high risk of physical deformity of the ear, cartilage destruction, ulceration, potential infection when ulceration is present, and potential hearing alterations. Segmental hemangiomas in this region have a higher rate of complications, and with them there can also be a risk of sensorineural and conductive hearing loss.

Potential Life-Threatening Complications

Airway Hemangiomas. Airway obstruction by an infantile hemangioma is a life-threatening complication that requires immediate evaluation and treatment. Airway hemangiomas can occur with or without the presence of cutaneous IHs. The highest risk occurs with hemangiomas located in a "beard distribution" specifically involving the left or right preauricular areas, chin, lower lip, and anterior part of the neck.[38] Involvement with IHs at four or more of these sites is associated with a 63% risk of a symptomatic airway hemangioma. A greater number of lesions in the beard distribution leads to a higher risk of airway involvement. Segmental hemangiomas involving facial segment 3 have a 29% risk of airway involvement. Clinically, airway hemangiomas present most commonly between 6 and 12 weeks of age with biphasic stridor or a hoarse, croup-like cry. The subglottis is the most common site of involvement, but the oral cavity, oropharynx, hypopharynx, larynx, and upper trachea can also be involved. Referral to a pediatric otolaryngologist is important for evaluation of the airway, and systemic treatment should be started immediately.[39] Treatment of airway hemangiomas may require a combination of multiple medical and surgical treatments depending on the extent of involvement, which may include propranolol, oral and intralesional corticosteroids, vincristine, interferon alpha, surgical excision, and laser therapy.

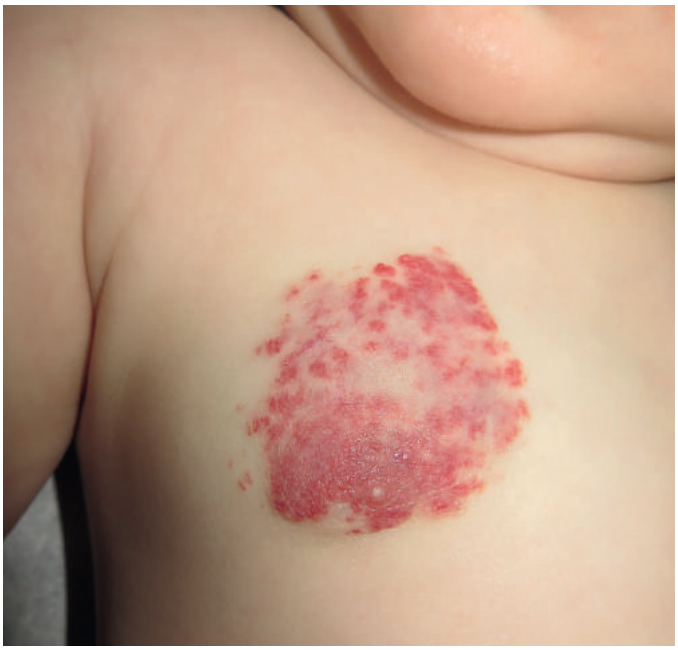
Hepatic Hemangiomas. The presence of an IH in the liver can lead to potential serious complications such as congestive heart failure and consumptive hypothyroidism. Individuals with five or more cutaneous IHs (Fig. 95.10) of any size and in any location should be screened for the possibility of hepatic IH.[40] This study showed that in patients with five or more cutaneous hemangiomas, 16% of them had evidence of a hepatic hemangioma versus none in the group with fewer than five cutaneous hemangiomas. Individuals with large or segmental IHs do not seem to be at greater risk of hepatic IHs.[41,42]

Parotid Hemangiomas. Parotid hemangiomas can be isolated to the parotid gland or can be present as part of a segmental IH in the maxillary distribution of facial segment 3. Parotid hemangiomas have a unique pattern of growth as they can have a longer proliferative growth phase than typical IHs. This can lead to longer treatment courses.[15] They have also been associated with functional complications relating to deformity of adjacent structures such as the ear and lip and conductive hearing loss caused by narrowing of the external auditory canal. Life-threatening complications can also arise, including an association with subglottic hemangiomas and, less commonly, congestive heart failure and consumptive hypothyroidism.[43,44]

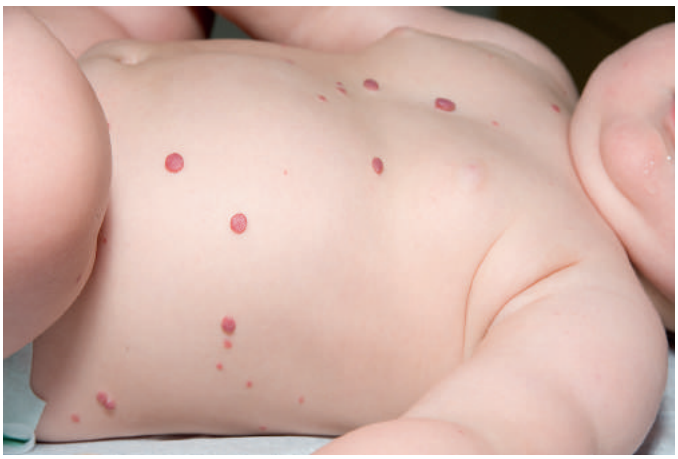
Treatment

Most IHs are uncomplicated and spontaneously regress, but there is a subset of approximately 10% to 15% of IHs that result in complications requiring treatment.[27,45] Treatment is indicated in cases complicated by disfigurement or risk of disfigurement, ulceration, or functional compromise. There are various treatment modalities, including topical or systemic medications, surgery, or laser therapy, that are chosen on the basis of various factors, including the stage of growth, location, potential complications, and associated conditions. The various treatment modalities are outlined in the following sections. In general, pharmacologic treatment is the first-line treatment, with surgical and laser therapies being considered as adjunctive or second-line treatment following medical interventions.

Active Nonintervention. For most lesions the initial treatment of choice is "active nonintervention." This is reserved for hemangiomas that are not at risk of causing disfigurement, ulceration, or causing any functional impairment. These often tend to be small focal hemangiomas in nonfacial locations. Anticipatory guidance should be discussed with the family in regard to the natural course of IHs: the initial period of rapid growth in the first few months of life and the slow rate of involution over many years.



• **Fig. 95.9** Infantile hemangioma of the breast involving the nipple and areola.



• **Fig. 95.10** Diffuse hemangiomatosis presenting with multifocal infantile hemangiomas on the skin and in the liver.

Demonstration of before-and-after photographs of growing and involuted hemangiomas in other children can help demonstrate the natural course and diminish parental concern. There is also a common concern of risk of significant hemorrhage. This is rare in IHs. Minor episodes of bleeding can result from trauma and, like any superficial wound, respond to short-term compression.

Although the large majority of IHs do not require treatment and spontaneously regress, the disfigurement associated with the residua of IHs should not be underestimated, and treatment should be considered on the basis of the location and size of the infantile hemangioma. Up to 40% of hemangiomas leave permanent skin changes that can be disfiguring.[46] Psychological and social problems may result from facial or other visible deformities. A small study found that patients with untreated involuted facial hemangiomas had higher levels of social anxiety and decreased social initiative as compared with children with treated facial IHs.[47] Therefore, early intervention should be considered for lesions with a higher potential for complications.

Topical Therapies. Topically administered timolol maleate is a nonselective β-adrenergic receptor blocker that was first reported to be used in IHs in 2010 and has been shown to be most effective for superficial and thin hemangiomas.[48] It is approved for use in pediatric glaucoma, but its use topically on the surface of the IHs is off-label. The preferred formulation is timolol gel forming solution, 0.5%. In general, timolol is well tolerated, without significant systemic side effects. However, a recent prospective study showed systemic absorption with use of one drop twice a day—although the concentrations detected were below 0.2 ng/mL, which is below the level at which systemic effects may begin to be seen.[49] Caution should be used in preterm infants with postmenstrual age less than 44 weeks and low birth weight infants weighing less than 2500 g at the time of initiation of treatment, as there have been a few case reports of symptomatic bradycardia in this group.[50] However, in these reported cases, the doses of timolol being used exceeded 0.2 ng/mL, and the treatment areas had variable or increased absorption (e.g., thin-skinned areas such as eyelids, mucosal surfaces, and ulcerated sites). Absorption within mucosal sites and ulcerated hemangiomas is variable, and therefore caution should be used, but there have been reports of use in these areas without any adverse side effects.[51] A recent randomized controlled trial showed that timolol, though well tolerated, did not differ from placebo at 24 weeks in terms of complete or nearly complete hemangioma resolution.[52]

Systemic Therapies

Propranolol. Propranolol is a nonselective β-adrenergic receptor blocker that in 2008 was discovered by chance to treat IHs.[53,54] It had been used at higher doses in children with cardiac disease for many decades previously. In 2014, Hemangeol (propranolol hydrochloride, Pierre Fabre, Parsippany, New Jersey, United States) was approved by the U.S. Food and Drug Administration as the only approved systemic treatment for IHs, and propranolol become first-line systemic treatment for IHs. The dosages for IHs range from 1 to 4 mg/kg per day in divided doses (two or three times daily), with a 98% response rate at a mean dosage of 2.1 mg/kg per day.[55] There have now been three randomized controlled trials[53,56,57] examining the efficacy of propranolol for IHs, with the largest trial showing the highest efficacy at dosages of 3 mg/kg per day for a 6-month course.[53] Despite this, most practitioners use maintenance dosages of 2 mg/kg per day because dosing regimens of more than 2 mg/kg per day did not show a significant increase in effect but did show an increase in the rate of adverse events.[58] The most common adverse side effects of the medication reported are gastrointestinal disturbance, sleep disturbance, and acrocyanosis. These side effects were overall mild and reversible. Serious adverse effects such as symptomatic hypotension, hypoglycemia, bradycardia, and bronchospasm occurred infrequently and, in a randomized control trial, were reported at a similar frequency in the placebo group.[53] Once the medication is discontinued, rebound growth can occur, but this was more likely if the hemangioma was treated for less than 9 months versus a course of 12 to 15 months.[59]

Corticosteroids. Oral corticosteroids were the first-line treatment before the discovery of propranolol. Prednisone or prednisolone dosages of 2 to 5 mg/kg per day were used, with the most optimal effects reported at a dosage of around 3 mg/kg per day.[9,27] Within 1 to 2 weeks, 30% of hemangiomas would show a dramatic response, but 40% would respond equivocally. Many side effects were noted in these patients, most notably cushingoid appearance, gastroesophageal reflux, insomnia, irritability, transient growth retardation, hyperglycemia, and, rarely, adrenal insufficiency, hypertension, and osteoporosis. Studies comparing propranolol with oral corticosteroids showed that clinical response to propranolol was more rapid and effective, with need for fewer surgical interventions, and that, overall, propranolol was better tolerated.[60] Corticosteroids as a monotherapy are no longer used for IHs but rather are now reserved for complex and refractory cases and can be used in conjunction with propranolol or other therapies.[61]

Intralesional corticosteroid injections may be used in small, localized hemangiomas in cosmetically sensitive areas with high rates of morbidity such as the lip, nasal tip, and eyelid.[62,63] This can be used as a therapy adjuvant to other topical or systemic treatments. Complications include cutaneous atrophy, skin necrosis, and, for intralesional periocular injections, ophthalmic artery occlusion and blindness. Thus, periocular intralesional steroid injection should be performed only by experienced pediatric ophthalmologists.

Other Therapies. Historically, interferon alpha and vincristine were used with variable effect for treatment of IHs. These therapies have potential adverse effects and are now reserved only for refractory lesions.[64–66] Orally administered sirolimus, a mammalian target of rapamycin (mTOR) inhibitor, has now been considered in a few such cases.[67]

Surgical Therapies

Laser Therapy. Pulsed dye laser treatment has been used in various settings with IHs. There have been some controversial data regarding its use as a monotherapy for uncomplicated hemangiomas, and it seems to be more efficacious when used in conjunction with propranolol or timolol.[68] It has also been used to heal ulcerations[69] and to decrease residual erythema and telangiectasias in an involuting hemangioma. However, a randomized prospective controlled trial of 121 infants found that pulsed dye laser treatment in uncomplicated hemangiomas is no better than watchful waiting.[70] Pulsed dye laser treatment cannot prevent the preprogrammed growth pattern of hemangiomas and has a limited role during the proliferative phase. One risk of laser use during the proliferative phase can be ulceration. In contrast, it can be useful during involution, if there is residual redness or telangiectasias, which may be resolved faster than with the natural course of involution but has no effect on fibrofatty residuum.

Surgical Excision. Surgical excision can be done in cases of medically refractory hemangiomas that are symptomatic and proliferating, in an emergency situation where there is life-threatening functional compromise, or in situations with recurrent profuse bleeding. It may also be beneficial in situations where there is a large disfiguring pedunculated lesion that will leave behind significant fibrofatty residual or scar.

Wound Care. Ulceration is a therapeutic challenge, but all ulcers benefit from local wound care and potential occlusive dressings. The type of dressing chosen depends on the amount

of exudate and on the location of ulceration. For sites that are difficult to dress, for example the diaper area, frequent and liberal application of petrolatum jelly is effective. Various dressing materials, including petrolatum-impregnated gauze and seaweed-derived alginate dressings, are often recommended. Off-label use of becaplermin gel, a recombinant human platelet-derived growth factor, has reportedly shown dramatic healing in a small case series. The product now carries a warning from the Food and Drug Administration, which must be taken into consideration before its use.[71,72] Agents for pain control should be considered, including topical anesthetics (being mindful of the percentage of body surface area covered) and oral analgesics such as acetaminophen or ibuprofen. A high index of suspicion should be maintained for secondary infection, with appropriate use of topical or oral antibiotics as needed. If conservative therapy is unsuccessful, pulsed dye laser treatment may relieve pain and speed reepithelialization.[26,73,74] The ulcers will heal but will inevitably leave scars.

Work-Up and Associations. A complete list of associations and the work-up needed for specific hemangiomas on the basis of their size and location is given in Table 95.3. Here we will highlight a few other special circumstances.

Visceral Involvement/Hepatic Hemangiomas. Diffuse neonatal hemangiomatosis (see Fig. 95.10) manifests itself as widely scattered, small superficial hemangiomas. Infants with this pattern of cutaneous involvement may have lesions limited to the skin, known as *benign neonatal hemangiomatosis*. However, associated hemangiomatosis of the liver, gastrointestinal tract, lungs, and/or central nervous system (CNS) can be complicated by visceral hemorrhage, hepatomegaly, high-output cardiac failure, or unexplained anemia or thrombocytopenia, with a significant mortality rate.[75] Congestive heart failure can also occur with a large, isolated hepatic hemangioma.[27]

Segmental Hemangiomas

PHACE Syndrome. PHACE syndrome is characterized by a large (>5 cm) segmental hemangioma of the face, scalp, or neck with associated anomalies and developmental defects.[76] The acronym PHACE is used to describe the constellation of anomalies related to this syndrome: posterior fossa malformations, hemangioma, arterial anomalies, cardiac anomalies/coarctation of the aorta, and eye abnormalities. Midline defects such as sternal cleft and supraumbilical raphe can also be seen, and therefore sometimes this disorder is referred to as *PHACES*. The diagnostic criteria for PHACE syndrome were formalized by a consensus panel, and clinical features were grouped into major criteria. Definitive diagnosis of PHACE syndrome requires a facial segmental IH or an IH larger than 5 cm on the face or scalp in addition to one major or two minor criteria.[77] This was revised in 2016 to also include a segmental hemangioma of the neck, upper trunk, or trunk and proximal upper extremity plus two major criteria.[78] The exact incidence of PHACE syndrome is unknown, but it may be more common than Sturge-Weber syndrome (SWS).[79] There is a striking female predominance in PHACE syndrome, with a female-to-male ratio of 9:1. A prospective study for PHACE syndrome found that 31% of infants with facial IHs with a surface area of 22 cm^2 or greater met the PHACE diagnostic criteria, which is based on expert consensus, and approximately 90% of affected infants had more than one extracutaneous finding.[80] Children with frontotemporal and frontonasal IHs (known as segments 1 and 4) have a higher correlation with structural cerebral and cerebrovascular anomalies. Those with mandibular (segment 3) or beard distribution lesions are at higher risk of cardiac abnormalities and IHs in the airway.[81] The maxillary face (segment 2) appears to be a lower-risk segment for association with PHACE syndrome. The most common and potentially devastating sequelae of PHACE

TABLE 95.3 Indications for Work-Up of Extracutaneous Anomalies Associated With Infantile Hemangiomas

Clinical Presentation of Hemangioma	Association	Evaluation
Large facial hemangioma/segmental facial hemangioma >5 cm	PHACE syndrome	MRI/MRA of the brain and neck, echocardiogram, ophthalmology evaluation
Multifocal hemangiomas ≥5 cm	Extracutaneous hemangiomas, especially hepatic hemangiomas	Abdominal ultrasound examination with Doppler imaging
Periocular hemangioma	Ocular complications	Ophthalmology evaluation
Parotid hemangioma	Consumptive hypothyroidism, congestive heart failure, airway hemangioma	
Lumbosacral hemangioma >2.5 cm	LUMBAR syndrome	MRI of lumbar spine and pelvis, neurosurgical evaluation
Large lower trunk or lower extremity hemangioma	PELVIS syndrome	MRI of lumbar spine and pelvis, neurosurgical evaluation, urologic evaluation
Beard distribution hemangiomas	Evaluation for respiratory distress	Otolaryngology evaluation and MRI of the neck
Breast hemangioma/large segmental chest hemangioma	Breast hypoplasia	Consider systemic treatment
Midline hemangiomas (with other cutaneous markers)	Spinal dysraphism	Spinal ultrasound examination at <3 months of age, MRI of the spine
Segmental proximal upper extremity hemangioma with extension onto the chest	PHACE syndrome or cardiac abnormality	Echocardiogram

MRA, Magnetic resonance angiography; *MRI*, magnetic resonance imaging.

syndrome are neurologic, including structural brain anomalies and abnormalities of cerebral vasculature. Progressive stenoses and occlusions of cerebral arteries can also be seen. Both moyamoya-like vasculopathy and arterial ischemic strokes have been reported.[82,83] Other comorbidities have recently been reported in patients with PHACE syndrome, including headaches, endocrine abnormalities, hearing abnormalities, speech delay, dysphagia, and dental anomalies.[78]

Because of the associated anomalies, patients suspected of having PHACE syndrome require the following:

- Echocardiogram to assess the patient for coarctation of the aorta or other structural cardiac abnormalities with possible cardiac MRI/magnetic resonance angiography (MRA) if the echocardiogram is abnormal
- MRI with contrast medium and MRA of the head and neck evaluating the patient for cerebrovascular and structural anomalies
- Ophthalmology examination of the retina
- Complete physical examination with particular focus on ventral midline defects
- Additional studies as indicated on the basis of signs and symptoms

Lumbar/Sacral/Pelvis Syndrome. Similarly to PHACE syndrome, large hemangiomas of the lower body may be associated with underlying structural abnormalities. Sacral and lumbar hemangiomas may reveal spinal dysraphism, including tethered spinal cord, lipomyelomeningocele, imperforate anus, renal anomalies, or abnormal external genitalia.[84–87] Many acronyms have been coined, all describing a similar entity of a segmental hemangioma in the lumbosacral or perineal region with associated regional abnormalities. These include LUMBAR, SACRAL, and PELVIS.[88–90]

Exact diagnostic criteria have not yet been defined, but IHs in the lumbosacral or perineal area that are large, midline, segmental, or present with other cutaneous markers (i.e., lipoma, gluteal cleft deviation, skin tag, aplasia cutis) should be screened for underlying abnormalities. One prospective study noted that 35% of patients with lumbosacral hemangiomas larger than 2.5 cm had evidence of spinal dysraphism on MRI.[91] Segmental hemangiomas in the lumbosacral region typically present with minimal or absent growth and persist as a patch with coarse telangiectasias, which can lead to delays in diagnosis. These are also at high risk of ulceration.

Congenital Hemangiomas. Congenital hemangiomas are an uncommon and distinct type of vascular proliferation that are fully formed at birth. They do not undergo the characteristic proliferative growth pattern in postnatal life as seen with IHs, but rather their proliferative phase occurs in utero. They are also GLUT1 negative, unlike IHs. Congenital hemangiomas can be divided into three major subtypes of rapidly involuting congenital hemangiomas (RICHs), noninvoluting congenital hemangiomas (NICH), and partially involuting congenital hemangiomas (PICHs) on the basis of their clinical progression. Congenital hemangiomas are usually solitary in nature and are more common on the extremities and head and neck.[92]

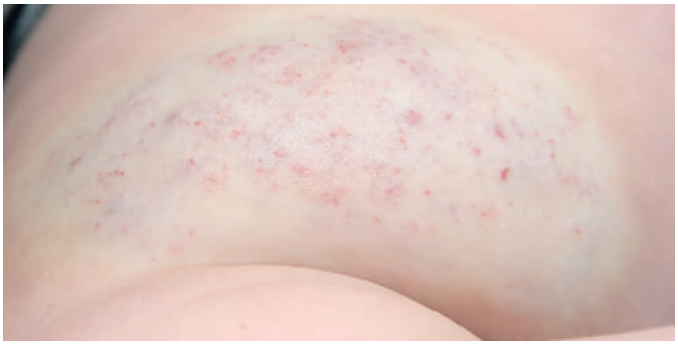
Rapidly Involuting Congenital Hemangiomas. RICHs can present clinically in three distinct ways: (1) a raised violaceous tumor with prominent peripheral vasculature, (2) a raised tumor with coarse overlying telangiectasias with a peripheral halo of vasoconstriction or pallor, or (3) a pink-purple tumor with deep infiltrative nodules. There can sometimes be overlying hypertrichosis.[93] Rapid involution often begins in the first few weeks of life and is completed by 14 months of age. After involution there may be some residual atrophy, telangiectasias, persistent and prominent vessels, or milia present. There is a rare subtype of RICH where complete involution occurs in utero called *RICH fetal involution type*. Complications of RICHs are that they can undergo ulceration and bleeding shortly after birth, which can be painful and leave a permanent scar in the area of ulceration. There have also been reports of transient coagulopathy with thrombocytopenia occurring. The decrease in platelet count is brief and not progressive as seen in KMP. The presence of this phenomenon can lead to misdiagnosis of RICH with tumors associated with KMP, such as tufted angiomas and kaposiform hemangioendothelioma, and, therefore, biopsy may be required to confirm the diagnosis.

Noninvoluting Congenital Hemangiomas. NICHs (Fig. 95.11) present as vascular patches, plaques, or nodules with blue or pink-purple color with overlying coarse telangiectasias and peripheral rim of pallor and vasoconstriction.[94] They persist over time and grow proportionally with the child, without spontaneous regression. Some can become more protuberant or develop an increase in draining veins over time. Symptoms may develop during pregnancy or puberty, most notably that of pain.[94]

Partially Involuting Congenital Hemangiomas. There is a small subset of congenital hemangiomas that begin a phase of involution that lasts until 12 to 30 months of age, at which time the involution halts.[95] The residual vascular lesion then persists lifelong morphologically, resembling a NICH. There have been no reported complications in patients with PICH. The existence of PICH suggests that all congenital hemangiomas may exist along a spectrum.

Kaposiform Hemangioendotheliomas and Tufted Angiomas. Kaposiform hemangioendotheliomas (KHEs) and tufted angiomas are rare vascular tumors with locally aggressive and benign growth potential, respectively. They are thought to exist along a spectrum and are associated with life-threatening KMP. KMP is a profound and life-threatening thrombocytopenia that results from intralesional platelet trapping with a consumptive coagulopathy evidenced by elevation of D-dimer levels, reduction in fibrinogen levels, and prolongation of prothrombin time and partial thromboplastin time. The severity of the coagulopathy is variable.

Tufted angiomas (Fig. 95.12) typically present in infancy or early childhood as a solitary dusky erythematous indurated vascular plaque with overlying hypertrichosis, hyperpigmentation, and/or telangiectasias.[96,97] Tufted angiomas tend to not be as aggressive and do not have as deep an infiltration as their counterpart kaposiform hemangioma. Spontaneous regression has been reported in some congenital cases or cases with earlier onset.[98]



• **Fig. 95.11** Noninvoluting congenital hemangioma presenting as an unchanged vascular plaque in a 1-year-old. There are coarse telangiectasias with a blue background and surrounding vasoconstrictive halo.

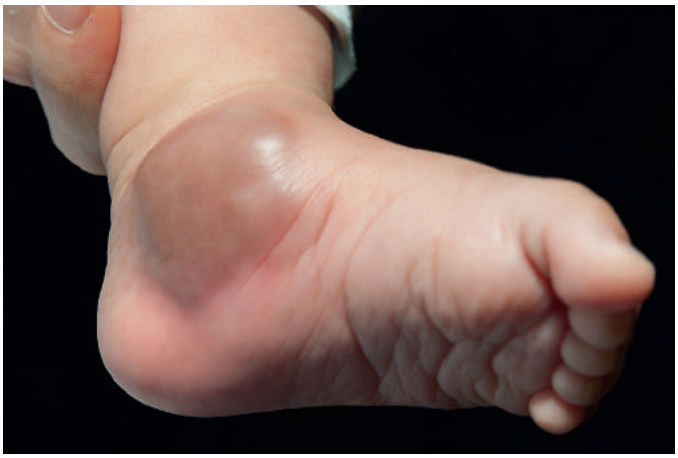
KHE presents as a solitary ill-defined red-purple plaque during infancy or early childhood. It can extend to involve the viscera, chest wall, and retroperitoneum and may present without cutaneous findings. KHE is more commonly seen in neonates and infants, as compared with older children, and may be associated with an increased risk of KMP.[99] Treatment can be either medical or surgical. A recent randomized controlled trial showed that sirolimus plus prednisolone was superior to sirolimus monotherapy for KHE complicated by Kasabach-Merritt phenomenon.[100]

Pyogenic Granulomas. Pyogenic granulomas, also known as *lobular capillary hemangiomas* because of their histologic appearance, are benign acquired vascular tumors that are commonly seen in infants and children (Fig. 95.13).[101] They have also been seen to develop within an existing port-wine stain. They present clinically as a rapidly growing solitary, red papule that can sometimes be exophytic or pedunculated in nature. They often present with a crusted or eroded surface because of their friable nature. They do not spontaneous involute, and treatment is often pursued because of recurrent episodes of bleeding and their friability. Treatment options include simple curettage or shave excision with electrocautery of the base, which is most definitive and curative. Other treatment options include topical therapies, such as topically administered timolol therapy, a beta blocker, imiquimod therapy, or pulsed dye laser treatment, which have variable success with prolonged treatment courses. Recurrence is possible.

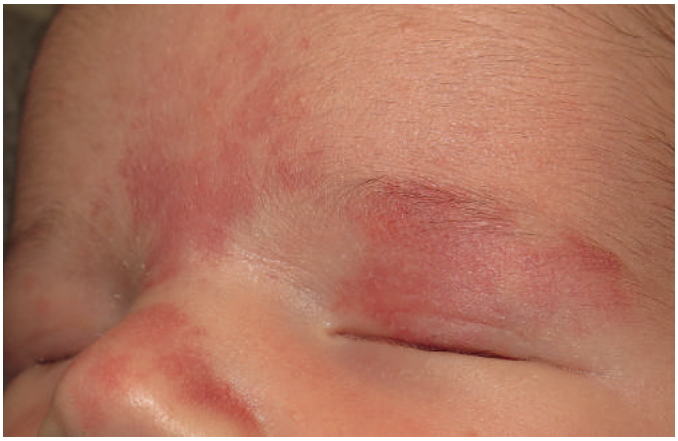
Vascular Malformations

Nevus Simplex

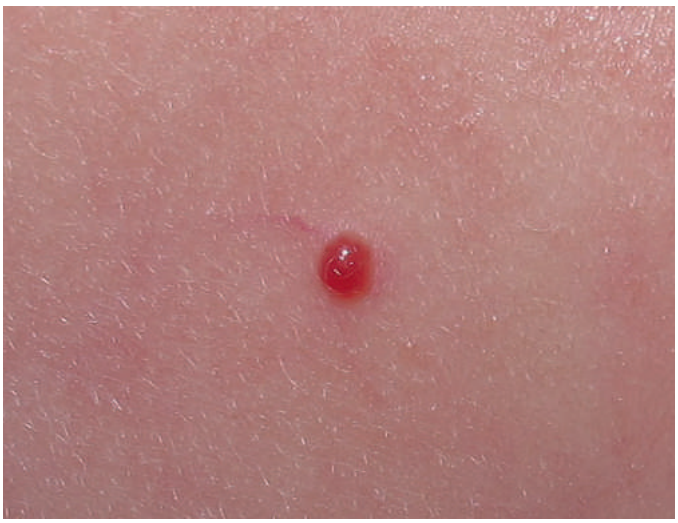
Nevus simplex, also known as a *salmon patch* or *fading capillary stain* and colloquially referred to as *angel's kiss* when it occurs on the forehead or eyelids and as *stork bite* when it occurs on the nape of the neck, is a very common capillary malformation that almost always spontaneously fades by 1 to 2 years of age (Fig. 95.14), although some malformations persist, most notably those at the nape of the neck (Fig. 95.15). It typically presents as a bilateral and symmetric faint pink patch with a feathery border and a midline predilection and most commonly occurs on the central forehead, glabella, upper eyelids, nose, upper lip, nape of the neck, posterior occiput, and the lower back. Nevus simplex located at the midline in the lumbosacral area is usually a benign finding in isolation and not associated with underlying spinal dysraphism, but if there are any other cutaneous findings in this area (i.e., lipoma, hypertrichosis, faun tail, aplasia cutis) or abnormalities noted (i.e., gluteal cleft deformity), then further work-up is



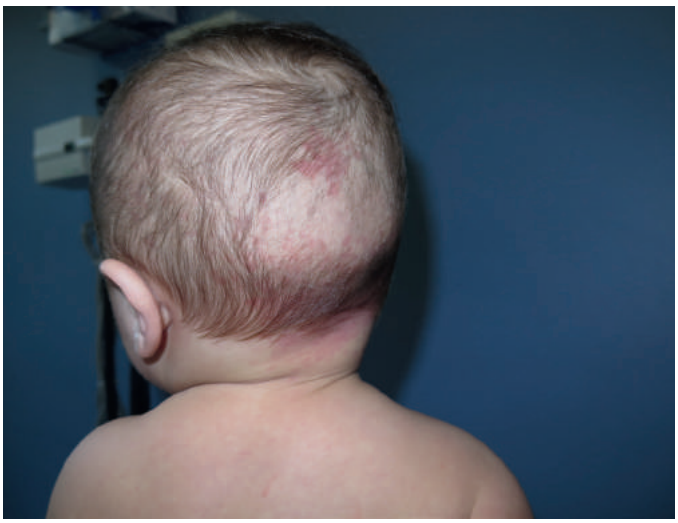
• **Fig. 95.12** Tufted angioma without Kasabach–Merritt phenomenon on the foot of a 4-month-old child. This was confirmed by biopsy.



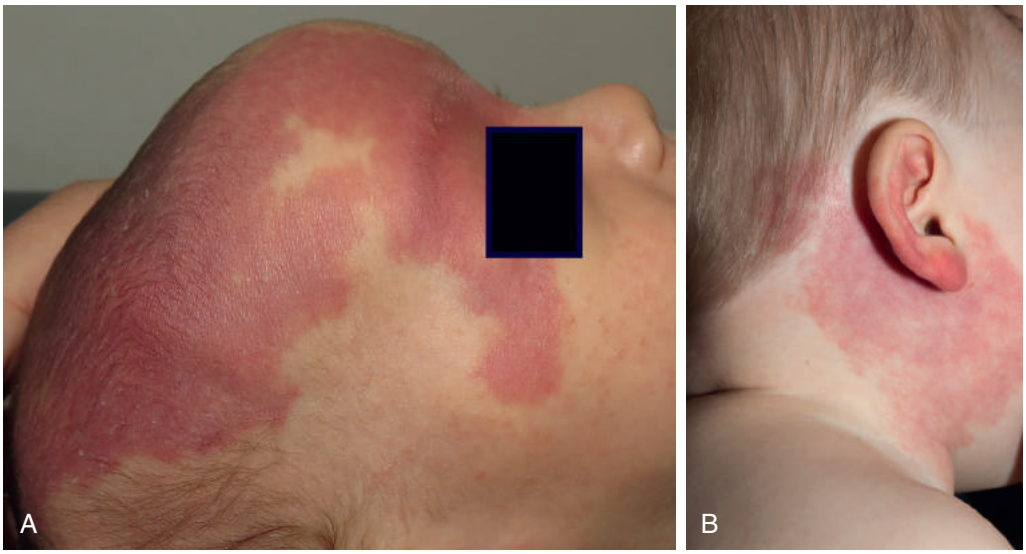
• **Fig. 95.14** Nevus simplex on the glabella, nose, and eyelids of a newborn presenting as faint pink patches with feathery borders.



• **Fig. 95.13** Pyogenic granuloma on the cheek of an 8-month-old infant presenting as a bright-red glistening papule.



• **Fig. 95.15** Nevus simplex on the posterior occiput. Nevus simplex in this location can often persist.



• **Fig. 95.16** (A) Port-wine stain of the periocular area, forehead, and scalp. (B) Port-wine stain involving the posterior auricular scalp, neck, cheek, and ear.

warranted. Prominent and persistent nevus simplex can be associated with underlying syndromes such as Beckwith–Wiedemann syndrome, macrocephaly–capillary malformation syndrome, and Nova syndrome (see Table 95.2).[102]

Capillary Malformation (Port-Wine Stain)

The terms *capillary malformation* and *port-wine stain* are used synonymously in the literature and refer to collections of malformed and ectatic dermal capillaries that persist over time (Fig. 95.16). They are present at birth and occur in 0.3% of neonates. They present as fairly well-demarcated red or pink patches that are often asymmetric, occur anywhere on the body, and may or may not be associated with localized overgrowth. They grow proportionally to the individual's somatic growth and over time can gradually darken from pink red to a darker red and deep purple. They also tend to thicken over time, developing a papulonodular surface change and can develop localized soft tissue or even bony hypertrophy. Nodular vascular growths called *pyogenic granulomas* (see earlier) can also develop within the existing port-wine stain and may require therapeutic intervention.

Port-wine stains can occur in isolation but also can be related to an underlying genetic disorder (see Table 95.2). Of these syndromes, SWS is the most common. SWS is a sporadic neurocutaneous syndrome characterized by facial capillary malformation most commonly involving the upper face and forehead, leptomeningeal vascular malformation that can lead to seizures, hemiparesis, developmental delay, malformations of the choroid, and ophthalmologic abnormalities, most commonly glaucoma. SWS occurs in less than 30% of infants with facial port-wine stains. The risk is increased in infants with more extensive CMs of the face, notably those that have hemifacial or median forehead patterns of involvement, previously thought to be located in the V1 trigeminal region. Patterns associated with SWS are now thought to correlate with patterns of mosaicism[103] rather than trigeminal nerve distribution. A recent study demonstrated that those children with smaller forehead capillary malformations (less than half of the "hemi-forehead") were at very low risk for brain involvement. This can help neonatal care providers in risk assessment.[104]

The degree of CNS involvement is variable in SWS, ranging from subclinical lesions to intractable seizures and intellectual impairment. Patients can still be at risk of glaucoma without SWS when the port-wine stain involves the forehead or upper or lower eyelid, V1 or V2 trigeminal pattern; therefore, complete ophthalmologic examination is indicated for affected infants. Both SWS and isolated CMs have been found to be due to an activating somatic mosaic mutation in the *GNAQ* gene.[105] In SWS, this mutation is also found in affected brain tissue, which is not seen in patients with isolated CMs. It is hypothesized that earlier timing of the mutation can lead to more extensive neurocutaneous involvement.

Treatment of an uncomplicated CM is aimed at minimizing disfigurement. With time these lesions can thicken and develop irregular surface changes, often with friable nodules. Most of the published data on laser treatment of port-wine stains in children come from studies using a pulsed dye laser.[106] Children require an average of 4 to 10 pulsed dye laser treatments for maximal lightening. The best results have been seen in children younger than 4 years, with studies recommending earlier onset of therapy to achieve the best results. Some studies describe use of laser treatment in patients as young as 4 weeks. In younger age groups, 20% can expect 95% clearing.[107] Pulsed dye laser therapy is less effective for facial port-wine stains that are close to the midline or those on the extremities.[108,109] Laser therapy can yield remarkable improvement for many port-wine stains, minimizing the emotional pain that accompanies facial disfigurement. Unfortunately, none of the currently available lasers is capable of permanently erasing port-wine stains in most patients. There are also reports of darkening of port-wine stains many years after effective lightening.[110] Laser treatment with the 1064-nm neodymium-doped yttrium aluminum garnet (Nd:YAG) laser and the 755-nm alexandrite laser has also been described to penetrate deeper vessels in more refractory port-wine stains on the basis of the theory that the longer wavelength of the second laser may be able to better target deeper vessels.[111] Recently, the use of topically administered rapamycin (an mTOR inhibitor) in conjunction with pulsed dye laser treatment has been used for refractory port-wine stains, with the hypothesized mechanism being decreased angiogenesis after laser therapy.[112]

Venous Malformations

Venous malformations are slow-flow vascular malformations composed of ill-defined venous channels with abnormal vessel walls that lack smooth muscle cells. They are often evident at birth, and present as soft, blue-purple tumors or plaques with tortuous vessels that increase in size with exertion or when in a dependent position (Fig. 95.17). Over time, they may become symptomatic and larger with increased dilation of vessels. They may affect the skin, mucous membranes, subcutaneous tissue, muscles, and joints, and can involve visceral organs. Most cases are sporadic, but familial cases have also been reported. Localized intravascular coagulation can occur within the venous malformation (VM) that leads to either formation of thrombi, which form into calcifications called *phleboliths*, which can be quite painful, or bleeding.[113] Glomuvenous malformations are a distinct subset of VMs that present more superficially as blue–purple, cobblestoned plaques and nodules commonly located on the extremities. Glomus cells distinctly line the malformed veins. Familial cases caused by mutations of the glomulin gene (*GLMN*) are more common than sporadic cases.

Lymphatic Malformations

Lymphatic malformations (LMs) are slow-flow vascular malformations. They can be classified as microcystic when individual malformed channels are smaller than 1 cm and macrocystic (i.e., cystic hygroma) when individual channels are larger than 1 cm, combined, or rarely generalized. LMs are most often seen in the head and neck region and can cause life-threatening airway compromise in neonates. They can occur as an isolated finding or can be seen in association with underlying syndromes (see Table 95.2). Some isolated LMs and those related to overgrowth syndromes are caused by a mutation in the gene encoding phosphatidylinositol 4,5-bisphosphate 3-kinase, catalytic subunit alpha (*PIK3CA*).[114] Therefore, in some cases, rapamycin (the mTOR inhibitor also used for refractory port-wine stain) has been used as a medical therapeutic option. Other possible therapeutic interventions include surgical excision for localized lesions, sclerotherapy, and laser treatment with a pulsed dye laser and a long-pulse Nd:YAG laser for superficial lesions (Fig. 95.18), but many lymphatic malformations, particularly cervicofacial lesions, require a multidisciplinary approach.[115]

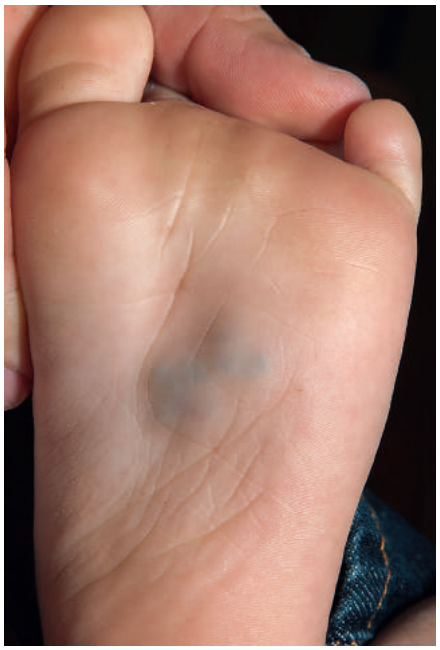
Arteriovenous Malformations

Arteriovenous malformations (AVMs) are fast-flow vascular malformations that are characterized by direct shunting of blood between the arteries and veins with bypassing of the capillaries. AVMs may present as a capillary malformation on the skin with pulsatility and significant warmth. The formation of vascular blebs that can easily bleed may also be seen. They can present within the skin but also viscerally anywhere in the body. The head and neck region, including the brain, are common locations. AVMs can occur in isolation or in association with various genetic syndromes (see Table 95.2). Recently, mutations in *RASA1*, associated with familial forms of AVMs, have been found in individuals with an AVM of an extremity (Parkes Weber syndrome) and in individuals with CM-AVM syndrome, where individuals present with multiple cutaneous CMs, many with a "thumb print"–like quality and potential AVMs in the brain and/or spine.

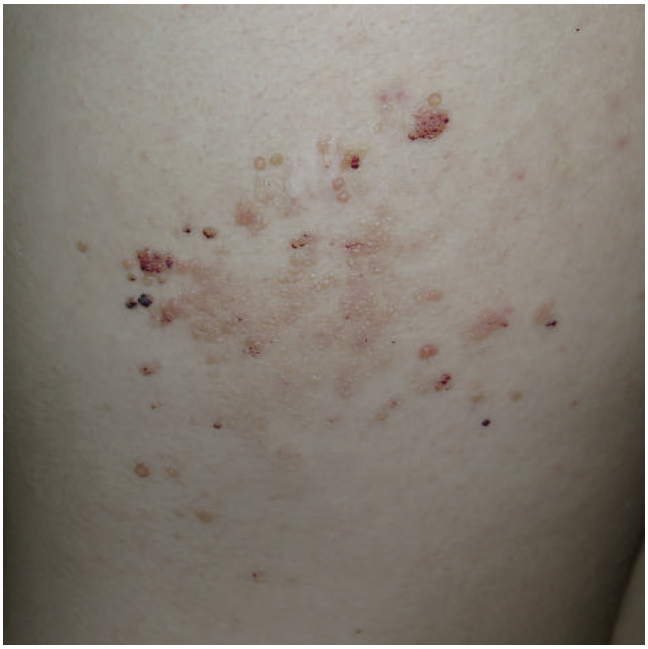
These lesions do not resolve spontaneously and are more likely to become larger and more symptomatic over time. Complications are related to the flow rate and extent of the lesion. Localized thrombosis and phlebitis occur in low-flow lesions; high-flow lesions can cause significant bleeding, destructive interosseous changes (Fig. 95.19), and high-output cardiac failure. Many centers now have collaborative multidisciplinary groups to help manage the most complicated vascular malformations and vascular tumors, which typically require treatment by physicians in many specialties.

Lymphedema

Lymphedema is a term used to describe diffuse soft tissue swelling characterized by firm, pitting edema. Lymphedema can occur in the setting of anomalous lymphatic drainage. Congenital variants have been reported. Females are affected more frequently than males. The lower limbs are the most commonly affected sites, but other sites may also be involved, and, rarely, chylothorax or ascites may be present. *Milroy disease* is an autosomal dominant

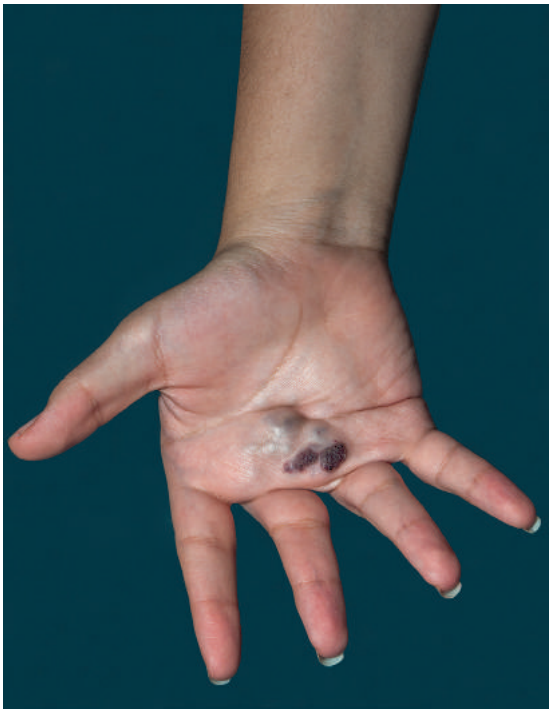


• **Fig. 95.17** A venous malformation on the sole of the foot.



• **Fig. 95.18** A superficial lymphatic malformation on the back presenting with characteristic hemorrhagic papules and vesicles.

condition that manifests itself with progressive lymphedema of the lower extremities. Lymphedema of the extremities also occurs in *Turner (XO) syndrome*. Disorders associated with lymphedema are listed in Table 95.4.



• **Fig. 95.19** Arteriovenous malformation of the hand causing distortion and contracture of the hand.

TABLE 95.4 Hereditary Lymphedema Syndromes (Primary Lymphedema)

Syndrome	Clinical Features	Genetics
Nonne-Milroy disease (primary congenital lymphedema)	Congenital lymphedema of the lower limbs Associated with enlarged veins of the leg, hydrocele and recurrent cellulitis	Mutation in *VEGFR3*, autosomal dominant
Meige disease/ lymphedema–distichiasis (pubertal-onset lymphedema)	Most common type of lymphedema Can be associated with distichiasis, yellow nails, ptosis, syndactyly, cleft palate, and cardiac septal defects	Mutation in *FOXC2*, autosomal dominant
Hypotrichosis–lymphedema–telangiectasia	Lymphedema, sparse hair, and telangiectasias of the skin	Mutation in *SOX18*, autosomal dominant
Hennekam syndrome (generalized lymphatic dysplasia)	Lymphedema and multiorgan involvement (intestinal lymphangiectasia, protein-losing enteropathy), developmental delay	Mutations in *CCBE1* (some cases), autosomal recessive

Disorders of Pigmentation

Hypopigmented Lesions

Localized areas of hypopigmentation on the skin of the newborn may be isolated phenomena, or they may be markers of extracutaneous abnormalities. The degree of hypopigmentation and the distribution of the defect help distinguish among the different conditions. To properly evaluate a patient with hypopigmentation, a distinction must first be made between complete depigmentation and hypopigmentation. A depigmenting condition produces pure white lesions that are devoid of normal melanocytes. Even in fair-skinned infants the lesions can often be easily seen in ordinary daylight. This group of disorders includes tyrosinase-negative oculocutaneous albinism, piebaldism, and vitiligo, all of which are rarely seen in infancy. A hypopigmented lesion is often subtly lighter in color than the surrounding skin. Histologic examination reveals a normal number of melanocytes. In fair-skinned children these lesions may require Wood lamp illumination to become obvious. This group includes anomalies with a deficient amount of melanin or hemoglobin caused by vasoconstriction, causing pallor and decreased skin pigment. Nevus anemicus and hemangioma precursors are two examples of areas of pallor that result from diminished superficial blood flow. Here we will discuss disorders of cutaneous mosaicism presenting as patterned pigmentation on the skin.

Nevus Depigmentosus (Nevus Achromicus)

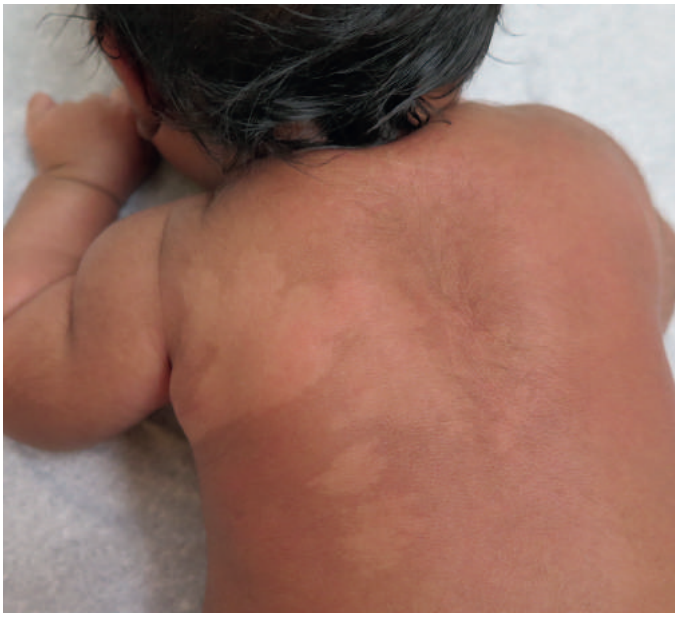
Nevus depigmentosus is an uncommon condition occurring in 0.4% of newborns.[116] It presents as a well-demarcated hypopigmented patch with irregular borders that can involve a small, isolated circular or rectangular area or a larger segmental region following the lines of Blaschko, which are embryonic lines of ectodermal cell migration (Fig. 95.20). The patches are not truly depigmented as the name suggests but actually have reduced melanin levels. Nevus depigmentosus is thought to be a form of cutaneous mosaicism and results in a postzygotic somatic mutation that leads to a population of cells with decreased melanogenesis potential. They usually present at birth or shortly thereafter and grow in proportion to the child's overall growth, thus maintaining its shape. They occur sporadically, with no familial pattern of inheritance. There have been case reports of seizures, cognitive delays, and ipsilateral extremity hypertrophy, but most affected individuals do not have any extracutaneous abnormalities.[117] Sometimes, lentigines can be seen within the hypopigmented area. These are thought to be areas within the nevus where there is resolution of the mutation and a return of pigmentation.[118]

• **Fig. 95.20** Nevus depigmentosus presenting as a well-demarcated hypopigmented patch that tends to be circular or oval.

Pigmentary Mosaicism

The term *pigmentary mosaicism* is representative of a group of heterogeneous disorders of hypopigmentation and/or hyperpigmentation that include hypomelanosis of Ito (incontinentia pigmenti achromians), linear and whorled nevoid hypermelanosis that present as unilateral or bilateral linear streaks, and whorls of cutaneous pigment change oriented along the lines of Blaschko (Fig. 95.21). They can also present in a block-like or checkerboard pattern or a phylloid or leaf-like pattern. Affected individuals may have areas that are hyperpigmented or hypopigmented, or both. The pattern may be congenital or may become apparent within the first 2 years after birth.

This condition is a form of somatic mosaicism. Various extracutaneous abnormalities (CNS, ocular, cardiac, and musculoskeletal)[119–122] have been reported in patients, but the vast majority of individuals have pigmentary changes confined only to the skin. CNS abnormalities, manifesting themselves as seizures or developmental delay, most often present by the age of 2 years.



• **Fig. 95.21** Pigmentary mosaicism presenting as a segmental block-like hypopigmented patch on the back of an infant.

Ash Leaf Macules

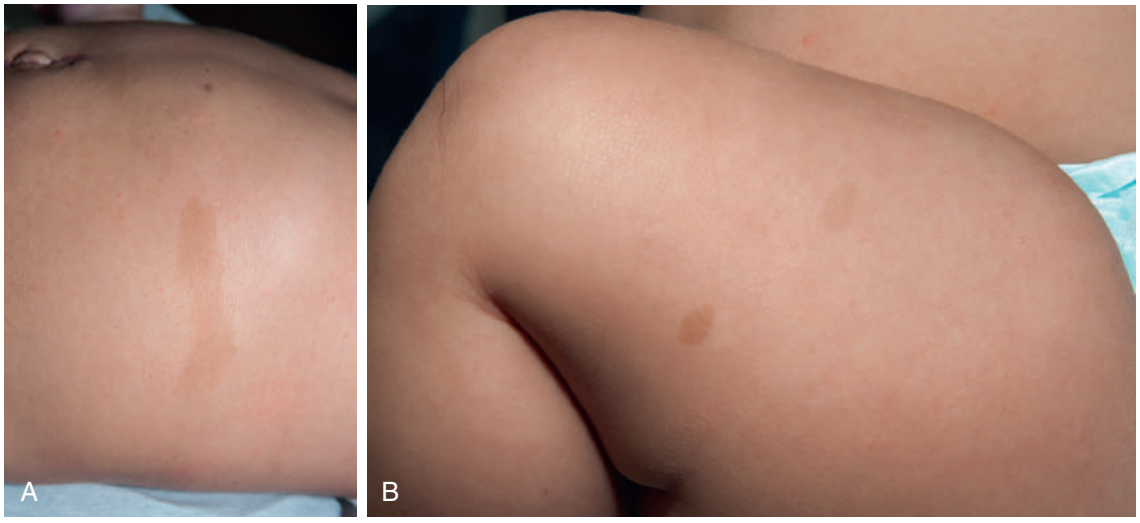
Ash leaf macules are small oval areas of hypopigmentation, named for their similarity in size and shape to a leaflet from a European mountain ash tree. They are one of the few congenital markers for infants with tuberous sclerosis. Tuberous sclerosis complex is a disorder of autosomal dominant inheritance with variable clinical manifestations characterized by the development of benign and malignant tumors in a variety of tissues, including the skin, CNS, and kidney.[123–125]

Hyperpigmented Lesions

Brown lesions usually reflect an increased number of melanocytic cells or an excess amount of melanin. Brown coloration can also be associated with a thickened epidermis. Most congenital brown lesions are isolated and benign, but it is important to recognize that some of them are syndrome associated and others may be potentially life threatening.

Café au Lait Macules

Café au lait macules (CALMs) are well-demarcated, oval or round, light brown macules or patches that differ in size, ranging from several millimeters to several centimeters in diameter (Fig. 95.22). They are commonly noted at birth or during infancy but may present in childhood as well. They cannot always be distinguished from melanocytic nevus on clinical grounds, but histologic examination is diagnostic, showing increased melanin levels within the basal keratinocytes, without melanocyte proliferation. CALMs differ within different ethnic groups, with a higher number and prevalence seen in darker-skinned individuals. The prevalence of CALMs is approximately 2.7% in the general population, 2% in white infants, and up to 12% in African-American infants. Large or segmental café au lait patches can be seen in McCune–Albright syndrome,[126–128] and multiple CALMs in the neonatal period may be an isolated finding but should alert the physician to the possibility of an associated syndrome, especially when six or more



• **Fig. 95.22** (A) Café au lait patch on the abdomen presenting as a tan brown well-demarcated patch. (B) Two café au lait spots presenting on the lateral thigh as well-demarcated tan brown macules.

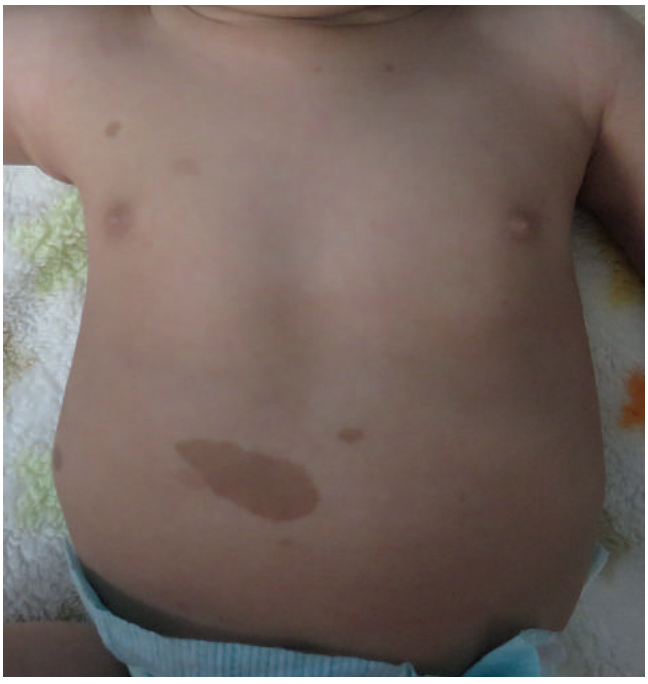
are present (Box 95.1; Fig. 95.23). Six or more cafe au lait lesions measuring 0.5 cm or greater in diameter in prepubertal children and 1.5 cm or greater in diameter in post pubertal children should alert the practitioner of the possibility of neurofibromatosis type 1 (NF1). Presence of cafe au lait lesions alone do not fulfill NF1 criteria.[129]

Lentigines

Lentigines are small tan-to-dark brown macules that most commonly appear sporadically in adulthood. They may be distinguished from other pigmented lesions by histologic examination that reveals elongated rete ridges, an increased number of singly dispersed melanocytes along the basal layer, and increased melanization of the basal keratinocytes. Multiple or congenital lentigines are features of several syndromes.

• BOX 95.1 Syndromes Associated With Multiple Café au Lait Macules

Neurofibromatosis type 1 and type 2
Legions syndrome
Watson syndrome
McCune–Albright syndrome
Noonan syndrome
Noonan syndrome with multiple lentigines (LEOPARD syndrome)
Tuberous sclerosis
Bloom syndrome
Russell–Silver syndrome
Turner syndrome
Bannayan–Riley–Ruvalcaba syndrome
Ataxia–telangiectasia



• Fig. 95.23 Multiple café au lait spots presenting on an infant with neurofibromatosis type 1. The presence of six of more café au lait spots larger than 0.5 cm in diameter on an infant and larger than 1.5 cm in adolescents is suggestive of the possibility of neurofibromatosis type 1. The presence of only café au lait spots does not meet the criteria for definitive diagnosis.

Congenital Dermal Melanocytosis (Mongolian Spots)

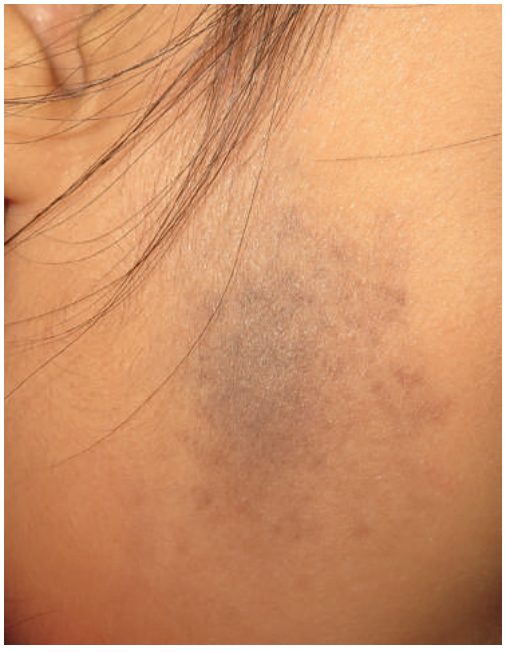
More than 90% of African Americans, 81% of Asians, and 10% of whites[130] are born with blue-gray macule of infancy, formerly known as *Mongolian spots*. These are brown, gray, or blue macules and patches, most commonly located in the lumbosacral area, but they can occur anywhere on the body. The macules may be single or multiple and range in size from a few millimeters to several centimeters in diameter. They often fade within the first few years after birth. Extensive lesions have been mistakenly attributed to abuse. Histologically, blue-gray macule of infancy is a collection of spindle-shaped melanocytes located deep in the dermis. Malignant change has never been reported.

Nevus of Ota/Ito

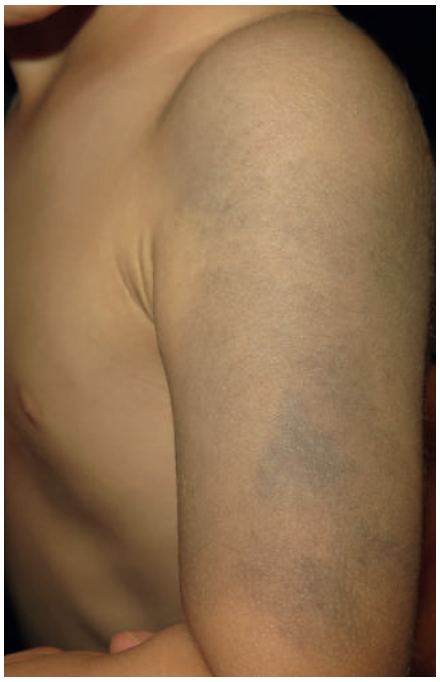
Nevus of Ota is a unilateral blue or gray discoloration involving the orbital and zygomatic areas, following the ophthalmic and zygomatic branches of the trigeminal nerve, including the sclera and fundus (Fig. 95.24). It is a sporadic condition, but it occurs with the highest frequency in Asians, affecting up to 1% of individuals in Japan,[131] and has a female predominance. The discoloration is detected at birth in 60% of cases. Glaucoma is a frequent complication, occurring in 10% of individuals. A similar lesion, located in the deltotrapezius area, is called *nevus of Ito* (Fig. 95.25). Spontaneous resolution does not occur, and over time these lesions can darken, increase in size, and potentially thicken. Individuals with periorbital involvement should undergo ophthalmologic examination. A rare association with malignant melanoma and uveal melanoma has been reported. Successful treatment has been achieved with Q-switched laser devices—ruby, alexandrite, and Nd:YAG.

Melanocytic Nevi

The category of nevocellular nevi includes congenital or acquired nevomelanocytic neoplasms. Nevomelanocytes are dendritic cells of neural crest origin. Nevocellular nevi have traditionally been categorized by the histologic position of the tumor nests within



• Fig. 95.24 Nevus of Ota on the lateral right cheek of a girl presenting as a blue-gray patch of discoloration.

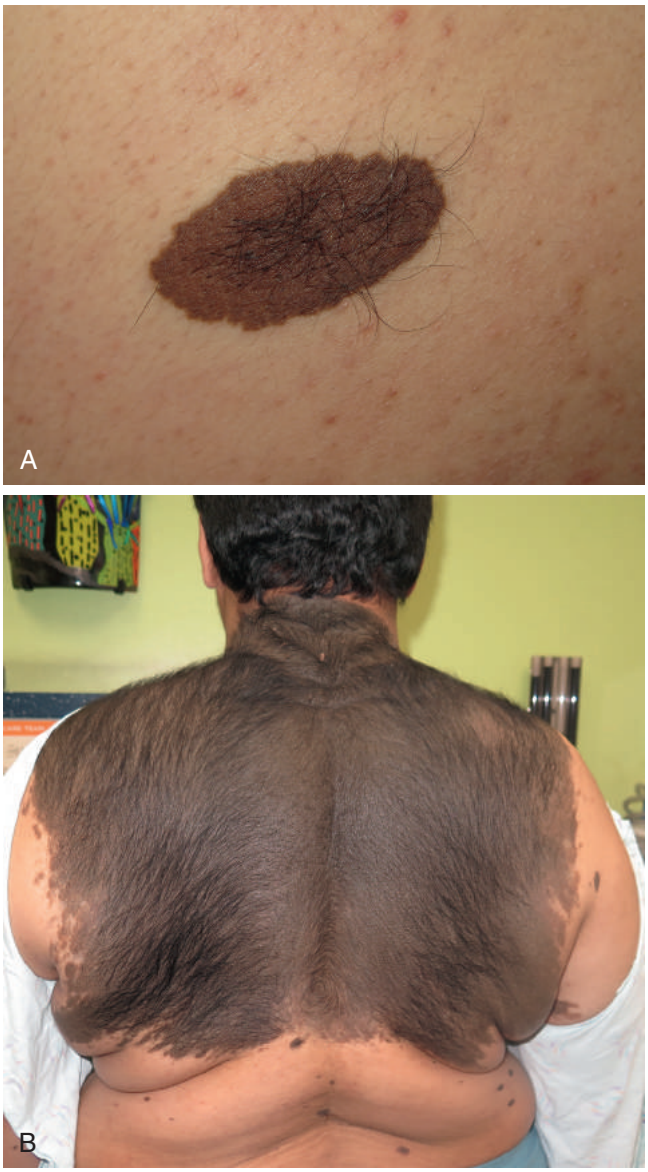


• **Fig. 95.25** Nevus of Ito on the left arm of a child presenting as a blue-gray patch.

the skin. *Junctional nevi* are the most superficial, located at the junction between the epidermis and dermis. These lesions appear clinically as macules. *Intradermal nevi* are located deep to the dermoepidermal junction and are usually papular. *"Blue" nevi* are a variant located in the deep dermis, made up of cells that have elongated, neural features. *Compound nevi* have both junctional and dermal nests of nevomelanocytes. Melanocytic nevi can be further subdivided by their time of onset: congenital, early onset (before the age of 2 years), and acquired.[132,133]

Congenital Melanocytic Nevi

Congenital melanocytic nevi (CMN) are hamartomas derived from neural crest cells that form in utero and are most often present at birth or within the first year (Fig. 95.26). They are classified by the largest diameter of their adult projected size. On the basis of size classification, they are categorized as small (<1.5 cm), intermediate sized (1.5 to 19.9 cm), large (20 to 40 cm), and giant (>40 cm). Delineations are based on adult data. Conversion factors have been developed to help predict ultimate size: for CMN on the head, multiply size by 1.7; CMN on the lower extremities, multiply size by 3.3; and for CMN on the trunk, upper extremities, and feet, multiply size by 2.8.[134] On the basis of these classifications, the estimated incidence of small and intermediate-sized CMN is approximately 1% to 6%, that of large CMN is approximately 1 in 20,000, and that of giant CMN is approximately 1 in 500,000.[135] Melanoma in small and intermediate-sized nevi tends to be approximately 1% lifetime risk or less, nearing the risk within the general population, with most cases occurring after puberty. In large and giant CMN, the risk of melanoma development ranges from 4.5% to 10%,[134,136–141] with 70% of melanomas diagnosed before the age of 10 years. Other malignancies have also been reported to arise from CMN. These include rhabdomyosarcoma, liposarcoma, malignant peripheral nerve sheath tumors, and other sarcomas. In addition to cutaneous malignancy, large and giant



• **Fig. 95.26** (A) Congenital melanocytic nevus with hypertrichosis, small size. (B) Large congenital melanocytic nevus involving the neck and back with multiple smaller "satellite" nevi on the arms and lower back.

CMN have been associated with the development of neurocutaneous melanosis (NCM), which is characterized by CNS proliferation of melanocytes.[142] Symptomatic NCM manifests itself with signs or symptoms of increased intracranial pressure and carries a very poor prognosis, with an increased incidence of CNS melanoma.[143,144] NCM often becomes symptomatic within the first few years after birth. MRI can aid in the diagnosis.[145] Although initial reports emphasized thickening of the leptomeninges, the most common MRI sign of NCM is the spin–lattice (T1) nuclear magnetic relaxation time in the parenchyma of the cerebellum or anterior temporal lobes (sometimes accompanied by the spin–spin [T2] relaxation time). Radiologic identification of malignant degeneration is difficult. Roughly half of asymptomatic infants and children with giant CMN have evidence of NCM on MRI.[143] The presence of asymptomatic NCM does not necessarily bode a poorer prognosis, and NCM can continue to be present and asymptomatic throughout life. The term *CMN syndrome* has now been used to broaden the scope of NCM to include more

extracutaneous manifestations associated with CMN. Patients with NCM are at risk of developing CNS melanomas, but this risk is significantly higher in individuals with symptomatic NCM. The risk of developing NCM has been correlated with the number of accompanying CMN, historically termed *satellite lesions*, with patients who have more than 20 such lesions carrying a five times greater risk of NCM. Neonatal care providers have the unique opportunity to consider a "feed and swaddle" MRI upon recognition of NCM risk, thereby potentially avoiding later exposure to general anesthesia.[146]

Conservative management of large congenital nevi by surveillance alone is complicated by the presence of features that may fit the screening ABCDE criteria (asymmetry, border irregularity, color variegate, diameter >6 mm, evolution) for a lesion that is suggestive of melanoma. Most of these nevi have an irregular surface appearance from birth and are variably thickened, hairy, verrucous, or nodular. Smaller, widely scattered satellite lesions are almost always present, but there have been no reports of melanoma in satellite lesions. Extracutaneous lesions have also been detected in several sites, including the meninges, lymph nodes, and placental villi.[147] Often these nevi have atypical histologic features as well. For children who develop malignant melanoma within a giant nevus, the prognosis is very poor. However, many of the lesions that have an alarming appearance at birth do not exhibit malignant behavior or produce widespread metastases or cause death. In fact, congenital melanoma is very rare and is associated with congenital nevi in less than 50% of reported cases.[141]

The management of CMN remains controversial, with advocates for and against prophylactic excision. Routine excision is generally not recommended and may not eliminate the risk of melanoma completely, given the presence of melanocytes deep with the fascia. Surgical removal is never an easy option. Multiple procedures are usually required, with the attendant high risks of significant morbidity, sometimes yielding results that are more disfiguring than the birthmark. Procedures performed in early childhood, such as tissue expansion[148] and partial-thickness resection,[149] may improve the aesthetic outcome but have associated stigma and pain. The efficacy of such approaches in the prevention of malignancy has never been documented, and it is unlikely to eliminate the risk of malignancy completely.

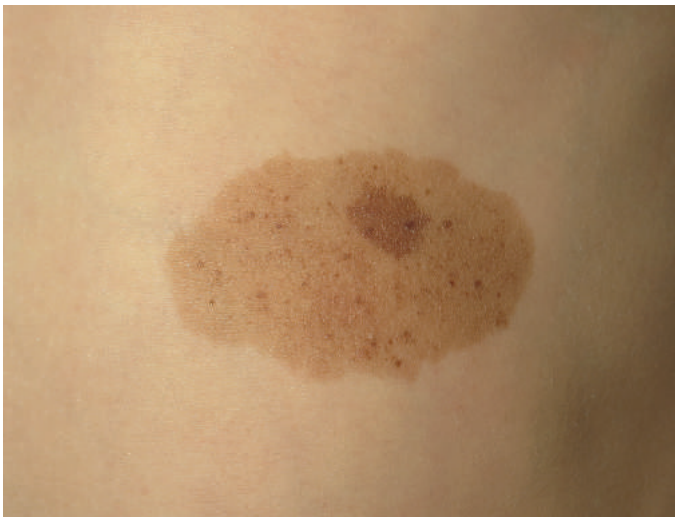
Nevus Spilus

Nevus spilus (speckled lentiginous nevus) is a hyperpigmented lesion that consists of focal proliferation of melanocytes along the basal layer of the epidermis (dark spots) within a café au lait spot in the background (Fig. 95.27). Nevus spilus is considered a distinct subtype of a congenital melanocytic nevus, with the associated risk of melanoma being lower than with traditional CMN. There are no other associated abnormalities.

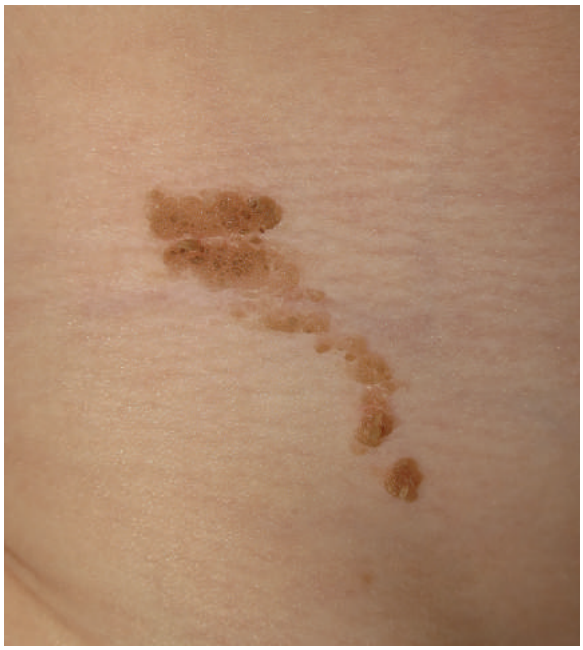
Congenital Tumors of Epithelial Origin

Epidermal Nevus

Epidermal nevus may manifest itself in the newborn period as a smooth hyperpigmented patch or rough, skin-colored plaque, most often on the trunk or extremities, frequently oriented along the lines of Blaschko (Fig. 95.28). With time, epidermal nevi may enlarge, usually within the first few years after birth, and most become verrucous and hypertrophic over time. Treatment is difficult because recurrence is common after destruction or excision.



• **Fig. 95.27** Nevus spilus presenting as a tan patch with multiple darker brown macules and papules within the patch.

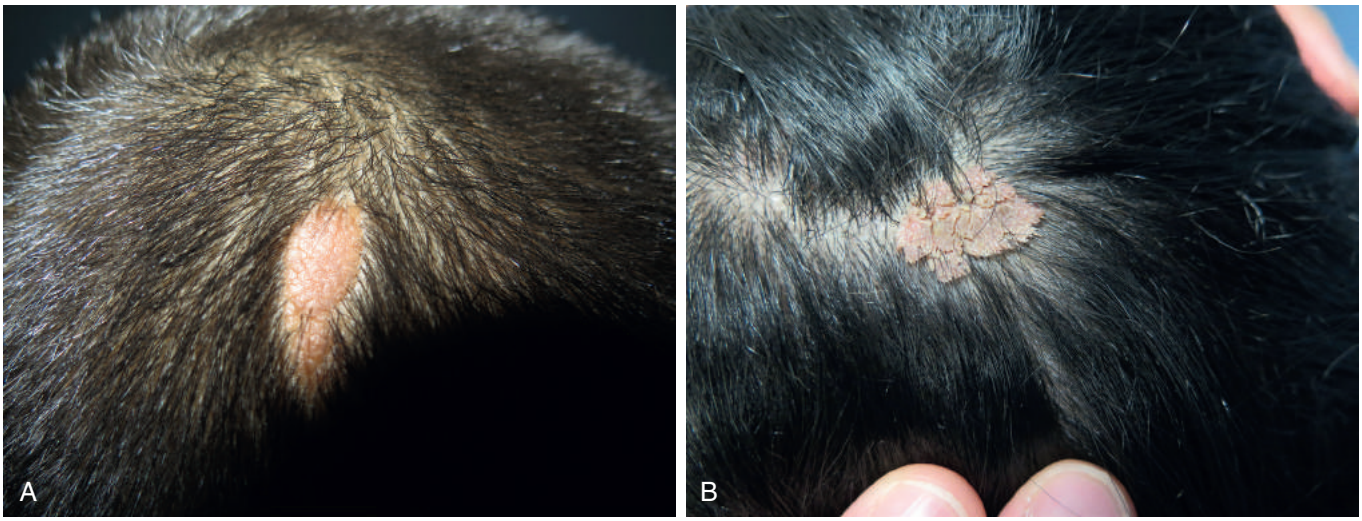


• **Fig. 95.28** Epidermal nevus presenting as verrucous tan brown papules coalescing in a curvilinear plaque.

Nevus Sebaceous

Nevus sebaceous presents as an alopecic tan, yellow- to salmon-colored plaque appearing most often on the scalp or face (Fig. 95.29). It may be nodular at birth and again after puberty, flattening during childhood. A variety of neoplasms, both benign and malignant, including basal cell carcinoma, develop in up to 15% of patients with sebaceous nevi. Development of neoplasms rarely occurs before puberty.

Like other mosaic disorders, epidermal and sebaceous nevi are believed to be localized manifestations of somatic genetic mutations that would be lethal if fully expressed. A subset of patients with epidermal nevi are genetic mosaics for an autosomal dominant form of ichthyosis called *epidermolytic hyperkeratosis* (or *bullous ichthyosiform erythroderma*).[150] These individuals may



• **Fig. 95.29** (A) Nevus sebaceous in an adolescent male presenting as a thickened yellow-pink alopecic plaque with wart-like changes. (B) Nevus sebaceous presenting as an alopecic plaque with verrucous or wart-like changes.

be at risk of having offspring with total body involvement. The striking appearance of epidermal nevi has inspired descriptive nomenclature. *Nevus verrucosus* is a solitary plaque. *Nevus unius lateris* is an extensive linear lesion that is unilateral, following the lines of Blaschko. Both keratinocytic and sebaceous components may occur in the same patient, the former more commonly involving the trunk and extremities and the latter more often involving the head and neck. The term *ichthyosis hystrix* refers to extensive, bilateral involvement with epidermal nevus.

Skin biopsy will rule out other conditions, distinguish between epidermal nevus and nevus sebaceous, and detect the diagnostic histologic features of epidermolytic hyperkeratosis, but is not typically performed. Small epidermal nevi do not require treatment. Nevus sebaceous carries a small risk of malignant degeneration generally later in childhood and adolescence. It may be excised at any time, depending on the size of the lesion, the benefits of surgical intervention versus the risks of anesthesia, and the preference of the patient and family, but rarely becomes symptomatic or much of an issue until after puberty. Recent studies suggest that the risk of basal cell carcinoma, the most common malignancy to arise in nevus sebaceous, is closer to 1%, much lower than was reported in the past.[151]

There is no optimal therapy for larger lesions or those that are disfiguring. Full-thickness excision, including the subcutaneous tissue, is recommended to decrease the risk of recurrence. Laser therapy has been performed with ablative lasers, and there are a few reports suggesting that the Q-switched Nd:YAG laser holds some promise as another therapeutic option. Topically applied keratolytic agents may be palliative. Genetic counseling about the risk for offspring of fully expressed disease should be considered for individuals with epidermal nevi that reveal the histologic features of epidermolytic hyperkeratosis.

Epidermal (Linear Sebaceous) Nevus Syndrome

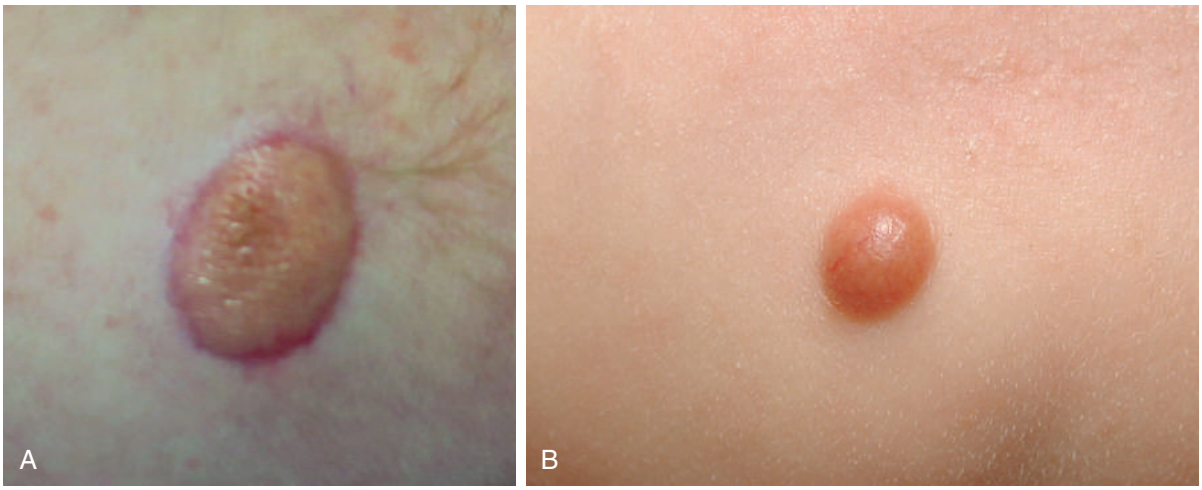
In less than 10% of affected people, epidermal nevi and sebaceous nevi (especially those involving the head) are associated with a variety of extracutaneous abnormalities, mainly ocular (in 33% of cases), neurologic (in 50%), and skeletal (in 70%), a condition referred to as *epidermal nevus syndrome*. Bone abnormalities include vertebral anomalies, kyphoscoliosis, limb shortening, and hemihypertrophy. CNS disorders include seizures, mental retardation, and hemiparesis; ocular abnormalities include eyelid/conjunctival nevus, coloboma, corneal opacity, and nystagmus. Malignancies also occur in this syndrome with a greater than expected frequency, including Wilms tumor, nephroblastoma, gastrointestinal carcinomas, and rhabdomyosarcoma.[152]

Congenital Tumors of Dermal and Subcutaneous Origin

Juvenile Xanthogranuloma

JXG is a benign, self-healing, non–Langerhans cell histiocytic tumor of infancy (Fig. 95.30). JXG is derived from dermal dendrocytes and is present in normolipemic individuals without abnormalities in their lipid metabolism.[153–155] JXG may be congenital in approximately 30% of cases, with 75% presenting within the first year of life.[156] Early on, cutaneous lesions present as erythematous papules with minimal yellow-orange color. As they mature, they become more characteristically yellow in color and may develop overlying telangiectasias. They often present on the head, neck, and trunk, and can be categorized by their size: "micronodular" (<10 mm) and "macronodular" (>10 mm) forms and rarely giant JXG, which can be up to 5 to 10 cm in size. They may present as a solitary lesion or as multiple lesions, with the solitary presentation being most common, occurring in up to 90% of patients. JXG may also be rarely localized to the eye or mucous membranes.[157] Skin biopsy is usually diagnostic, revealing characteristic foamy histiocytes and Touton giant cells within the dermis.

The vast majority of infants with JXG are otherwise healthy. Giant JXG can have an alarming appearance and may be confused with other types of histiocytic tumors.[158] JXGs have two clinically significant associations. The first is ocular JXG and its associated complications. Less than 0.5% of children with skin lesions have ocular involvement, but half of children with ocular JXG have cutaneous lesions.[159] Ocular examination in individuals younger than 2 years with multiple JXGs is recommended. Ocular tumors may manifest themselves as unilateral glaucoma, uveitis, heterochromia iridis, or proptosis, and ocular JXG is the most common

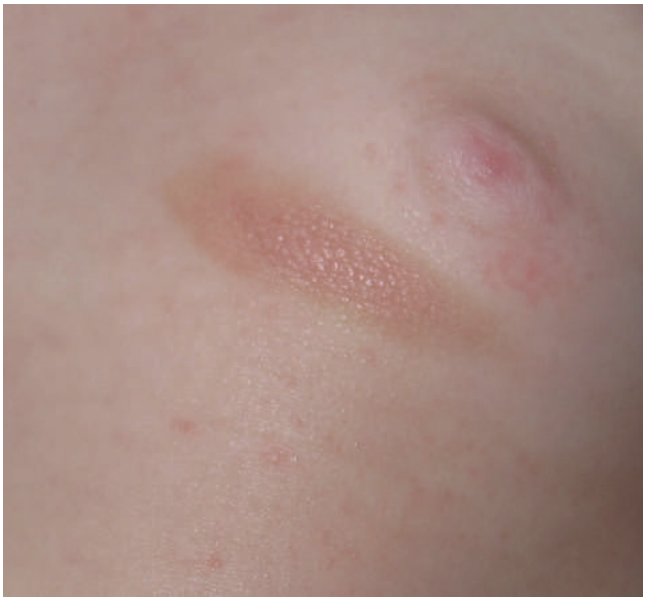


• **Fig. 95.30** (A) Juvenile xanthogranuloma with characteristic tan yellow color and rim of erythema in an infant. (B) Juvenile xanthogranuloma presenting as a yellow-orange papule.

cause of hyphema in infancy.[160,161] The iris is the most frequently affected ocular tissue.[162]

The second significant association is related to reports of a rare triad of JXG, juvenile myelomonocytic leukemia (JMML), and neurofibromatosis type 1 (NF-1). The appearance of JXG usually precedes the diagnosis of leukemia or occurs concurrently at the time of diagnosis, and there are often multiple JXGs.[163,164] Routine screening for JMML is not recommended in NF-1 patients with or without JXG, but evidence of hepatosplenomegaly, lymphadenopathy, or pallor should prompt appropriate work-up.[165] Fewer than 20 patients with intracranial JXG without cutaneous manifestations have been described.[166,167]

Most cases of JXG are asymptomatic and self-limited, but ulceration and bleeding of cutaneous lesions can occur. Giant lesions have a similar prognosis.[158] Ophthalmologic evaluation is indicated for children who present in the first 2 years after birth with multiple lesions. Parents should be provided with anticipatory guidance about the ocular complications.[159] JXG typically involutes within 3 to 6 years.[168] Cutaneous lesions can leave behind residual anetoderma, mild atrophy, and/or hyperpigmentation. Recurrence has been documented after surgical excision, a therapy that is indicated only for lesions that are frequently traumatized, symptomatic, or are more disfiguring than the resultant scar would be.



• **Fig. 95.31** Solitary mastocytoma presenting as a tan brown plaque with characteristic peau d'orange (orange peel–like) surface change.

Mastocytosis

Mastocytosis comprises a group of disorders characterized by increased numbers of tissue mast cells (Fig. 95.31).[169] The skin is the most common site of involvement, but the lymphoreticular system, gastrointestinal tract, and bone marrow also may be affected. Symptoms result from the local or generalized effects of the release of histamine and other mast cell mediators. Pruritus, edema, blistering, and flushing are common. Abdominal pain, diarrhea, and vomiting are unusual. Hypotension is rare.[170] If rubbed or traumatized, skin affected by mastocytosis will develop a diagnostic wheal (Darier sign). The site may blister or become hemorrhagic in a neonate.

Urticaria pigmentosa is the name given to the most common form of mastocytosis in infants, featuring multiple, small (1 to 3 cm in diameter) papules usually located on the trunk. The disease may be congenital but usually manifests itself within the first 6 months after birth.[170] A single, localized lesion is known as a *solitary mastocytoma*. These tumors can range in size from approximately 2 to 6 cm. Diffuse cutaneous mastocytosis is an unusual condition that may manifest itself at birth with widespread blistering or diffuse thickening of the skin. Systemic mastocytosis is more commonly seen in adults and is defined by multifocal lesions in the bone marrow or other extracutaneous organs, together with signs of systemic disease. It is further subdivided into indolent systemic mastocytosis, systemic mastocytosis with an associated clonal hematologic non-mast cell lineage disease, aggressive systemic mastocytosis, and mast cell leukemia.[171]

The diagnosis may be confirmed by a skin biopsy, which reveals mast cell hyperplasia within the dermis. Aminocaproate esterase is the most specific enzyme marker for identification of mast cells. Immunohistochemical stains for tryptase and c-Kit are also sensitive and specific markers for mast cells.[172] Mutations in *KIT*, the gene encoding the receptor for stem cell factor, may

play a significant role in the biology of mast cell malignancies.[173] Plasma histamine levels are elevated in most children with mastocytosis, sometimes to remarkably high levels. Further work-up for evidence of systemic involvement should be limited to pediatric patients with extracutaneous signs and symptoms or those who require general anesthesia.[170]

Caregivers should be educated to avoid exposing infants to factors that trigger mast cell degranulation, such as friction, pressure, temperature extremes, and substances that promote mast cell degranulation (aspirin, alcohol, narcotics, amphotericin B, or iodine-containing contrast media). If general anesthesia is required, perioperative administration of histamine receptor blockers is recommended.[174]

For patients with limited skin involvement, application of potent topical corticosteroids may hasten involution of lesions. Symptomatic patients may benefit from a classic histamine H_1 receptor blocker such as hydroxyzine or cyproheptadine. An H_2 blocker, such as ranitidine, or orally administered disodium cromoglycate may be added in the presence of gastrointestinal symptoms. Hypotension requires corticosteroids in addition to H_1 and H_2 antihistamines and intensive supportive care. Solitary mastocytomas usually involute by school age. Lesions of urticaria pigmentosa typically resolve by puberty.

Connective Tissue Nevus (Connective Tissue Hamartoma)

Connective tissue nevi are hamartomas comprising dermal collagen, elastic fibers, or a combination of the two. They are benign lesions that can occur in isolation or in relation to a genetic syndrome such as tuberous sclerosis or Proteus syndrome.[128] They present as flesh-colored dermal plaques that can be subtle on examination to hypertrophic with a cobblestoned and cerebriform appearance. They are usually asymptomatic and do not often require any intervention. Diagnosis is often made clinically, but if the diagnosis is unclear, a skin biopsy can be performed.

Neurofibroma

Neurofibromas are benign tumors that are composed of neuromesenchymal tissue. They appear as brown or red-brown soft papules or papulonodules. They often present in young adulthood, but there is a variant of a plexiform neurofibroma that can present earlier in infancy and can resemble a congenital melanocytic nevus. Neurofibromas can occur as isolated tumors in an association with an underlying genetic disorder, such as NF-1. They can be surgically excised if they become symptomatic or are cosmetically disfiguring.

Developmental Anomalies of the Skin

Developmental anomalies are present at birth and represent a heterogeneous group of disorders that are caused by a disruption in the formation of vital structures within the skin. They may occur in isolation or they may be a marker of extracutaneous abnormalities and thus require further evaluation.

Midline Anomalies

Congenital midline defects are a distinct group of diagnostically and therapeutically challenging conditions. These anomalies can be markers for potential neural tube dysraphism occurring at the cranial or caudal midline. These markers include dimples, sinuses, skin tags, capillary malformation, hemangiomas, nodules, lipomas, dermoid cysts or sinus, and midline circumscribed or annular hypertrichosis that may represent a marker for an underlying CNS problem or an intracranial connection.[175,176] A midline mass in the nasal area may represent a dermoid cyst or sinus, encephalocele, or glioma.[177] Midline scalp lesions include aplasia cutis congenita, dermoid cyst or sinus, encephalocele, meningocele, and heterotopic brain tissue. The "hair collar sign" may mark ectopic neural tissue and underlying CNS malformations.[178,179] Biopsy of a midline mass should not be performed unless an imaging study, computed tomography, or MRI has been performed to help clarify the nature of the lesion and any intracranial connections, although small intracranial connections may still be missed. If the possibility of an intracranial connection exists, referral to a neurosurgeon is indicated.[176]

Markers of occult spinal dysraphism exist in the lumbosacral region and include various cutaneous midline lesions such as lipomas, skin tags, pseudotails and tails, dimples and sinuses, hypertrichosis, aplasia cutis congenital, dermoid cyst or sinus, pigmentary changes, hemangiomas, CMs, and telangiectasias. The presence of two or more stigmata indicates a higher risk of the presence of occult spinal dysraphism. Box 95.2 stratifies findings on the basis of their risk for occult spinal dysraphism.[180] There are no evidence-based guidelines for imaging recommendations for spinal dysraphism. In general, in high-risk situations, MRI is the preferred imaging modality but is limited by its cost, availability, and need for sedation. In infants up to 6 months old, high-resolution ultrasonography may be performed before the ossification of the vertebral bodies, but it has decreased sensitivity when compared with MRI and has large variability in accuracy depending on the person performing the study. If the ultrasound results are abnormal or inconclusive, MRI should be performed. It is suggested that simple dimples[181,182] and isolated low-risk lesions do not require a screening ultrasound examination.[183] On the neck, a midline pit or nodule may represent a congenital midline cervical cleft[184] or

• BOX 95.2 Risk Stratification of Markers of Occult Spinal Dysraphism

High Risk
- Two or more cutaneous stigmata
- Lipoma
- Acrochordon, pseudotail, true tail
- Aplasia cutis and congenital scars
- Dermoid cyst or dermal sinus
- Infantile hemangioma ≥2.5 cm

Intermediate Risk
- Atypical dimple >5 mm in diameter, >2.5 cm from the anal verge
- Infantile hemangioma <2.5 cm
- Hypertrichosis (faun tail or silky down)

Low Risk
- Hyperpigmentation or hypopigmentation
- Melanocytic nevi
- Simple dimple ≤5 mm in diameter, ≤2.5 cm from the anal verge
- Teratomas
- Port-wine stain (capillary malformation) or telangiectasias

Modified from Sewell MJ, Chiu YE, Drolet BA. Neural tube dysraphism: review of cutaneous markers and imaging. *Pediatr Dermatol.* 2015;32:161–170.

thyroglossal duct cyst or sinus. A bronchogenic cyst or sinus cyst presents as a nodule or pit at the suprasternal notch at birth and is due to remnant respiratory epithelium. Branchial cleft anomalies, cysts, or sinuses do not present at the midline and are located in the preauricular region and lateral neck. They are at risk of infection and therefore can be surgically removed.

Preauricular Pits and Sinuses

Preauricular pits are common congenital abnormalities that present as small depressions or dells, often 1 to 3 mm in size located adjacent to the external ear, usually at the anterior margin of the ascending limb of the helix, but have also been reported along the lateral surface of the crus of the helix and the superior posterior margin of the helix, tragus, or lobule. There may be a sinus tract connected to the pit in the skin, in which case fluid or pus may drain from the opening. Preauricular sinuses are usually asymptomatic and isolated but can be multiple and bilateral in 25% to 50% cases. The bilateral sinuses tend to be inherited in an autosomal dominant pattern. There have been associations with a few genetic syndromes as well (Box 95.3).

Preauricular sinuses are rarely associated with deafness or renal problems. Renal ultrasound screening is recommended only if there are other worrisome features present, such as the presence of dimorphic features, a family history of deafness, a maternal history of gestation diabetes, and auricular and/or renal malformations.[185] Hearing impairment occurs at a higher rate in individuals with accessory tragi, and screening is recommended if a newborn hearing screen was not performed.[186] Symptomatic sinuses that drain fluid or become infected should be cultured and treated with appropriate antibiotics. If recurrent infection occurs, surgical excision by an experienced surgeon is recommended because the sinus can extend to the periosteum with the auditory canal.[187]

Accessory Tragus

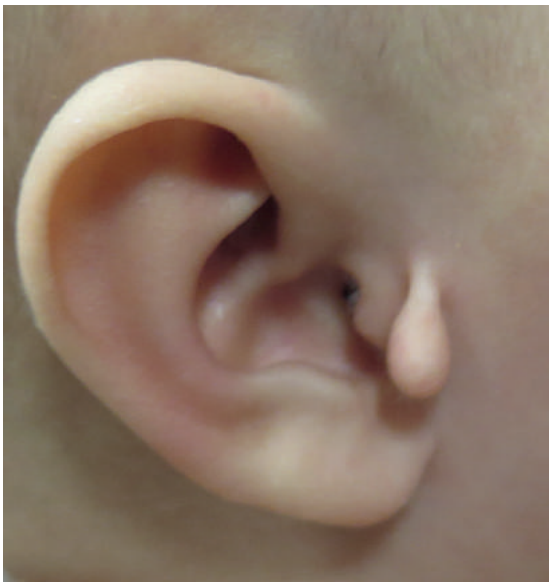
Accessory tragi, inaccurately referred to as *preauricular tags*, are relatively common congenital malformations of the external ear. They present as soft, flesh-colored pedunculated papules that can occur anywhere from the preauricular area to the angle of the mouth following the embryonic fusion line of the mandibular and maxillary branches of the first branchial arch (Fig. 95.32). They are present at birth and can be multiple and/or bilateral. In most cases they occur as an isolated developmental defect, but association with other abnormalities of the first and second branchial arch or branchial arch syndromes can occur (Box 95.4).[188] Association with deafness and renal abnormalities is controversial. The renal ultrasound and hearing screening guidelines are the same as mentioned with preauricular pits and sinuses earlier. Accessory tragi can be limited to the dermis but can also contain cartilage or can be contiguous with the external ear canal. Therefore, if surgical excision is performed, it should be done by an experienced surgeon. Suture ligation should not be done, and can result in complications.

• BOX 95.3 Preauricular Pits and Sinuses and Associated Genetic Syndromes

Branchiootorenal syndrome
Branchiootic syndrome
Branchiootoureteral syndrome
Branchiooculofacial syndrome
Branchiootocostal syndrome
Waardenburg syndrome
Goldenhar syndrome (oculoauriculovertebral syndrome)
Cat eye syndrome

Congenital Cartilaginous Rests of the Neck (Cervical Tabs, Wattles)

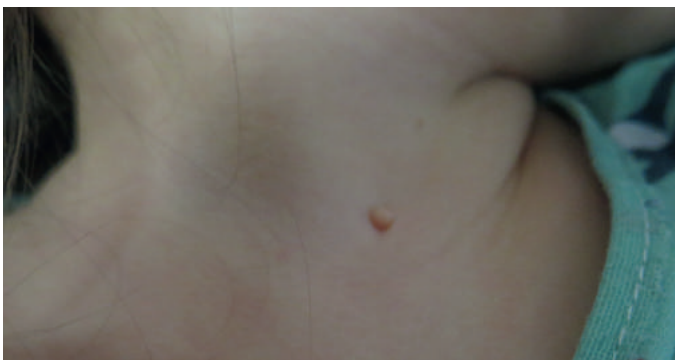
Congenital cartilaginous rests of the neck, also known as *cervical tabs* or *wattles*, are present at birth and are formed from a remnant of the branchial arch or ectopic auricular tissue (Figs. 95.33 and 95.34). They present as soft, flesh-colored papules or nodules that



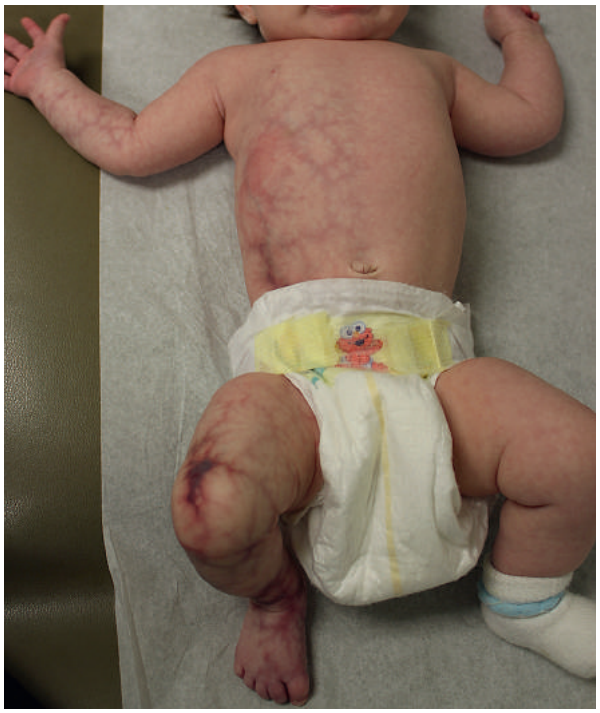
• Fig. 95.32 Accessory tragus.

• BOX 95.4 Accessory Tragi and Associated Genetic Syndromes

Goldenhar syndrome (oculoauriculovertebral syndrome)
Treacher Collins syndrome
Townes–Brocks syndrome
VACTERL syndrome
Wolf–Hirschhorn syndrome



• Fig. 95.33 Congenital cartilaginous rests of the neck, aka *wattles*, presenting as a small flesh-colored papule on the anterior part of the neck.



• **Fig. 95.34** Cutis marmorata telangiectasia congenita.

can occur anywhere on the neck, but most commonly appear over the lower half of the sternocleidomastoid muscle. They may contain cartilage and can be multiple and bilateral. They do not have a deeper connection or sinus or tract associated with them and, therefore, do not require further imaging or treatment. Surgical excision could be considered, but largely for aesthetic purposes.

Supernumerary Digits (Rudimentary Polydactyly)

Supernumerary digits can range in their presentation from subtle small pedunculated papules to full-sized digits. They arise from the lateral surface of the normal digit and may occur on any digit, with the ulnar surface of the fifth finger being the most common site. They often contain cartilage, nerves, and nail. Therefore surgical excision with nerve dissection can be undertaken to remove these accessory digits. Ligation of the supernumerary digit without complete dissection of the nerve can result in a traumatic neuroma, skin necrosis, and infection.[189,190]

Supernumerary Nipples (Polythelia, Accessory Nipples)

Supernumerary nipples can be found anywhere along the embryologic milk lines that course from the axilla to the inner thigh. They present as brown or reddish-brown pedunculated papules and are often bilateral. In the newborn period their clinical presentation is very subtle, as a small light-brown macule. They may present with or without true extramammary tissue and mammary glands. They can be mistaken for melanocytic nevi, warts, neurofibromas, or acrochordons. Usually no further evaluation or treatment in necessary unless glandular tissue is present, in which case complete surgical excision is recommended because of enlargement at puberty causing pain and social stigma.

Median Raphe Cysts (Congenital Sinus and Cysts of Genitoperineal Raphe, Mucous Cysts of the Penile Skin, Parameatal Cysts)

Median raphe cysts present as small white to flesh-colored papules that can be found anywhere on the ventral penis, scrotum, and perineum in a midline position. They can be solitary or multiple. They are believed to result from congenital alterations in embryologic development of male genitalia and are due to incomplete closure of the urethral and/or genital folds, or result from outgrowths of the embryologic epithelium after primary closure of the folds.[191] They are asymptomatic in most cases, and there have been reports of spontaneous regression over time. If they persist, become symptomatic, or infected, simple excision with primary closure can be undertaken.[192]

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96 Eye and Vision Disorders

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KEY POINTS

- Early visual experience drives the architecture of the visual brain.
- Screening eye examinations are important in all infants, regardless of gestational age. All neonates should have an examination of the red reflex before discharge from the newborn nursery.
- The absence of visual responsiveness by 2 months of age should prompt an urgent ophthalmologic evaluation.
- Most full-term infants establish normal ocular alignment within the first 2 months.
- Nystagmus can be a manifestation of a congenital motor entity, secondary to visual pathway defects or neurologic disease. Nystagmus caused by defective vision does not develop until approximately 3 months of age.
- Successful treatment of congenital cataracts is highly dependent on early diagnosis and prompt referral.
- The successful management of retinoblastoma depends on the ability to detect the disease while it is still intraocular; disease stage correlates with delay in diagnosis.
- Neonatal care providers have both a clinical and a medicolegal responsibility to arrange timely diagnostic retinopathy of prematurity (ROP) examinations for preterm babies in the neonatal intensive care unit and to communicate to parents the vital importance of keeping outpatient ophthalmology appointments. Even a one-week delay can result in adverse outcomes.
- Improvements in neonatal care and ophthalmic surgical techniques have led to improved outcomes for infants who develop ROP.
- There are limitations to current ROP screening guidelines, and incorporation of additional factors such as postnatal weight gain can result in more precise risk stratification.
- Newer technologies for ROP screening that include telemedicine and artificial intelligence have promise but face multiple logistical barriers before being widely adopted.
- Retinal laser ablation remains the standard of care for ROP requiring treatment, but advances in our understanding of intravitreal anti-VEGF agents have led to increased use and benefits over lasers in certain cases.

The fast pace of development of the visual system in the neonatal period makes the recognition of ocular abnormalities extremely important. As early as 4 to 6 months after birth, some visual functions are permanently set and if impaired cannot be fully restored to normalcy. For example, a visually significant congenital cataract must be surgically addressed before the third month of life to avoid potentially irreversible vision loss. This urgency makes the neonatologist an invaluable player in the recognition and management of neonatal eye diseases. Screening eye examinations are important in all infants, regardless of gestational age and whether they occur in the neonatal intensive care unit (NICU), the newborn nursery, or the primary care provider's office. Healthcare professionals caring for newborns need to be familiar with indications for referral to a pediatric ophthalmologist, and in premature infants, the added risk of retinopathy of prematurity (ROP) mandates that neonatologists ensure timely diagnostic examinations by an ophthalmologist with expertise in ROP.

General Examination Techniques

The Newborn Eye Examination: Approach and Equipment

Screening eye examinations are important in all infants, regardless of gestational age. All neonates should have an examination of the red reflex before discharge from the newborn nursery.

A more thorough eye examination, although necessary, can be stressful and sometimes painful for a newborn or a young infant. ROP examinations in particular, which often necessitate the use of an eyelid speculum to retract the eyelids and scleral indentation to visualize the peripheral retina, have been associated with an increase in pain.[1] The procedure has also been associated with other adverse effects, including episodes of desaturations, bradycardia, hypertension, and prolonged crying times.[2] Swaddling, nesting, nonnutritive sucking, and oral administration of sucrose before, during, and after an examination can be very helpful in this regard,[3–5] particularly for premature babies undergoing serial ROP examinations. The most distressing aspects of the eye examination are generally the bright light of the ophthalmoscope and the insertion of the speculum. The use of a topical anesthetic, such as proparacaine hydrochloride 0.5% or tetracaine 0.5% drops, reduces the discomfort but is not always sufficient and should be supplemented with other measures, such as sucrose, pacifiers, and nesting.[6,7] In addition, NICU and office staff should be aware that the repetitive use of topical anesthetics can result in corneal ulceration and melting, so bottles should be properly disposed of and not confused with other medications, such as topical lubricants or antibiotics. The use of an indirect ophthalmoscope without a speculum may produce less pain response than that noted during examination with a speculum or with a contact fundus camera, such as the RetCam (Clarity Medical Systems, Pleasanton, California, United States).[8] Nevertheless, a speculum is often necessary to adequately visualize ocular structures and should always be used if adequate visualization of the fundus is otherwise not

possible. Assistance with comforting the baby and monitoring vital signs is important. In addition, the head and body of the infant may need to be securely held to allow both a detailed and a safe examination. Finally, a particular note should be made of the oculocardiac reflex, a dysrhythmia, typically bradycardia, resulting from direct manipulation of the eye during and immediately after the examination; monitoring by an assistant during and after the examination is therefore important. Nevertheless, it is extremely rare for a properly supported infant to be unable to tolerate a quick fundus examination, the discomfort of any observers notwithstanding. Eye examinations should not be postponed unilaterally without first discussing with the ophthalmologist the risk of ROP in each specific infant.

Pharmacologic dilation with mydriatic eye drops is commonly performed by consulting ophthalmologists, typically on all new patient evaluations and at all ROP examinations. Occasionally, a pediatrician may find it helpful to dilate the pupils to better visualize the red reflex or optic nerve head. Use of drops is better deferred if an ophthalmology consultation will be requested, because dilating the pupils may make it difficult or impossible to accurately assess the pupils, ocular alignment, intraocular pressure, and iris. Dilating eye drops include sympathomimetic drugs (e.g., phenylephrine) and anticholinergic drugs (e.g., tropicamide, cyclopentolate, and atropine). Potential side effects of these drugs include elevated blood pressure, increased heart rate, cardiac arrhythmias, feeding intolerance, slowed gastric emptying, urticaria, contact dermatitis, and seizures.[9–11] Bradycardic and apneic episodes following administration of these eye drops are pain response reactions and not a direct effect of the drugs. Cohen et al.[12] analyzed masked videotapes from before, during, and after eye drop administration and found that one-third of infants have a significant pain response to mydriatic eye drops. Supportive measures, therefore, can also be used at the time of administration of dilating eye drops. Adverse effects are potentially of greater concern in preterm infants, who are of lower weight and typically require multiple doses to achieve adequate dilation, as do many children with dark irides. Therefore it may be prudent to use reduced concentrations of mydriatics in premature infants, particularly cyclopentolate. A randomized masked trial[10] concluded that cyclopentolate 0.2% with phenylephrine 2.5% is the mydriatic of choice in preterm infants with dark irides because higher concentrations of cyclopentolate (0.5%, 1.0%) were more likely to result in increased mean blood pressure or feeding intolerance. In all children, systemic absorption of eye drops can be minimized by compression of the lacrimal sac for 1 to 2 minutes after instillation. It is also recommended to wipe all the overflow drops from the skin as local vasoconstriction and pallor of the periorbital skin are frequently observed in premature neonates in whom the epidermal permeability barrier is still incompetent.[13] These authors typically use three drops of Cyclomydril (cyclopentolate hydrochloride 0.2% and phenylephrine hydrochloride 1%) or tropicamide 1% and phenylephrine 2.5% separated by 5 minutes in each eye, 30 to 60 minutes before the examination. Multiple doses are necessary because, in premature and newborn babies, adequate pupil dilation is often difficult to obtain because of the immaturity of the dilator muscle of the pupil; the pupils of babies with dark irides, in particular, may be more difficult to dilate because of pigment binding of the mydriatic drugs, and higher concentration or more frequent administration may be required. We recommend avoiding the use of cyclopentolate 1% in children younger than 6 months.

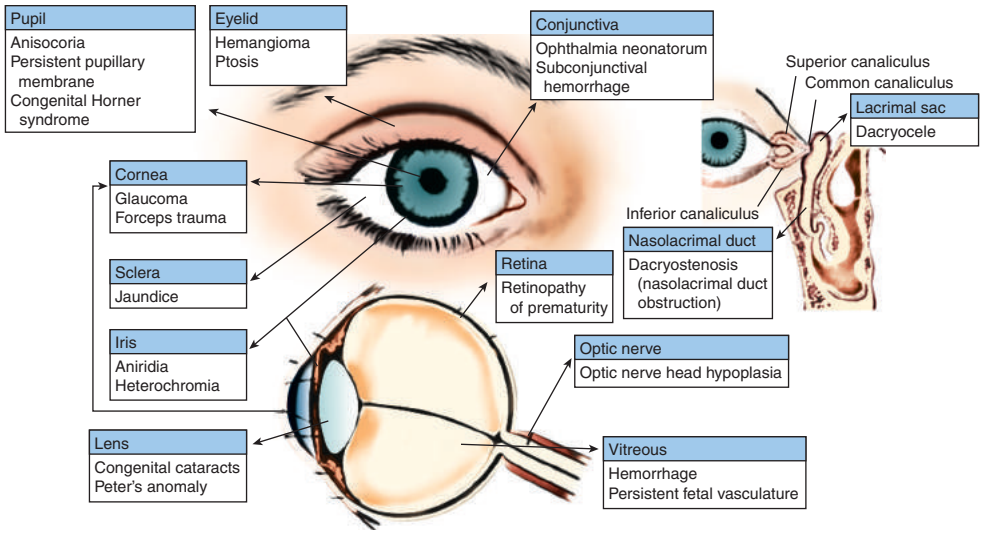
Red reflex testing with a direct ophthalmoscope (described in detail later) is a mandatory element of all newborn and well-baby physical examinations. Numerous vision-threatening and even life-threatening ocular diseases are primarily identified by the checking of red reflexes. The direct ophthalmoscope may, of course, be used to directly visualize the optic nerve head and retina, but particular considerations and limitations must be kept in mind. Pupil size must be maximal; typically this means pharmacologic dilation (see above). If mydriatics are not used, dimming ambient light will maximize pupil dilation. The examiner must be very close to the infant (a few centimeters at most). Approaching from a slightly lateral angle and following the "arrows" of branching vessels back to the nerve head will help to identify the optic disc. Most important, the field of view or "spot size" of the direct ophthalmoscope is approximately the size of the optic nerve head, which represents but a tiny fraction of the ocular fundus. Therefore it is not possible to adequately evaluate the retina for ROP or other peripheral retinal diseases with a direct ophthalmoscope; instead, an indirect ophthalmoscope is needed. This instrument requires both greater skill and a handheld lens to use but provides binocular viewing with depth perception and a much wider field of view. Pharmacologically dilated funduscopic examination with an indirect ophthalmoscope is required for ROP and other retinal diagnostic examinations, such as for retinoblastoma or retinal hemorrhage (RH) in suspected abusive head trauma (AHT).

In addition to red reflex testing, the eyes should be closely examined with proper lighting and magnification as part of a newborn general exam. The periocular and ocular structures should be approached in a systematic manner. One option is to begin with the external structures and work inward and posteriorly, looking at each eye carefully and comparing the two eyes with each other. As a guideline, any abnormality or asymmetry noted on examination should be referred to a pediatric ophthalmologist for further management. The urgency with which to seek consultation depends on the specific finding, and guidelines appear throughout this chapter. However, one should err on the side of urgency, because the neonatal period is a critical period in visual development. To adequately inspect the eyes, familiarity with normal anatomy is required. A general overview of these structures follows (Fig. 96.1).

The eyelids protect the eyes. The eyelids contain numerous glands, which produce tears to keep the ocular surface well lubricated. Blinking spreads the tears and actively pumps the tears into the lacrimal drainage system. An inability to adequately close the eyes presents a major problem and can result rapidly in surface drying, corneal epithelial breakdown, and vision- or eye-threatening complications, such as ulceration, infection, or scarring. Use of an overhead heater should be avoided for infants who have poor eyelid closure (e.g., congenital eyelid abnormalities, neurologic problems), as the ocular surface can rapidly decompensate in such cases; alternative sources of maintaining body temperature should be used, and an ophthalmology consultation should be requested to manage the ocular surface.

The nasolacrimal duct provides a means of egress for tears, which pass through the puncta, canaliculi, and lacrimal sac to the duct. The duct is blocked at birth in 5% to 20% of newborns, resulting in epiphora and discharge in an otherwise white and quiet eye; more than 90% of such blockages clear by 1 year of age.[14] Of note, congenital glaucoma can manifest itself with epiphora as well (see Corneal Clouding).

The conjunctiva is a translucent membrane that overlies the surface of the eye and the inside of the eyelids. The sclera is the white, fibrous wall of the eye. It is relatively flexible at birth and gradually toughens over the first few years of life. The cornea is continuous with the sclera, which it meets at the limbus, and is a



• **Fig. 96.1** Anatomic structures and common neonatal diseases by anatomic site.

clear dome-shaped structure in the center of the globe (eye). The iris is a donut-shaped structure posterior to the cornea. The anterior chamber of the eye lies between the cornea and the iris and is best visualized with slit lamp examination but this is often not possible in neonates and infants. The iris is an immature structure at birth. The color tends to be gray, blue, or light brown and may become darker as the pigmented layer of the iris stroma becomes more fully developed, which typically occurs by about 6 months of age. *Heterochromia* refers to differences in the color of the iris between or within eyes and can be seen in congenital Horner syndrome (with mild ptosis and a miotic pupil), as well as syndromic conditions such as Waardenburg syndrome or Hirschsprung disease. Behind the iris lies the crystalline lens. An opacity in the lens is referred to as a cataract (see Leukocoria and Abnormal Red Reflex). The uvea is composed of the iris, the ciliary body, and the choroid. The retina is a multilayered, complex, highly metabolically active structure that lines the inside surface of the globe and contains photoreceptors that receive light and generate neuronal signals that are ultimately perceived as visual images. The macula is the central, posterior retina, between the superior and inferior temporal retinal vascular arcades, and the fovea is the very central retina containing the highest concentration of photoreceptors and producing central, high-resolution vision. Visual signals are transmitted through the optic nerve, whose cell bodies lie in the most anterior retina and which is composed of approximately 1 million individual nerve fibers. The proximal end of the optic nerve is visible as a normally golden disc approximately 15 degrees nasal and just superior to the fovea. The optic nerves lead to the optic chiasm and continuing visual and pupillary pathways in the brain.

Knowledge of normal eye structures and certain growth parameters in the newborn is important because a deviation from the averages can be associated with significant disease. For example, in congenital glaucoma, the corneal diameter is increased, and the axial length (sagittal length) of the eye is a parameter that is carefully followed by the ophthalmologist, with the aid of an ultrasound examination, to determine if the intraocular pressure is adequately controlled. At birth, the eyeball is 70% of the adult size (the average axial length in a newborn is 17 mm) and reaches 95% of the adult size by age 3 years. The corneal horizontal diameter is usually 9.5 mm at birth, which is 80% of the adult diameter. Corneal diameter can be assessed by simple bedside examination.

The visual system is immature at birth. The fovea is not completely differentiated until 15 to 45 months,[15] and myelination of the optic nerve is not completed until about 1 year of age.[16] The eye continues to develop synapses in the visual cortex during the first 10 years after birth, and although visual acuity reaches normal adult ranges by 2 years of age, this period continues to be important because any abnormality can lead to amblyopia (see the definition later). Color vision improves greatly during the first 3 months after birth, and most normal 3-month-olds have at least some color vision; color visual processing mechanisms continue to mature throughout the first year of life.[17,18]

Because of the immaturity of the system at birth, qualitatively estimating the vision of a newborn is seldom attempted, and other clues to the status of visual function are more commonly used. However, clinical and laboratory techniques can be used to estimate vision in special situations. These include eliciting a nystagmus response with optokinetic targets (striped patterns), Teller cards for preferential forced looking (a test that depends on an infant's preference to look at patterns—grating of black and white stripes—rather than homogeneous fields), visual evoked potentials, and electroretinogram. Each of these techniques uses different stimuli and yields somewhat different results, but they all demonstrate a dramatic improvement in "acuity" during the first year of life. Most commonly, visual function in a newborn is assessed by the detection of light aversion, which implies light perception. A bright light is shone into each eye or even through the thin eyelids to elicit the closing or squeezing of the lids. Although visual fixation may be intermittently present soon after birth, it is not well developed until after the second month; in contrast, a blink response to light is already present at 26 weeks' postmenstrual age (PMA),[19] and head turning to a diffuse light starts around 32 weeks' PMA. A blink in response to an approaching object (visual threat response) does not develop until approximately 16 weeks' postnatal age. An infant should fix on and follow a face or object by 2 to 3 months of age. A clever technique that is based on the observation that neonates and infants will attend to the reflection of their own faces in a mirror has been described.[20] Measurement of the "mirror distance" is a simple and reliable technique to estimate visual acuity in infants that can be used as a screening test similar to the traditional "fix and follow" (see later) and is a useful additional tool for detecting impaired visual

function at this early age.[20] Generally, the absence of visual responsiveness by a developmental or corrected age of 2 months should be taken seriously and prompt an urgent ophthalmologic evaluation.

Poor vision or blindness should be suspected in any infant with absent or poor pupillary responses, paradoxical pupillary response (initial brisk constriction of the pupil when the lights are turned off),[21] and nystagmus or roving eye movements, although these are not usually present until 2 to 3 months of age. Constant poking or rubbing of the eyes can also be a sign of poor vision.

Causes of congenital blindness or poor vision include Leber congenital amaurosis (an early and severe form of retinitis pigmentosa), other retinal dystrophies (achromatopsia and congenital stationary night blindness), congenital cataract, glaucoma, aniridia, albinism, optic nerve abnormalities (hypoplasia and coloboma), chorioretinal colobomas, high refractive errors, and congenital infections. In most babies, the cause of poor vision is obvious after a complete ophthalmologic examination. Occasionally, further investigation is necessary and may include electrophysiologic testing and neuroimaging. Babies with cerebral (central or cortical) visual impairment (CVI) have normal eye examination findings, including normal pupillary responses and no nystagmus. CVI is used to describe children with visual impairment as a result of neurologic disease, which may be congenital or acquired. Perinatal causes include intrauterine infection, cerebral dysgenesis, asphyxia, hypoglycemia, intracranial hemorrhage, periventricular leukomalacia, hydrocephalus, trauma, meningitis, and encephalitis. In developed countries, CVI is the single greatest cause of visual impairment in children, and most of these children have an associated neurologic deficit (usually epilepsy or cerebral palsy), which places a major burden on children's special services in these countries.

The prevention of all causes of blindness in infants and children is considered a high priority within the World Health Organization. This is because many causes of blindness in childhood are preventable or treatable and many of the conditions associated with blindness in children are also causes of child mortality (e.g., premature birth, congenital rubella syndrome, Vitamin A deficiency). Prevention of blindness in children is therefore closely linked to child survival, in addition to decreasing the economic, emotional, and social burden of a lifetime of blindness. This, however, imposes particular challenges as children are born with an immature visual system and, for normal visual development to occur, they need clear, focused images to be transmitted to the higher visual centers in the brain. Failure of normal visual maturation (amblyopia) cannot be corrected in adult life, so there is a level of urgency in treating childhood eye diseases.[22]

Amblyopia is defined as a reduction in best-corrected vision that cannot be attributed directly to any structural abnormality of the eye or proximal visual pathway. Amblyopia is the maldevelopment of the visual centers of the brain as a result of abnormal visual experiences early in life. It includes three etiologic categories, which often overlap. Deprivational amblyopia results from obstruction at any point in the visual axis that causes the retina to perceive poor-quality, distorted, or no images. The causes of deprivational amblyopia include congenital or acquired cataracts, corneal opacity, ptosis, and vitreous hemorrhage. Strabismic amblyopia results from a child's preferring one eye over the other when the visual axes are misaligned. Refractive amblyopia is a consequence of either a significant inequality of the refractive error in each eye or very high refractive errors in both eyes. Any of these forms of amblyopia can be encountered in the first few months of postnatal life. Because amblyopia is responsible for more cases of unilaterally reduced vision in childhood than all other causes combined, and because it is highly preventable with early detection and treatment, all newborns and infants suspected of having any of these conditions should be referred promptly to an ophthalmologist.

The onset of the pupillary light reflex (constriction in response to light) occurs around 30 to 34 weeks' gestation[19] and is not fully developed until the first month after birth. The pupils should be examined for size, shape, symmetry, reactivity to light, and afferent defects.

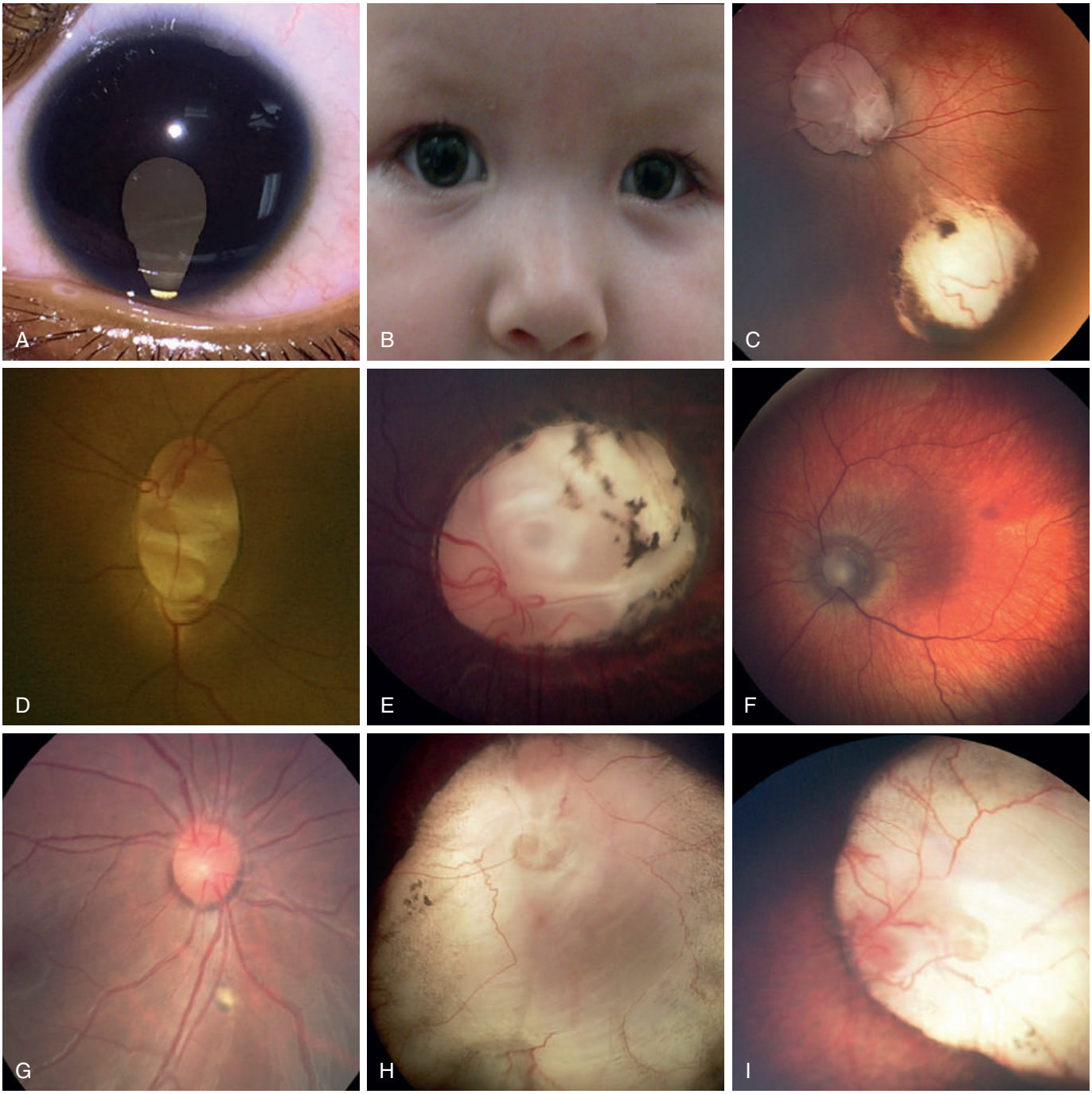
As the light passes through the pupils and is reflected through the normal clear media of the anterior and posterior segments of the eye, a characteristic red reflex is produced. The reflex is generated not from the retina, which is transparent, but from the choroidal pigmentation and vasculature. The red reflex test is vital for the early detection of vision and potentially life-threatening conditions such as cataracts, glaucoma, retinoblastoma, retinal abnormalities, systemic diseases with ocular manifestations, and high refractive errors. Red reflex assessment is an essential component of every neonatal and infant physical examination. The American Academy of Pediatrics currently recommends that all neonates have an examination of the red reflex before discharge from the neonatal nursery. In addition, the test should be performed during all subsequent routine health supervision visits.[23,24]

The red reflex test should be performed in a darkened room, projecting the largest white-light circle light of the ophthalmoscope onto both eyes of the infant—first simultaneously, from approximately 18 inches away, while looking through the aperture of the ophthalmoscope. Once the reflexes have been assessed together to allow comparison, specific abnormalities can be more closely inspected by examination of each eye separately at a nearer distance. Since neonates and infants are frequently asleep and it is difficult to open their eyes and keep them open, the red reflex test may be challenging. A useful technique to encourage a neonate to spontaneously open the eyes is the use of a primitive reflex seen in neurologically healthy children up to 6 months old: the child is supported by a hand on the thorax at approximately a 45-degree angle from the horizontal with use of the other hand to jiggle the child's bottom.[25]

It is not necessary to pharmacologically dilate the pupils, although the reflexes are easier to assess with larger pupils; indeed, the most common cause of an absent reflex is small pupils. Darkening the room or changing the light beam of the ophthalmoscope will usually overcome this problem. Although traditionally the normal reflex has been described as "red," it can often be yellow, orange, or maroon, depending on the amount of skin/eye pigmentation (i.e., light orange-yellow in lightly pigmented blue eyes or dark red in darkly pigmented brown eyes). A common cause of misdiagnosis is the inability to appreciate variations of normal due to a reference comparison to the color reflected from a blonde fundus. Darkly pigmented individuals often have a duller red reflex due to the increased amount of melanin in their fundus.[26] Therefore, the symmetry of the color, clarity, and intensity of the red reflex between the eyes is usually more useful than the qualitative assessment of each red reflex independently. A markedly diminished or dim reflex, the presence of a white reflex (leukocoria), dark spots in the reflex, or an asymmetric reflex between the eyes is an indication for immediate referral to an ophthalmologist experienced in the examination of children.[23] An exception to this rule is a transient opacity in the tear film from mucus or a foreign body that is mobile and completely disappears with blinking or manual opening and closing of the eyelids, after which the

red reflex should appear normal. Unequal or high-refractive errors (need for glasses) and strabismus may also produce abnormal or asymmetric red reflexes. An additional observation is the position of the light reflex on the corneal surface. Asymmetric positioning of this reflex can indicate misalignment of the eyes (strabismus; see later).

In addition, the shape and regularity of each pupillary aperture should be assessed to look for colobomas (Fig. 96.2A) and other congenital abnormalities (see Common Diagnostic Problems). Asymmetry of pupil size (anisocoria) can be a sign of Horner syndrome, trauma, or a congenital third nerve palsy. The tunica vasculosa lentis is a plexus of vessels that crosses the pupil, visible in preterm babies up to 34 weeks' PMA. The extent of this anterior lens capsule vascularity can be used to estimate gestational age in babies between 27 and 34 weeks' gestation.[27–29] A failure of these vessels to regress can occasionally be seen as a persistent pupillary membrane, which appears as a regular arrangement of vessels looping into the pupillary axis in front of or behind the lens.

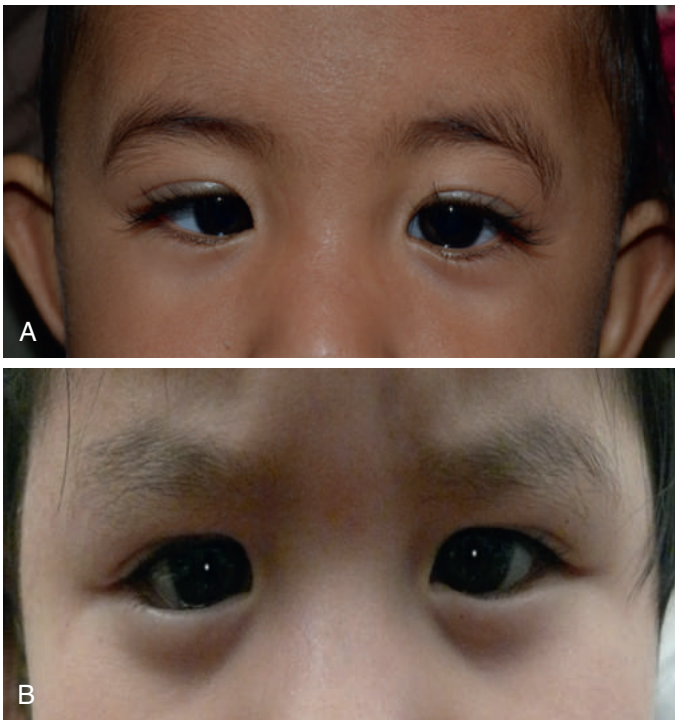


• **Fig. 96.2** Wide spectrum in presentation of colobomas of the eye. (A) Iris coloboma, notice the difference in red reflex at the lens equator. (B) Microphthalmos with coloboma (the left eye is smaller because of a uveal coloboma). (C) Noncontiguous optic nerve and chorioretinal coloboma. (D–F) Optic nerve colobomas. (G) Small chorioretinal coloboma inferior to the optic disc. (H) Large chorioretinal coloboma involving most of the posterior pole. (I) Large chorioretinal coloboma involving the macula and optic disc.

Although the extraocular muscles are formed by 12 weeks' gestation and fetal eye movements can be detected as early as 16 weeks' gestation, the supranuclear eye movement system is not fully developed until after birth in full-term neonates. The eyes of a neonate commonly appear misaligned. It is not unusual to see the eyes shift from straight (orthotropia) to crossed inward (esotropia) to outward (exotropia). Transient deviations (neonatal ocular misalignments) occur very commonly in the first month of life in visually normal infants. At this age, it is not possible to distinguish those infants who will progress to develop pathologic strabismus from those who will develop normal binocular vision.[30] Exotropia is commonly observed in newborn nurseries and has been reported to occur in up to 33% of infants;[31] however, most exodeviations will usually resolve with the development of the fixation reflex and are rarely observed beyond 6 months of age.[32] In contrast, transient esodeviations in patients who do not go on to develop infantile esotropia do not usually persist beyond 10 weeks of age.[32,33] Most full-term infants establish normal ocular alignment within the first 8 weeks of life. In some babies the epicanthal folds of the eyelids hide the medial aspect of the sclera, creating the appearance of strabismus; however, in these cases, the visual axes are not misaligned. This common condition is referred to as *pseudostrabismus* (Fig. 96.3B) and can be confirmed by the directing of a bright light to both eyes simultaneously and observing that the reflection of the light on the corneas appears symmetric between the eyes with respect to the center of the pupil; the reflexes will appear asymmetric between the eyes if a true misalignment exists (Fig. 96.3A).

Most cases of strabismus in infants are not paralytic in origin, but congenital third, fourth, and sixth nerve palsies can occur, as well as early acquired cranial neuropathies due to trauma, infection, and other central nervous system abnormalities. When there is doubt, any apparent misalignment after 3 to 4 months of age should be considered pathologic and referred for evaluation.[23] This is especially important as, in some cases, strabismus can also be the first sign of serious ocular or systemic disorders. Premature and low birth weight infants are at increased risk of developing strabismus and other amblyogenic conditions throughout their childhood. Perinatal stroke is also associated with a high incidence of strabismus, early head turn, visual field cuts, and other vision abnormalities. Such high-risk infants as well as those with a strong family history of strabismus or amblyopia should be referred for evaluation.

It is recommended that all newborns have an ocular motility assessment.[24] In addition, since vision in young nonverbal children is mostly assessed by evaluation of the child's ability to fix on and follow an object, this test provides information on the status of the visual and extraocular muscle systems. A standard assessment strategy is to determine whether each eye can independently fixate on the object, maintain fixation on it for a short period, and then follow it as it is moved in various directions. Neonates and young infants particularly fix and follow the human face or its likeness. This assessment should be performed binocularly and then monocularly in an awake and alert child. In neonates, the following may be a jerky saccadic pursuit movement, which represents a series of hypometric saccades to localize the target. If following cannot be demonstrated, it should be verified that the motor system is intact. Range of motion and the ability to generate a saccade may be assessed by the inducement of vestibular nystagmus by rotation of the child. If poor fixation and following are noted after 3 months of age, a significant ocular or neurologic abnormality is suspected and should be referred for evaluation.[24]



• **Fig. 96.3** (A) Inward misalignment of the eyes in a premature child with infantile esotropia. (B) Pseudoesotropia or pseudostrabismus (a wide nasal bridge and prominent epicanthal folds give the appearance of esotropia; note the position of the corneal light reflex centered in the pupil of both eyes).

Common Diagnostic Problems

Leukocoria and Abnormal Red Reflex

The term *leukocoria* means "white pupil." It is often used more broadly to encompass a spectrum of opacities and abnormalities. On inspection, the pediatrician may grossly visualize a white lesion in the pupillary space or identify an abnormal red reflex. A white or abnormal reflex may also be identified in recreational photographs taken by family members.[34–36] The differential diagnosis for leukocoria includes vision- and life-threatening conditions, and leukocoria in an infant or older child requires urgent ophthalmologic evaluation. These conditions include cataract, retinoblastoma, chorioretinal or optic nerve head coloboma, retinal detachment, vitreous hemorrhage, advanced ROP, persistent fetal vasculature, Coats disease, familial exudative vitreoretinopathy, toxocariasis, and uveitis. The distribution differs widely with the population studied. In one series, 60% of 71 children who presented to a tertiary referral center with leukocoria had cataracts, 28% had retinoblastoma, and 12% had other retinal abnormalities.[37]

Cataract

A cataract is any opacification of the normally clear crystalline lens of the eye. Although congenital cataract is much less common than age-related cataract, it is among the top 3 causes of preventable childhood blindness and is responsible for approximately 10% of childhood blindness worldwide.[22] Congenital cataracts may be isolated, seen in association with another ocular

developmental abnormality, or associated with systemic diseases. The incidence of congenital cataract is approximately 2 to 4 in 10,000 live births.[22,38] Some subtypes of congenital cataracts are small and nonprogressive; dense central opacities larger than 3 mm are considered visually significant. Successful treatment of congenital cataracts may be extremely difficult, and intervention must occur very early in life; therefore early diagnosis is essential. Useful vision can be restored if the surgery is completed within the first 6 weeks after birth. Beyond this time, visual restoration becomes progressively more difficult because of irreversible deprivation amblyopia.[39,40] The appearance of nystagmus before surgery is an ominous sign of poor visual outcome and adds further urgency for surgical intervention.[41] Therefore it is essential that all newborns and young infants have screening eye examinations by a pediatrician because the visual prognosis is directly tied to timely ophthalmologic referral. Cataracts can develop or progress with time, so examination for an abnormal red reflex should be repeated at each well-child visit even if prior examination findings appeared normal.

Most patients with isolated nonsyndromic cataracts have no identifiable cause. Although teratogenic agents (e.g., rubella) may account for a proportion of cases, such insults normally give rise to other systemic malformations in addition to cataracts. Genetic mutation is likely to be the most common cause, particularly for bilateral cataracts;[42,43] it is estimated that hereditary cataracts constitute 22% of global childhood cataracts.[44] Genetic inheritance is most commonly autosomal dominant and rarely autosomal or X-linked recessive.[42,45] Multiple genes are involved in lens development; mutation screening of inherited congenital cataracts have identified nearly 200 locus and more than 100 causative genes.[38]

Marked variability can be present even within the same pedigree, and children with a family history of infantile or juvenile cataracts should be examined early by a pediatric ophthalmologist. The same is true of children with one of the numerous systemic conditions associated with cataracts: intrauterine infections (rubella, varicella); metabolic and endocrine disorders (galactosemia, neonatal hypoglycemia, diabetes mellitus, and hypoparathyroidism); fetal alcohol syndrome; chromosomal disorders (trisomy 21, Turner syndrome, trisomies 13 and 15); dermatologic diseases (congenital ichthyosis, ectodermal dysplasia); skeletal and connective tissue disorders (Smith-Lemli-Opitz, Marfan, Conradi, and Weill-Marchesani syndromes); renal disorders (Lowe, Alport, and Hallermann-Streiff-François syndromes); neurofibromatosis; and myotonic dystrophy. A selective diagnostic evaluation may be pursued in infants with cataracts, particularly bilateral cataracts, and may include TORCH titers (including syphilis); urine tests for reducing substance (galactosemia); plasma urea, electrolyte, and urinary amino acid levels (Lowe syndrome); complete blood count and ferritin, blood glucose, calcium, and phosphate levels; quantitative amino acid levels and red blood cell enzyme levels (galactokinase, galactose1-phosphate uridyltransferase); genetic consultation; chromosome analysis and next-generation sequencing and ocular examination of parents and siblings. Infants with isolated unilateral cataracts often do not have a family history and rarely have associated systemic disorders.[42]

Retinoblastoma

Retinoblastoma is the most common ocular malignancy of childhood and accounts for 3% of all childhood cancers. The average age-adjusted incidence rate of retinoblastoma in the United States and Europe is 2 to 5 per million children or 1 in 14,000 to 18,000 live births[46] but is higher in India and Africa (resulting in approximately 9000 new cases per year, of which fewer than 300 are in the United States). Seventy-five percent of patients have unilateral retinoblastoma, and 25% have bilateral retinoblastoma. The successful management of retinoblastoma depends on the ability to detect the disease while it is still intraocular; disease stage correlates with delay in diagnosis. Untreated retinoblastoma is almost uniformly fatal, and the long-term survival rate for disease diagnosed after it has spread outside the eye is less than 50%. In contrast, 5-year survival rates are greater than 90% when timely recognition and referral to centers specializing in retinoblastoma treatment occur (http://www.1rbw.org).

Two-thirds of patients receive a diagnosis before 2 years of age, and 95% receive a diagnosis before the age of 5 years.[46] The earliest age at diagnosis reported is 21 weeks' gestation by prenatal ultrasound examination.[47] Abramson et al.[48] reviewed 1831 consecutive cases of retinoblastoma. The most common presenting sign was leukocoria (54%), followed by strabismus (19%), poor vision (4%), family history with request for early examination (5%), and red eye (5%). The presenting sign was identified first by a family member or friend in 80% of cases, a pediatrician in 8% of cases, and an ophthalmologist in 10% of cases. Among patients presenting with leukocoria, the sign was first identified by a family member in 90% of cases. These findings stress the importance of routine red reflex testing for all children seen by pediatricians, beginning with newborns. Any child found to have leukocoria should be referred to an ophthalmologist for urgent evaluation.

The retinoblastoma gene, *RB1*, located on chromosomal region 13q14, was the first tumor suppressor gene to be described. Five percent of patients with bilateral disease carry a large deletion involving the 13q14 locus. In those cases, retinoblastoma is part of a more complex syndrome characterized by facial dysmorphic features (thick anteverted earlobes, high and broad forehead, prominent philtrum, and short nose), skeletal abnormalities, mental retardation, and motor impairment. Children with germline mutations are at increased risk of developing nonocular tumors. Genetic counseling of affected parents is critical to estimate the risk of transmitting the disease to their children. Regardless of the clinical presentation, it is recommended that all patients undergo genetic testing.

Persistent Hyperplastic Primary Vitreous (Persistent Fetal Vasculature)

PFV is a congenital ocular dysgenesis in which the hyaloid embryonal vasculature does not regress completely. During embryogenesis and fetal development, the "primary vitreous" contains the hyaloid vasculature system, which fills the posterior segment of the eye and comes forward to surround the lens. This system normally disappears, and a spectrum of abnormalities can be seen when these structures fail to regress, ranging from persistent pupillary strands to a vascular stalk remnant to a retrolenticular membrane and retinal disorganization or detachment. Involved eyes are typically microphthalmic, and an abnormal red reflex or leukocoria may be identifiable. Depending on the extent, surgical intervention may help to avoid recurrent hemorrhage, glaucoma, and phthisis bulbi (atrophy and degeneration of a blind eye, which can become painful), and in some cases, useful vision can be achieved.[49] Persistent hyperplastic primary vitreous (PHPV) is the most common retinoblastoma-simulating lesion, followed by Coats disease and presumed ocular toxocariasis.[50]

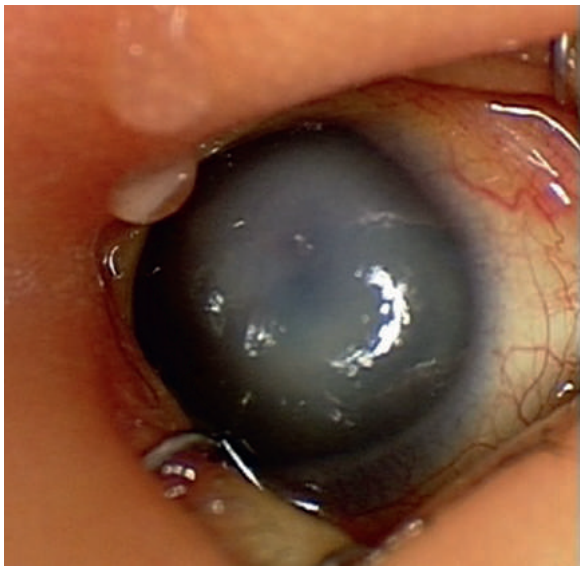
Coloboma

An ocular coloboma is a congenital anatomic defect or cleft that results from the failure of the optic fissure to close during embryogenesis.[51] The result is essentially an area of missing tissue in the eye, most commonly in the inferonasal quadrant. Depending on the population studied, its incidence ranges from 0.5 to 7.5 per 10,000 births and accounts for 3% to 11% of blind children worldwide.[51] Involved structures can include the iris, ciliary body, retina, choroid, and optic nerve (see Fig. 96.2). An iris coloboma appears as an irregular "keyhole," or "cat's-eye" pupil (see Fig. 96.2A). A chorioretinal or optic nerve head coloboma, depending on the size, will appear as an abnormal red reflex or leukocoria. The affected eye may be microphthalmic (see Fig. 96.2B). The visual prognosis depends on whether the central macula is involved, and children may have good central vision despite upper visual field defects if the macula is spared. Long term, there is a variable risk of complicating retinal detachment or choroidal neovascularization associated with retinal and optic nerve colobomas.[51] An ocular coloboma can be isolated or syndromic. There are numerous ocular abnormalities and systemic findings associated with coloboma, and more than 200 syndromes have been described. Examples include the CHARGE association, 22q11 deletion, and Treacher Collins, Walker-Warburg, and Aicardi syndromes. The systemic diagnostic testing in patients with apparently isolated bilateral or unilateral uveal coloboma should include a kidney ultrasound examination, audiometry, and spine radiographs. In addition, an echocardiogram and neuroimaging could be considered.[52,53]

Corneal Clouding

Slightly cloudy corneas can be seen in otherwise normal healthy newborns and normally clear within 1 to 2 days. Clouding is commonly seen in small for gestational age and premature infants, and it is proportional to the degree of prematurity. At 26 weeks' gestation the degree of cloudiness is significant enough to prevent the evaluation of the iris details underneath, and along with an immature hyaloid vascular system, it prevents a detailed view of the posterior segment structures (retina and optic nerve). This is an important consideration when the ophthalmologist is asked to evaluate premature infants soon after birth for an intraocular spread of systemic infections (endophthalmitis) or structural anomalies in suspected genetic diseases, etc. Slowly, as the child matures, the cornea establishes transparency and most of the time is sufficiently clear to allow retinal examinations for ROP by 30 to 32 weeks' postconceptional age.

Persistent opacification of the cornea in a newborn may be the result of congenital glaucoma, corneal dystrophies, developmental abnormalities of the cornea and or other anterior segment structures (Fig. 96.4), infection, iatrogenic trauma, or metabolic disorders. Close inspection with magnification will identify opacification in a focal, regional, or diffuse pattern, depending on the cause. The cornea is normally clear all the way to its border with the sclera (the limbus), with iris details easily visible underneath; these details will be obscured when the cornea is opacified. A large or central opacity will also result in an abnormal red reflex. When identified, congenital corneal opacification requires urgent ophthalmologic evaluation to rule out congenital glaucoma, infection—which could become eye or life threatening—or an opacification that is amenable to surgical correction during the early critical period in visual development.



• **Fig. 96.4** Cloudy cornea secondary to anterior segment dysgenesis.

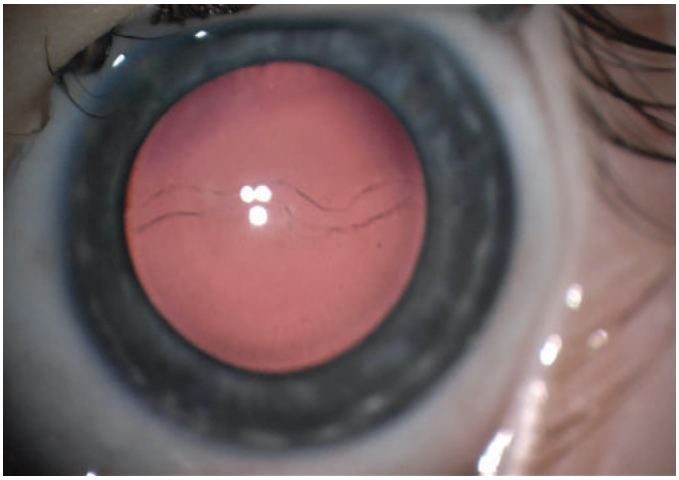
Glaucoma is an optic neuropathy usually associated with raised intraocular pressure. In contrast with adult- or juvenile-onset glaucoma, which may be successfully managed medically, congenital glaucoma is a surgical disease that requires prompt intervention, frequently in the neonatal period. Vision loss from glaucoma is typically irreversible. Key signs to identify include corneal clouding (not always), corneal and eye enlargement (buphthalmos), tearing, blepharospasm (blinking), Haab striae (tears in the Descemet membrane, seen as lines in the red reflex), and photophobia. It is worth highlighting that tearing is a sign of glaucoma, not just of a blocked tear duct. Finally, congenital glaucoma has multiple systemic associations, such as Sturge-Weber syndrome, neurofibromatosis, Lowe syndrome, congenital rubella, and Rubenstein-Taybi disease.

Sclerocornea is characterized by a cornea that is opacified and white like the sclera, with which it is developmentally continuous. Typically, these opaque areas are located at an indistinct corneoscleral limbus (border), can extend centrally, and contain superficial vascularization. It can occur in isolation or can be associated with cataracts, microphthalmos, and/or infantile glaucoma.[54] In contrast to sclerocornea, *Peters anomaly* classically has a central corneal opacity with clear cornea peripherally, absence of posterior corneal stroma, and variable attachments between the posterior corneal surface and the iris and or lens.[55] The encompassing term *anterior segment dysgenesis* is sometimes preferred to describe many of these developmental anomalies of the cornea and anterior segment that result in congenital corneal opacities and congenital glaucoma as there is significant overlap and variability in their presentation (see Fig. 96.4).

Infectious keratitis can be herpetic with characteristic epithelial dendritic ulcers and corneal stromal inflammation. *Bacterial keratitis* is less common in countries that practice routine administration of conjunctivitis prophylaxis at birth. However, a bacterial corneal ulcer can begin as a corneal abrasion (see the later discussion) and then quickly enlarge, resulting in corneal thinning or perforation, endophthalmitis, and even bacterial sepsis. Aggressive topical and sometimes systemic antibiotics are required, and any infant with a red eye and corneal opacity or abnormality should be referred for immediate ophthalmologic evaluation.

Iatrogenic trauma may result in corneal injury and may be the result of amniocentesis or forceps delivery. Injuries from the latter are characterized by linear breaks in the Descemet membrane, which are more likely to be vertical or diagonal than the horizontal breaks seen with glaucoma, and may be accompanied by other signs of trauma (Fig. 96.5).

Corneal dystrophies, such as congenital hereditary endothelial dystrophy and posterior polymorphous dystrophy, may also result in cloudy corneas. The former always manifests itself at birth, whereas the latter may or may not be present at birth.[54] Metabolic disorders, such as some mucopolysaccharidoses, may cause progressive clouding but often do so later in life and rarely during the neonatal period.



• **Fig. 96.5** Horizontal corneal endothelial breaks consistent with Haab striae in primary congenital glaucoma.

Red Eye/Eye Discharge

The most common and important causes of a red eye in neonates include infectious conjunctivitis, subconjunctival hemorrhage, foreign bodies, and vascular malformations.

The incidence of neonatal conjunctivitis (ophthalmia neonatorum) has decreased dramatically since the introduction of prophylaxis in 1881. Despite this, ophthalmia neonatorum still blinds thousands of babies annually worldwide.[56] Current prophylaxis practices vary globally, with many countries no longer considering it to be an important public health intervention, and some countries continuing with prophylaxis. Policy regarding prophylaxis should be informed by the prevalence of *Neisseria* and *Chlamydia*, their resistance profile against specific antibiotics, and access to prenatal care.[56]

The etiologic cause of conjunctivitis in the newborn can be chemical, bacterial, or viral (Table 96.1). Although infections are usually transmitted to the infant by direct contact during passage through the birth canal, organisms can ascend to the uterus, so even infants born via cesarean delivery can be infected, particularly in the setting of prolonged rupture of membranes but even with intact membranes.[57] Prophylactic agents include 1% silver nitrate, 0.5% erythromycin ointment, 1% tetracycline ointment, and 2.5% povidone-iodine with no agent shown to have superior effectiveness.[56] No prophylactic agent completely eliminates the risk of developing an infection, and a high index of suspicion

TABLE 96.1 Diagnostic Features and Management of Neonatal Conjunctivitis

Etiologic Agent	Onset	Clinical Characteristics	Diagnosis	Treatment
Chemical	24 h	Noninfectious Lid edema, watery discharge	History of exposure, self-limited in <48 h	None
Gonococcal (see Fig. 96.5)	3–4 days	Bilateral, hyperacute purulent conjunctivitis, marked lid edema, copious discharge Can perforate cornea	Cell culture and Gram stain Gram-negative intracellular diplococci	Ceftriaxone 25–50 mg/kg daily intravenously Topical irrigation Topical antibiotics useful only if corneal ulcer present
Chlamydia trachomatis (most common)	5–7 days	Mild mucopurulent nonfollicular conjunctivitis, lid edema, pseudomembrane formation Pneumonitis after 3–12 weeks	Cell culture, Giemsa stain, direct immunofluorescent assay, enzyme-linked immunoassay, PCR Basophilic intracytoplasmic inclusions in epithelial cells	Orally administered erythromycin, 12.5 mg/kg every 6 h for 2 weeks or azithromycin suspension, 20 mg/kg orally daily for 3 days
Staphylococcus, Streptococcus, and other bacteria	5–14 days	Nosocomial, mucoid discharge, conjunctival hyperemia, and chemosis	Cell cultures, Gram stain	Broad-spectrum topical antibiotic (e.g., polymyxin B–trimethoprim, one drop every 4 h for 7 days)
Herpes simplex virus	6–14 days	Unilateral or bilateral conjunctivitis (nonfollicular), serous discharge, associated lid vesicles, and, occasionally, corneal epithelial dendritic defects that stain with fluorescein with or without systemic involvement	Cell cultures, direct fluorescent antibody staining, enzyme immunoassay detection, PCR Multinucleated giant cells with intracytoplasmic inclusions	Acyclovir, 60 mg/kg/day intravenously in three divided doses for 2 weeks (3 weeks if CNS or disseminated disease) plus topical drops (1% trifluridine, 0.1% iododeoxyuridine, or 3% vidarabine)

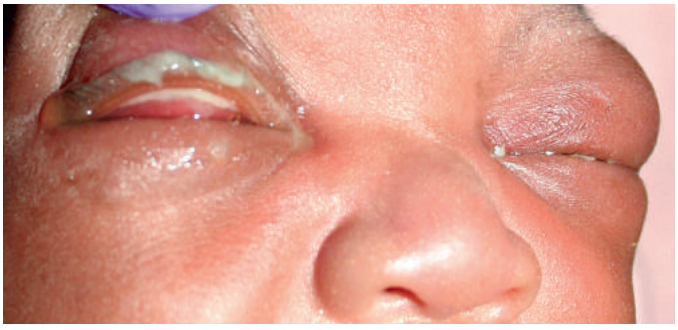
CNS, Central nervous system; *PCR*, polymerase chain reaction.

should be maintained, in particular in those patients with risk factors (maternal infection, lack of prenatal care, or premature rupture of membranes). Gentamicin ointment should be avoided, as it is associated with periocular ulcerative dermatitis when used on the newborn eye.[58]

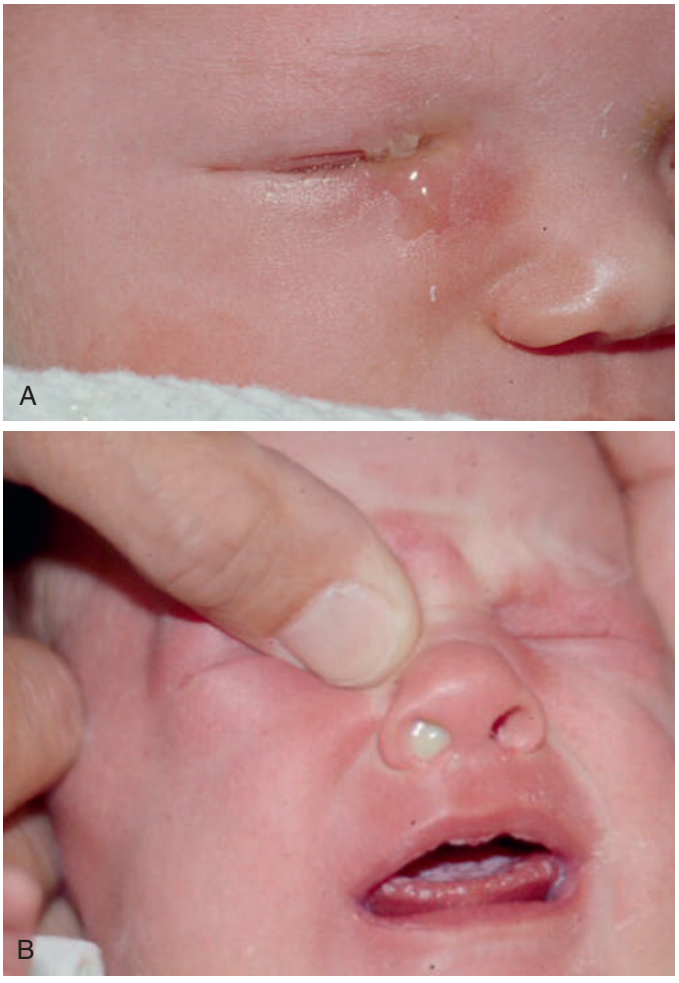
Despite common teaching, the timing of the onset of conjunctivitis is not a reliable diagnostic clue, because significant overlap exists among the different etiologic agents. For this reason, conjunctival cultures (in Thayer-Martin agar, blood agar, and chocolate agar) and conjunctival scraping for Gram and Giemsa staining are mandatory and should be performed without delay. It is not necessary to wait for an ophthalmology consultation to initiate laboratory investigation and treatment, because delays in treatment of gonococcal conjunctivitis can have devastating consequences. *Neisseria gonorrhoeae* can penetrate an intact corneal epithelium, rapidly leading to perforation of the globe within hours if treatment is not initiated; therefore all forms of conjunctivitis must be considered bacterial until proven otherwise. Appropriate treatment should be instituted once the results of cultures are known but should never be delayed (Fig. 96.6).

The infant with mucopurulent discharge must be distinguished from the infant who exhibits only excessive tearing (epiphora) and a relatively white eye. Congenital nasolacrimal duct obstruction is the most common cause of neonatal epiphora, although the possibility of congenital glaucoma must always be ruled out. Congenital obstruction of the nasolacrimal duct is present in about 5% to 20% of infants but usually resolves spontaneously by 12 months of age.[59] It is more common in premature infants and in patients with Down syndrome; cesarean section has been inconsistently found to be a risk factor.[59] Bilateral involvement is present in about one-third of cases. Usually, the obstruction is due to failure of the nasolacrimal duct canalization, most commonly occurring at the distal end of the nasolacrimal duct (membrane or valve of Hasner). Symptoms become manifest by 1 month of age in 80% of cases, with tearing, tear stagnation (increased tear lake), intermittent mucopurulent discharge, and crusting of the eyelashes. As a consequence of chronic obstruction, secondary infection in the lacrimal sac may occur, a condition known as *dacryocystitis*. Pressure on the lacrimal sac causes a reflux of mucopurulent material from the punctum. This tends to be a low-grade chronic infection, although occasionally it can progress to blepharoconjunctivitis and cellulitis (Fig. 96.7). The most common organism in culture-positive specimens are *Haemophilus haemolyticus* (20%) and *Haemophilus influenzae* (20%).[60] In contrast, acute dacryocystitis, more commonly seen with congenital dacryocystoceles, is a more severe infection that requires prompt intravenous antibiotics (see later). Simple nasolacrimal duct obstruction (NLDO) usually resolves spontaneously within the first year of life in more than 80% of cases; only conservative management is indicated in most cases. This consists of a digital massage downward from the lacrimal sac over the nasolacrimal duct on the side of the nose. The massage empties the sac, reducing the opportunity for bacterial growth. Topical broad-spectrum antibiotic drops or ointment, such as bacitracin zinc and polymyxin B sulfate ophthalmic ointment or drops, can be used if there is conjunctival injection and discharge. Referral to an ophthalmologist should be considered if the condition has not resolved toward the end of the first year, because probing and/or intubation of the lacrimal sac is likely necessary to relieve the obstruction, although the optimal timing of surgery remains controversial. Recent studies report an age-dependent decrease in the success rate of initial probing,[61] while others report no age-related decline in the success rate of surgical treatment for congenital NLDO in children treated up to 36 months of age.[62]

Preseptal and orbital cellulitis can also manifest with conjunctival injection, chemosis, and discharge. In addition, orbital cellulitis may cause altered ocular motility and pupillary reflexes and proptosis. Both conditions are typically unilateral and can rarely occur in the first month of life, sometimes secondary to acute dacryocystitis from an infected dacryocystocele (see Ptosis and Other Eyelid and Lacrimal Abnormalities).



• **Fig. 96.6** A 6-day-old newborn with gonococcal conjunctivitis. Note the marked lid edema and copious purulent eye discharge.



• **Fig. 96.7** (A) Dacryocystocele in a newborn. (B) Manual decompression of mucopurulent material. (Photo courtesy Robert Kersten, University California, San Francisco.)

A subconjunctival hemorrhage is seen as a bright red discoloration under the conjunctiva, obscuring the white scleral background, and is common in the perinatal period. In most cases, it is caused by elevated venous pressure in the head and neck produced by compression during uterine contractions. Later in life, subconjunctival hemorrhage may be a feature of child abuse, although it can also occur spontaneously. A subconjunctival hemorrhage in isolation is completely innocuous and usually resolves in 10 to 14 days.

Motility Abnormalities and Nystagmus

Various eye motility and alignment abnormalities can be present in the first month of life. The most common form of strabismus in early infancy is infantile or essential esotropia (see Fig. 96.3A). This form of convergent strabismus is rarely actually congenital, so this term has been abandoned. It is usually present by age 3 to 4 months and is characterized by a large-angle deviation. These children tend to cross-fixate (use the left eye to view the right visual field and vice versa), simulating an abduction deficit because it is hard to get the child to follow an object to the ipsilateral field. This can easily be mistaken for bilateral sixth nerve palsy. However, sixth nerve palsies in the neonatal period are extremely rare, and an abduction movement can sometimes be elicited by the patching of one of the child's eyes to force the other eye to abduct in search of an object or by use of the doll's-eye (the eyes lag behind the turning of the head from side to side) or optokinetic nystagmus maneuvers. Infantile esotropia is a condition that is not likely to resolve spontaneously without surgical correction. Because of a high associated risk of amblyopia, prompt referral of these patients to a pediatric ophthalmologist within the first few months of life is appropriate. Even if the child's age or systemic condition is not appropriate for surgical correction, patching and other treatments should be initiated as early as possible. Ideally, surgical correction is accomplished early, by 6 to 12 months of age.

Congenital motor nystagmus (infantile nystagmus syndrome) is an involuntary, bilateral, conjugate oscillation of the eyes that develops within the first 6 months of life. Despite the name, eye movement abnormalities are rarely noticed at birth. Disorders of the anterior visual pathways resulting in blindness or severe visual deprivation very early in life can also result in nystagmus (sensory deprivation nystagmus), which can be manifest as large-amplitude "wandering" eye movements or with a smaller-amplitude, faster movement that resembles the congenital motor form. Nystagmus caused by a visual deficit does not develop until about 3 months of age. Any form of bilateral (and sometimes unilateral) visual deprivation, including cataracts, corneal abnormalities, glaucoma, optic nerve problems, and chorioretinal colobomas, can manifest themselves with nystagmus, and a prompt ophthalmic evaluation is needed to rule out these conditions, which are treatable in some cases. Other conditions such as albinism, achromatopsia (congenital absence of the retinal cones), and aniridia can also result in nystagmus, which is often very similar to motor nystagmus. Since the ocular findings in these conditions are more subtle and easily missed, an electroretinogram is usually recommended before the diagnosis is confirmed. Because of the overlap with identifiable causes, motor nystagmus should always be considered a diagnosis of exclusion, as other ocular and neurologic disorders can manifest with the same clinical characteristics. Certain specific forms of nystagmus are commonly associated with neurologic dysfunction; these include upbeat nystagmus, see-saw nystagmus, and even monocular nystagmus. However, these forms have all been associated with sensory loss, and an ophthalmologic examination is necessary even when neuroimaging studies rule out intracranial disease.

Other transient disorders of the ocular motor system include flutter-like, high-frequency, small-amplitude movements that in newborns may be self-limiting and resolve spontaneously within the first few weeks of life. This saccadic oscillation should not be confused with nystagmus. Some infants can also exhibit transient downward deviation of the eyes.[63] This disorder can be distinguished from the more serious sun-setting sign, associated with hydrocephalus, by demonstration of intact upgaze movements using vestibular–ocular responses. Neuroimaging fails to demonstrate any underlying neurologic disorder, and the deviation tends to reduce gradually over the following months. A true upgaze palsy and convergent strabismus can be seen in premature infants who sustained intraventricular hemorrhage.[64] Paroxysmal tonic upgaze of childhood is a rare and distinctive syndrome characterized by episodes of sustained conjugate upward deviation of the eyes. Symptoms normally appear in babies younger than 1 year and are characterized by an upward stare with the eyes rolled back, while the chin is held low (possibly to compensate for the abnormal eye position). The horizontal eye movements are normal, as are the rest of the neurologic examination and imaging findings. The condition tends to improve with time.[65]

Ptosis and Other Eyelid and Lacrimal Abnormalities

External inspection of the eyes and eyelids should be performed in every newborn. The eyelids are fused usually until 25 weeks' gestation, but they may rarely remain fused until 30 weeks' gestation. Eyelid colobomas are rare congenital full-thickness defects of a missing eyelid, usually involving the upper eyelid border. Eyelid colobomas may be associated with Goldenhar syndrome. The consequent lack of ocular surface coverage places the infant at high risk of corneal abrasion, ulcer, scarring, and vision loss. Such infants require urgent ophthalmology consultation and should not be placed under overhead heaters without adequate ocular protection.

Most cases of congenital ptosis are caused by an isolated developmental anomaly of the levator palpebrae muscle. Other causes include congenital third nerve palsy, Horner syndrome, blepharophimosis syndrome, and Marcus Gunn jaw-winking ptosis, which is a synkinesis between the levator palpebrae and the muscles of mastication. This type of ptosis is characterized by elevation of the lid associated with sucking or chewing.

Because form deprivation amblyopia can occur as a result of complete obstruction of the visual axis, this condition must be managed aggressively by an ophthalmologist familiar with the treatment of eyelid disorders and amblyopia. Even though this type of amblyopia is the most severe and concerning to the neonatologist, it is important to remember that even without the complete obstruction of the pupil, amblyopia can occur secondary to the blur induced by astigmatism produced by the ptotic eyelid on the cornea. If vision is not threatened, surgery may be deferred until 4 or 5 years of age, although close follow-up to detect amblyopia is necessary, and many surgeons have argued the benefits of earlier surgery.[66]

A number of eyelid tumors can be present at birth or shortly thereafter. Capillary hemangiomas are usually not present at birth and appear in the first couple of weeks of life. The lesions can have a superficial component, which is red and sometimes dimpled, resembling a strawberry, and a deeper component, which appears

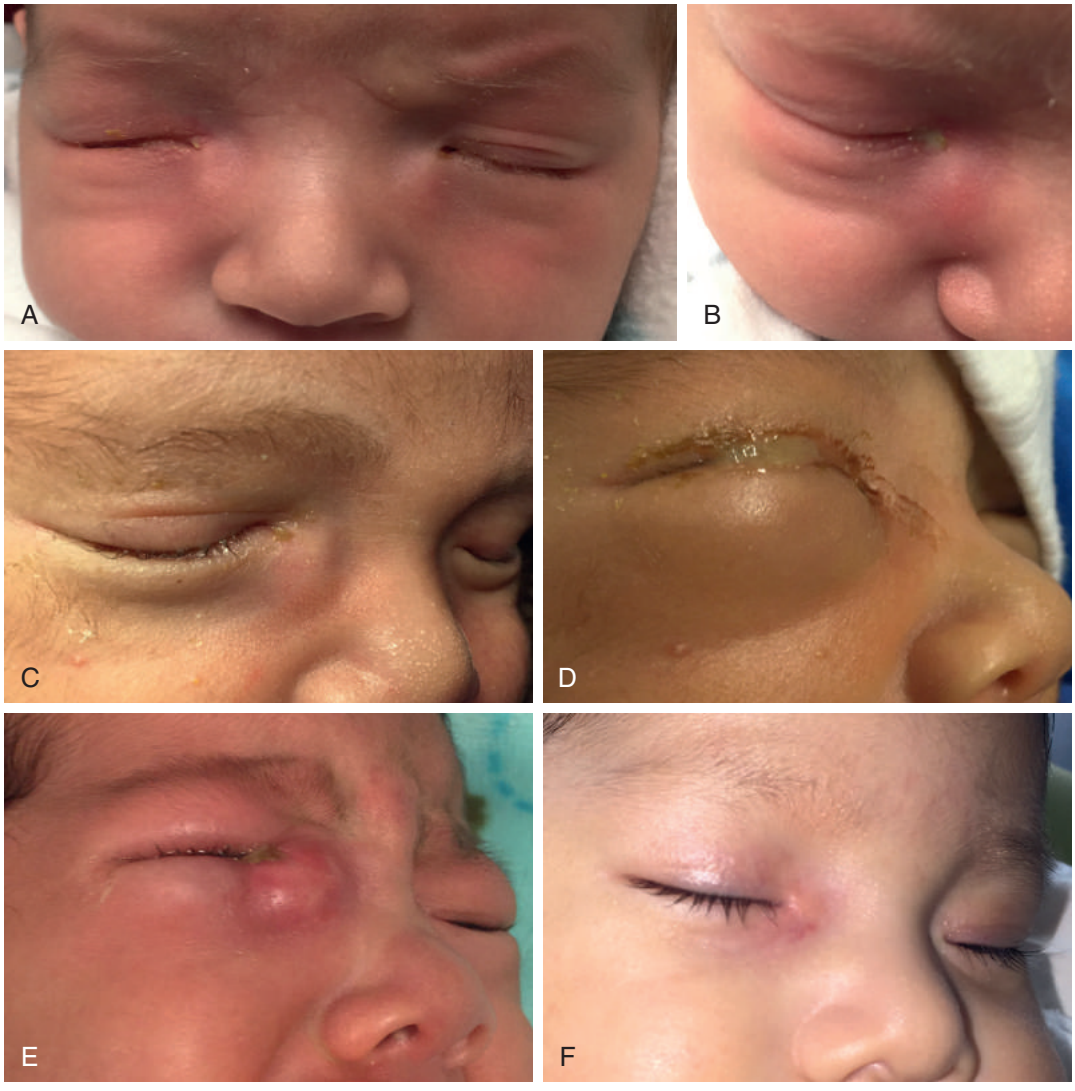
as a deep, diffuse purplish mass. The hemangioma grows during the first 6 to 8 months of life and then stabilizes, regresses, and involutes over several years. These lesions are amblyogenic because they can cause mechanical ptosis, obstruct the visual axis, or cause significant astigmatism. Systemic nonselective beta-blockers (e.g., propranolol) have become first-line treatment, showing remarkable effectiveness in causing regression of these tumors.[67,68] Topically administered timolol can be effective for superficial lesions as well.[69] Other treatment options include topical, intralesional, or systemic steroids; interferon alfa; vincristine; and surgical resection.

Capillary hemangiomas should be distinguished from other capillary vascular malformations (such as port wine stains) that are present at birth and do not exhibit regression. These are sharply circumscribed lesions that are usually unilateral. In these patients, Sturge-Weber syndrome should be suspected. When the skin lesion involves the eyelid, an ophthalmologic consultation should be requested to rule out glaucoma (occurring in about 50% of patients) and vascular abnormalities of the choroid.

A dacryocystocele is formed when a proximal and a distal obstruction coexist in the lacrimal sac and the lacrimal sac becomes distended.[70] Clinically it is manifested as a bluish, nontender mass just inferior and nasal to the medial canthus (Fig. 96.8), is not uncommon, and can have potentially serious consequences in a neonate, including meningitis and septicemia. Intravenous antibiotics should be administered followed by decompression of the sac after 24 to 48 hours if the dacryocystitis shows no abatement. A dacryocystocele should be distinguished from a frontal encephalocele and can occasionally be confused with a hemangioma or a dermoid cyst.

Ocular Trauma in the Neonatal Period

Newborns may experience injuries to the eye as a result of birth, more commonly difficult births, such as those involving prolonged delivery, cephalopelvic disproportion, vacuum extraction, or forceps use. Intrauterine injuries may result from amniocentesis, with delayed discovery at birth; severe eye injuries have also



• **Fig. 96.8** (A) Bilateral dacryocystoceles. (B) Infected dacryocystocele with erythema in medial canthal area and a firm tender mass. (C, D) Dacryocystocele with progression to preseptal cellulitis. (E, F) Dacryocystitis with localized abscess before and after nasolacrimal duct probing with endonasal cyst marsupialization.

been reported to occur during antenatal fetal procedures.[71] Some common injuries will heal without long-term visual or ocular sequelae.[72] However, identification of birth-related eye trauma necessitates immediate ophthalmologic consultation to perform a complete dilated examination, identify all injuries, and assess the need for treatment.

Subconjunctival hemorrhage results from the rupture of blood vessels on the surface of the eye. It is distinguished from conjunctival injection due to an inflammatory or infectious cause by characteristically well-demarcated borders between spots of blood underneath the conjunctiva and completely white sclera directly adjacent to the blood. Subconjunctival hemorrhages will resolve spontaneously with time and do not require treatment. However, large diffuse hemorrhages can be a sign of more significant trauma underneath the hemorrhage or within the eye, and a complete eye examination, including dilated funduscopic examination, may be indicated. Recurrent subconjunctival hemorrhage could be a sign of a coagulopathy or platelet abnormality, and an appropriate work-up should be pursued in such cases.

Hyphema is the presence of red blood cells in the anterior chamber of the eye (the space between the cornea and the iris), most commonly the result of trauma, including birth trauma. It is often associated with other ocular injuries. Clot or hemorrhage of differing magnitude may be seen on close inspection, ranging from a subtle crescent of blood at the corneoscleral limbus to an eye completely filled with blood, obscuring any view of the structures underneath. The red reflex is commonly abnormal. Hyphema is a potentially vision-threatening condition and requires close management by an ophthalmologist. The immediate concern is obstruction of the aqueous fluid drainage angle structures, resulting in an acute rise in intraocular pressure (IOP) or acute glaucoma. High IOP is painful enough to cause vomiting in children and adults and may manifest as crying, irritability, grimacing, poor feeding, or emesis in an infant. If the IOP is high enough for any prolonged period, irreversible optic nerve damage can ensue, with permanent vision loss. Corneal blood staining may also occur, which takes many months to clear, and can result in amblyopia.

An open globe injury (ruptured globe) refers to a full-thickness break in the eyewall (e.g., sclera, cornea). These injuries can occur as a laceration or as a rupture, in which pressure is applied to the front of the eye, and the eye wall breaks open at its weakest points, such as the corneoscleral limbus or extraocular muscle insertion sites. Key signs include an obvious break with protrusion of intraocular contents (e.g., the iris), which typically appear brown; extensive subconjunctival hemorrhage; a flat anterior chamber (the iris and cornea have come together); hyphema; vitreous hemorrhage, which may be seen as a poor red reflex; and abnormal eye contour or intraocular foreign body on orbital imaging. An open globe is a surgical emergency.

A *corneal abrasion* is an epithelial defect on the corneal surface. Staining with fluorescein will identify areas of missing epithelium, which appear green under a blue light source and magnification. Treatment includes topical antibiotic ointment and close follow-up to ensure complete healing and to monitor the patient for the development of bacterial keratitis (see the earlier discussion of corneal clouding). In a neonate, a corneal abrasion must be taken very seriously, because complications can include the sequential development of keratitis, endophthalmitis, septicemia, and meningitis.[73]

Other injuries include RHs (see the later discussion); corneal trauma from forceps, resulting in tears of the Descemet membrane, visible as vertical or circumferential lines on red reflex examination, corneal thickening and opacification from edema, and corneal scarring; eyelid or adnexal injuries requiring surgical repair; choroidal rupture, which requires dilated fundus examination for diagnosis; and even traumatic optic neuropathy.[71]

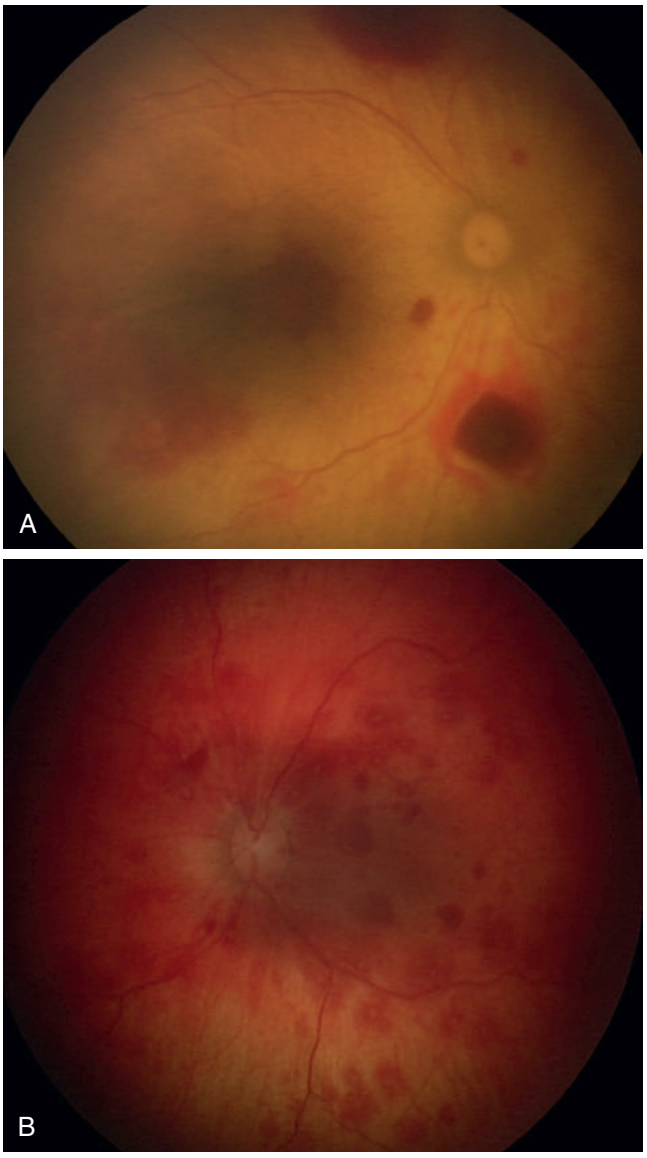
Retinal Hemorrhages and Abusive Head Injury

RH from the birth process is very common. The incidence is estimated to be between 10% and 40% of all newborns, depending on the examiner, time from birth, and the specific population studied.[74–76] The rate in infants delivered with vacuum assistance is even higher, at 75% to 78%.[74,75] The association between vacuum-assisted delivery and RH supports a role for mechanical trauma to the retinal vessels (e.g., from direct compression of the globe); however, additional factors are likely involved, because head circumference and the duration of labor are not significant factors, and RH occurs after cesarean delivery, albeit at a decreased frequency (7%).[74,76] RHs are usually identified by indirect ophthalmoscopy, although an abnormal red reflex may be noted in some severe cases.

The long-term visual consequences of birth-related hemorrhages remain unknown. Central or large hemorrhages can interfere with visual development in some cases (Fig. 96.9A), but the occurrence of significant visual deficits caused by RH is probably low. It is important to understand the characteristics that distinguish birth-related RH from those associated with AHT, as there may be considerable overlap. The great majority of birth-related RHs are intraretinal; subretinal and preretinal hemorrhages are less frequent. Such hemorrhages include superficial flame and deeper blot hemorrhages, and larger hemorrhages may frequently contain white centers, which are a completely nonspecific finding.[74–76] Although commonly located in the posterior pole of the eye, they may also extend to the mid- and far-retinal peripheries and range in number from a few to too numerous to count.[74,75] They may be bilateral, unilateral, or asymmetric.

The preceding characteristics are similar to those of the RH seen in AHT (see the later discussion), although the severity is usually greater in AHT. A key characteristic, therefore, is the time to resolution of birth-related RH. Most intraretinal hemorrhages clear within the first 2 or 3 weeks after birth, many within the first few days.[74,75] In most series, all intraretinal hemorrhages have cleared by age 4 weeks, whereas preretinal or subretinal hemorrhages may take 6 weeks or more to clear.[74] Therefore multiple intraretinal hemorrhages present past 1 month of age should be considered not related to birth. Isolated, resolving intraretinal hemorrhage or persistent preretinal or subretinal hemorrhage past 1 month of age may rarely be related to birth, but this pattern of RH would not at any age by itself be considered specific for any cause, including abuse.

Pediatric AHT is a leading cause of death in infancy. Previous names have included *shaken baby syndrome, inflicted or nonaccidental head trauma*, and *inflicted childhood neurotrauma*. AHT is characterized by intracranial hemorrhage with or without RHs and or additional injuries, including bony fractures. Affected children are less than 3 years of age, with the great majority younger than 1 year.[77,78] The mechanism of trauma is believed to be repetitive acceleration-deceleration of an infant's head, with or without blunt impact. RHs are present in 50% to 100% of victims of AHT.[77,78] The presence of any RH in an infant is highly associated with abuse, and increasing RH severity correlates with an increasing likelihood



• **Fig. 96.9** Retinal hemorrhages in neonates. (A) Birth-related hemorrhages. Note the large hemorrhage covering the central macula in this neonate with thrombocytopenia. This patient developed severe myopia and amblyopia. (B) Diffuse intraretinal hemorrhages (dot blot, flame shape, and white centered) secondary to nonaccidental head trauma.

of abuse.[78,79] In AHT, RH may range from none to a few intraretinal hemorrhages confined to the posterior pole to bilateral, "too numerous to count," intraretinal, subretinal, and preretinal hemorrhages, extending to the far periphery or ora serrata (termination of the retina).[80,81] The hemorrhages may be unilateral or markedly asymmetric. Macular retinal folds and hemorrhagic macular retinoschisis (splitting of the retinal layers) may be seen at the severe end of the spectrum. In comparison, RHs associated with accidental head trauma are typically few in number, intraretinal, and limited to the posterior pole (see Fig. 96.9).[78,82] However, more severe RH and even retinal folds may be seen with unambiguous severe accidental trauma, such as fatal motor vehicle crashes.[83]

In addition to accidental head trauma, the differential diagnosis of RH in infancy includes birth-related RH (see the earlier discussion), coagulopathy, septicemia, leukemia, anemia, and glutaric aciduria. These conditions may be recognized with the use of various diagnostic tests and are often distinguishable from the patterns of RH seen in AHT. Studies have demonstrated that prolonged chest compression with cardiopulmonary resuscitation rarely results in RH, and when present, these RHs are a few isolated posterior pole intraretinal hemorrhages. RHs associated with raised intracranial pressure are limited to small splinter hemorrhages on a swollen optic disc or superficial, flame-shaped, intraretinal hemorrhages adjacent to a swollen optic disc.[84] Vaccination injections, convulsions, and severe coughing do not cause RH.[85]

Children with AHT may present with a history of minor blunt head trauma, such as a short fall, or no trauma history at all and exhibit lethargy, seizures, increased or decreased tone, vomiting, poor feeding, breathing difficulties, or apnea.[77] Head CT identifies subdural or subarachnoid hemorrhage, sometimes with a combination of chronic and acute features, but very rarely the findings may initially be normal; brain MRI provides a better look at the soft tissue and brain parenchyma to identify features such as hypoxic-ischemic injury; plain film radiographs may reveal a skull fracture; a skeletal survey is a critical modality for identifying other bony injuries. Because the history provided is often vague or unreliable, a high index of suspicion and a low threshold for obtaining diagnostic tests and specialist consultation must be maintained.

An ophthalmologic examination should occur within 48 hours, preferably within 24 hours, because RH can begin to resolve within days.[86] A dilated fundus examination with an indirect ophthalmoscope is required to adequately visualize the retina. Ophthalmologic consultation should not be delayed because of an inability to pharmacologically dilate the pupils because of neurologic pupil examination checks; the ophthalmologist can still attempt to view a portion of the retina and return for a dilated examination later. It is very important to document the type(s), number, location(s), and laterality of all RHs, by the use of explicit descriptive terms and thorough use of diagrams.

Fundus photographs, for example, those taken with a RetCam camera, should be obtained when possible. Such photographs provide important documentation and are often easily obtained at the time of sedation administered for MRI or other tests. However, the authors stress that the presence of RH is not descriptive enough, and the extent and detailed characteristics of the hemorrhages are vitally important. Cameras may identify and characterize posterior pole RH, but they do not always provide adequate visualization of the retinal periphery and are not a substitute for an indirect examination. RHs have been reported as a result of RetCam use in a premature infant undergoing ROP screening, but not in other circumstances.[87] Notably, this 25-week-gestational-age infant was less than 34 weeks' PMA at the time of the examination and had immature retinal vasculature, without smooth muscle, elastin, or collagen layers and poor autoregulation, unlike the mature vasculature of a few-months-old infant evaluated for AHT.[87] Further, other investigators have failed to identify any RHs with the routine use of the RetCam for ROP screening.[88] Nevertheless, whenever possible an indirect ophthalmoscopic examination should be performed and documented before a contact fundus camera is used.

Visual impairment in children with AHT is thought to be due, most often, to cortical damage. However, persistent macular or vitreous hemorrhage, retinoschisis, and other scarring conditions may result in significant deprivation amblyopia, induced myopia, and anisometropic amblyopia, or photoreceptor damage limiting visual function. The overall mortality rate in AHT has been

reported to be between 13% and 36%.[89] Approximately two-thirds of the survivors have long-term neurologic deficits. A prospective study of 25 children with a mean follow-up of 5 years, found that 68% had neurologic and cognitive impairment, and half of these had severe disabilities and were totally dependent.[89]

Common Ophthalmic Manifestations of Systemic Diseases

See Tables 96.2–96.4.

TABLE 96.2 Ophthalmic Manifestations of Systemic Diseases With Neonatal Findings

Etiologic Disorder	Inheritance	Ophthalmic Manifestations
Aicardi syndrome	X-linked dominant disorder characterized by triad of callosal agenesis, infantile spasms, and chorioretinal lacunae OMIM 304050	Bilateral or unilateral, multiple, chorioretinal lacunae (most constant feature), optic disk/retinal/iris colobomas, optic nerve head hypoplasia, microphthalmos, retinal detachment[168]
Alagille syndrome (1 and 2)	Autosomal dominant disorder characterized by neonatal jaundice secondary to hepatic cholestasis OMIM 118450 and 610205	Posterior embryotoxon, optic disc drusen, and retinal pigmentary changes sometimes in associations with rod-cone dystrophy
Albinism	Heterogenous disorder of melanin metabolism associated with abnormal development of the retina and visual pathways Ocular forms: type 1 (X-linked) Oculocutaneous forms: types 1–4, Hermansky-Pudlak and Chediak-Higashi syndromes (autosomal recessive, autosomal dominant) More than 13 genes involved	Reduced vision, delayed visual maturation, nystagmus, strabismus, iris transillumination, fundus hypopigmentation, foveal hypoplasia, misrouting of optic nerve fibers, anomalous optic chiasm
CHARGE syndrome (*c*oloboma, *h*eart defect, choanal *a*tresia, *r*etarded growth, *g*enital hypoplasia, and *e*ar anomalies)	Nonrandom cluster of congenital abnormalities Mutation of *CHD7* on 8q12.1[169,170] OMIM 214800	Unilateral or bilateral uveal colobomas (retinal more frequent than iris), microphthalmos, Bell palsy
22q11.2 deletion syndrome	Contiguous gene deletion syndrome (encompasses DiGeorge syndrome, velocardiofacial syndrome, conotruncal anomaly–face syndromes) Autosomal dominant	Posterior embryotoxon, tortuous retinal vessels, eyelid hooding, strabismus, ptosis, sclerocornea[171,172]
Down syndrome	Trisomy 21 OMIM 19605	NLDO, refractive errors, nystagmus, strabismus, retinal abnormalities, keratoconus, cataracts[173]
De Morsier syndrome (septo-optic dysplasia)	Heterogeneous disorder defined by the combination of optic nerve hypoplasia, pituitary dysfunction, and midline abnormalities of the brain (absence of the corpus callosum and septum pellucidum) OMIM 182230	Optic nerve hypoplasia (unilateral or bilateral), strabismus, nystagmus, optic chiasm hypoplasia. Severe cases may have microphthalmos or anophthalmos
Galactosemia (classic)	Metabolic disorder caused by mutation in the galactose 1-phosphate uridyltransferase gene OMIM 230400	Congenital cataracts ("oil-droplet cataracts")
Goldenhar syndrome (oculoauriculovertebral dysplasia, hemifacial microsomia)	Craniofacial birth defect involving first and second branchial arch derivatives OMIM 164210	Epibulbar dermoid (unilateral or bilateral), coloboma of the upper lid, ptosis, strabismus, microphthalmos, nasolacrimal duct obstructions
Homocystinuria	Autosomal recessive metabolic disorder caused by cystathionine β-synthetase deficiency OMIM 236200	Progressive ectopia lentis (lens subluxation), pupil block glaucoma, progressive myopia, optic atrophy, retinal detachment[174]
Kabuki syndrome	Congenital mental retardation syndrome OMIM 147920	Long palpebral fissures with eversion of the lateral third of the lower eyelids, ptosis, strabismus
Möbius sequence	Sporadic disorder characterized by congenital facial weakness and abduction deficits OMIM 157900	Abnormal tearing, esotropia with limited abduction (unilateral or bilateral CN VI palsy), horizontal gaze palsy, other CN palsies (III, IV, V), ptosis
Myasthenic syndromes (congenital and neonatal myasthenia gravis)	Neuromuscular disorders. The congenital form is a nonautoimmune autosomal dominant disorder. The neonatal form is secondary to maternal antibodies	Bilateral or unilateral ptosis, any pattern of strabismus, limited ocular motility

Continued

TABLE 96.2 Ophthalmic Manifestations of Systemic Diseases With Neonatal Findings—cont'd

Etiologic Disorder	Inheritance	Ophthalmic Manifestations
Incontinentia pigmenti (Bloch-Sulzberger syndrome)	X-linked dominant disorder with characteristic skin lesions OMIM 308300	Strabismus, nystagmus, cataracts, optic atrophy, corneal abnormalities, retinovascular abnormalities (may resemble ROP), retinal detachment
PHACES syndrome (*p*osterior fossa brain malformations, *h*emangiomas of the face, *a*rterial cerebrovascular anomalies, *c*ardiovascular anomalies, *e*ye anomalies, *s*ternal defects)	Heterogeneous associations that occur in patients with large segmental cervicofacial hemangiomas OMIM 606519	Microphthalmos, Horner syndrome, retinal vascular abnormalities, optic nerve atrophy, iris hypoplasia, cataracts, sclerocornea, lens coloboma, strabismus, choroidal hemangiomas, congenital third nerve palsy, morning glory deformity, and glaucoma[175,176]
Stickler syndrome (hereditary arthro-ophthalmopathy) (1 and 2)	Autosomal dominant disorder of collagen synthesis OMIM 97300 and 604841	Congenital myopia, vitreous abnormalities, cataracts, retinal detachment
Trisomy 13	Chromosomal abnormality most consistently associated with severe ocular defects	Anophthalmos, cyclopia, microphthalmos, uveal colobomas, cataracts, corneal opacities, retinal dysplasia, intraocular cartilage
Trisomy 18	Chromosomal abnormality with frequent ocular defects	Microphthalmos, short palpebral fissures, ptosis, hypertelorism, iris coloboma, corneal opacities, cataracts
Tuberous sclerosis	Autosomal dominant multisystem disease characterized by hamartomatous growths in multiple organs OMIM 191100 and 605284	Retinal hamartomas, vitreous hemorrhage, chorioretinal punched-out lesions, papilledema, optic nerve atrophy, strabismus[177]

CN, Cranial nerve; *NLDO*, nasolacrimal duct obstruction; *OMIM*, Online Mendelian Inheritance in Man; *ROP*, retinopathy of prematurity.

TABLE 96.3 Eye Manifestations of Intrauterine and Perinatal Infections

Infection	Ophthalmic Findings		Diagnosis
Toxoplasmosis *(Toxoplasma gondii)*	Retinitis, vitritis, choroiditis and anterior uveitis; flat atrophic retinal scars	Present in 75% of newborns with toxoplasmosis, 10% have only ocular findings	Clinical ELISA Positive IgM assay result (maternal IgM does not cross the placenta)
Syphilis *(Treponema pallidum)*	Chorioretinitis (salt-and-pepper appearance or pseudoretinitis pigmentosa), anterior uveitis, glaucoma, interstitial keratitis	Bilateral interstitial keratitis is the classic ophthalmic finding; it occurs in 10% of patients but manifests itself later in childhood or adulthood	Positive VDRL and FTA-ABS test results
Rubella	Nuclear cataracts, glaucoma, uveitis, microphthalmos, retinopathy with salt-and-pepper appearance, pseudoretinitis pigmentosa	50% of children with rubella have ocular findings, bilateral 70% Severe postoperative inflammation after cataract surgery	
Cytomegalovirus infection	Retinochoroiditis, optic nerve atrophy, microphthalmos, cataracts, uveitis, strabismus[178]	Most common intrauterine viral infection in the United States Only 10% of infants show clinical features of CMV infection, with ophthalmologic abnormalities in less than 30% of these	CMV IgM, urine culture, PCR detection of CMV DNA in blood
Herpesvirus infection (HSV 1 and 2, EBV)	Vesicular skin lesions, keratoconjunctivitis, retinochoroiditis, cataracts		Cultures from conjunctival or corneal swabs

CMV, Cytomegalovirus; *EBV*, Epstein–Barr virus; *ELISA*, enzyme-linked immunosorbent assay; *FTA-ABS*, fluorescent treponemal antibody absorption; *HSV*, herpes simplex virus; *IgM*, immunoglobulin M; *PCR*, polymerase chain reaction; *VDRL*, venereal disease research laboratory.

TABLE 96.4 Eye Findings After in Utero Exposure to Teratogens

Teratogen	Ophthalmic Manifestations
Alcohol (fetal alcohol syndrome)	Short palpebral fissures, microphthalmos, epicanthal folds, optic nerve head hypoplasia, tortuosity of retinal vasculature, strabismus, cataracts[179]
Cocaine	Optic nerve abnormalities, delayed visual maturation, ROP-like fundus abnormalities,[180] prolonged eyelid edema[181]
Anticonvulsants (fetal hydantoin syndrome)	Ptosis, trichomegaly, hypertelorism, strabismus, retinal coloboma, microphthalmos, optic nerve head hypoplasia[182,183]
Methadone	Nystagmus, delayed visual development, abnormal VEPs at birth[184,185]
Warfarin and coumadin	Optic atrophy, cataracts, anterior segment dysgenesis, microphthalmos[186]
Thalidomide	Strabismus, Duane syndrome, ptosis, paradoxic gustolacrimal tearing, Möbius sequence[187]
Misoprostol	Möbius sequence[187]

ROP, Retinopathy of prematurity; *VEP*, visual evoked potential.

Role of the Neonatal Healthcare Provider

Neonatal and primary pediatric healthcare providers clearly play a central role in the ophthalmologic care of newborns and young infants. The first few weeks after birth constitute a critical period of visual development in the brain, and the opportunity and responsibility to screen the newborn for ocular disease rests in the hands of those providers, whether neonatologist, pediatrician, family physician, or nurse practitioner. A referral to a pediatric ophthalmologist should be made for any abnormality or asymmetry in an atomic structure or visual function. Some conditions require a particularly urgent referral, such as an abnormal red reflex, cloudy cornea, and ocular infection or trauma. Identification of a sight-threatening cataract or life-threatening retinoblastoma is most commonly made by an astute pediatric clinician in the NICU, nursery, or primary care office who carefully evaluates the red reflex in both eyes. In the case of neonatal conjunctivitis, diagnostic cultures and treatment should be undertaken without delay, but ophthalmology consultation is still necessary to rule out corneal or intraocular involvement. Premature infants at risk of ROP are a particularly vulnerable population. It is the neonatologist's clinical and medicolegal responsibility to arrange timely diagnostic ROP examinations in the NICU and to communicate to parents the absolute necessity of keeping ophthalmologist outpatient appointments after the infant has been discharged from the hospital. Such appointments should be scheduled before discharge. A delay in care by even 1 week could result in a disastrous visual outcome.

The neonatal healthcare and primary care pediatric teams should provide ongoing eye-related education and support. Reducing the risk of pediatric AHT is one important topic to be discussed with all parents and caregivers. Viewing of educational videos on shaken baby syndrome is mandatory in an increasing number of municipalities, and reductions in AHT incidence have been demonstrated. Pediatricians should discuss crying and parental stress at office visits during the first few months of life; the Period of PURPLE Crying educational materials (https://www.dontshake.org/purple-crying) are very useful in this regard. Pediatricians can also provide support for the families of children with visual impairment, ensuring early, anticipatory referral to state commissions for the blind and early intervention services, and ongoing encouragement for parents to maximally utilize such resources when they are available.

Rapid visual development occurs in the months following the neonatal period. Primary care eye examinations and vision assessments continue to be vital for the detection of conditions that may result in visual impairment or blindness, lead to problems with school or social performance, signal the presence of a serious systemic disease, or threaten the child's life. Children should have an eye examination at each well-child visit. Children at high risk of eye disease should be referred for examination by a pediatric ophthalmologist, including children with a history of prematurity or metabolic or genetic diseases; significant developmental delay or neurologic problems; systemic diseases associated with eye abnormalities; a family history of retinoblastoma, childhood cataracts or glaucoma, inherited retinal disorders, or blindness in childhood; and those whose parents needed glasses at a very young age.

Retinopathy of Prematurity

ROP, a disease of the developing retinal vasculature, first became a significant cause of blindness in children in the 1950s with increased survival of premature infants as a result of improved neonatal care, in particular the use of supplemental oxygen in industrialized countries. With the restriction of oxygen use in the mid-1950s, there was a reduction in the incidence of blindness from ROP, but this was associated with increased rates of death and cerebral palsy in premature babies. During the late 1960s, despite more accurate methods to monitor oxygen supplementation and improved management of perinatal complications, blindness from ROP began to reemerge because smaller and less mature babies were surviving. Surgical treatment of established disease and improved neonatal care are probably the major factors responsible for the reduction of the incidence of blinding ROP observed since the 1980s in developed high-income countries.[90] However, ROP-related blindness continues to be an important public health problem among middle-income countries, in which the capability to save small babies has developed, but oxygen use is often not controlled, and the resources to screen babies for and treat ROP are lacking.[91] From the perspective of the neonatologist, a fundamental understanding of the current classification, screening, and treatment guidelines for ROP can lead to better outcomes for premature infants.

Pathogenesis of Retinopathy of Prematurity

ROP develops as a result of incomplete retinal vascularization, resulting retinal hypoxia and pathologic neovascularization, and eventually tractional retinal detachment and blindness. With premature birth, the relative hyperoxia of the extrauterine environment and exogenous oxygen supplementation damage existing retinal blood vessels and suppresses the retinal secretion of vascular endothelial growth factor (VEGF), a hypoxia-induced vasoactive molecule responsible for normal blood vessel development in the body.[92–95] Premature birth also results in a loss of maternal insulin-like growth factor (IGF)-1, a somatic growth factor

secreted by the liver but derived primarily from maternal sources until the third trimester.[96] As retinal development progresses, relative hypoxia ensues, but VEGF-induced vessel growth is temporarily suppressed by low serum levels of IGF-1.[93] Finally, as the infant's innate production of IGF-1 rises after 30 to 33 weeks' PMA, heightened local concentrations of VEGF are activated, causing visible pathologic neovascularization.

Classification of Retinopathy of Prematurity

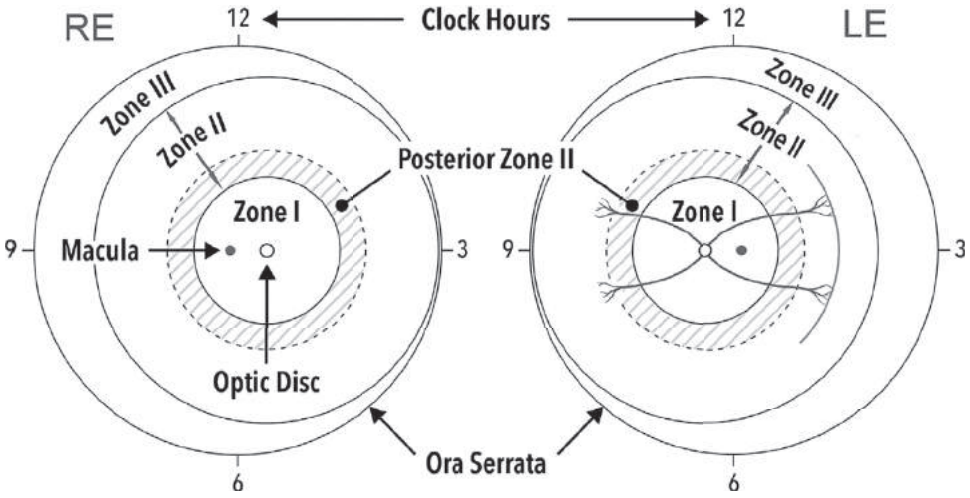
The International Classification of Retinopathy of Prematurity (ICROP) first was published in 1984,[97] expanded in 1987,[98] and was recently updated in a third version,[99] providing a standard nomenclature for the clinical findings and staging of ROP. As shown in Table 96.5, this classification takes into account four components of the ocular findings: anterior-posterior location of the retinopathy (zone), severity (stage), the extent of the disease at the circumference of the vascularized retina (in clock hours), and the presence or absence of "plus disease." *Plus disease* occurs along a spectrum of vascular changes with increasing dilation and tortuosity of the vessels of the posterior pole of the eye and is an important sign of disease severity and risk of imminent progression to retinal detachment. In eyes with ROP in multiple zones and stages, ROP status is determined by the highest stage and the lowest zone observed, along with noting the presence or absence of plus disease.

In ICROP, the retina is divided into three concentric "zones" centered on the optic nerve with zone I including the central retina, zone III including the far peripheral retina, and zone II inclusive of the retina between zones I and III (Fig. 96.10). The newer classification subdivides zone II into an anterior and posterior portion. The majority of retinopathy seen in the United States occurs in zone II, though generally more aggressive retinopathy may occur in zone I or posterior zone II. ROP that occurs in zone III tends to be milder. The severity of retinopathy is designated on a scale of 1 (least severe) to 5 (most severe) by "staging" the appearance of the junction between the vascular and avascular retina (Fig. 96.11). Stages 1 and 2 represent mild and moderate disease, and stage 3, characterized by fibrovascular proliferation off the retina into the vitreous indicates more serious disease. Stages 4 and 5 denote the presence of a retinal detachment. The term *plus disease*, a clinical diagnosis made based on a comparison with reference photographs used in clinical trials in the United States,[100] indicates marked tortuosity and/or dilation of the arterioles and venules in the posterior pole in at least two quadrants. *Pre-plus disease* designates the presence of abnormal vessel changes insufficiently severe

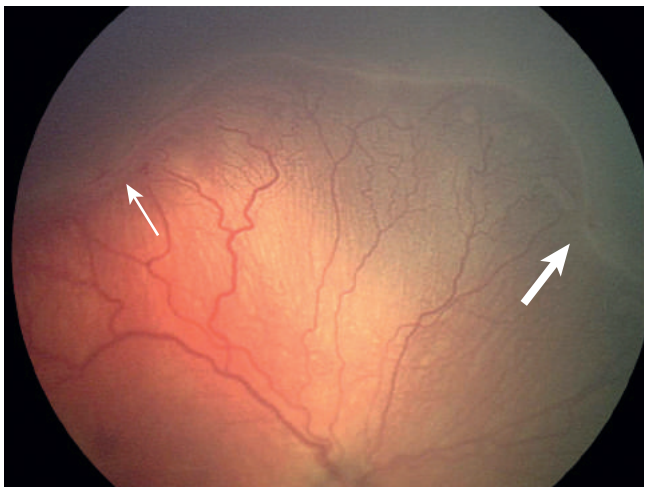
TABLE 96.5 International Classification of Retinopathy of Prematurity, Third Edition (2021)

Anterior–posterior location of vascularization	**Zone** (most posterior retinal vascularization or ROP lesion)	**Zone I:** most posterior; retinal area within a circle centered on the disc and with a radius of twice the estimated disc–foveal distance **Zone II:** ring-shaped retinal area extending nasally from the edge of zone I to the nasal ora serrata and with the same radius distance temporally, superiorly and inferiorly; **posterior zone II** is a region of 2 disc diameters peripheral to the zone I border **Zone III:** residual crescent-shaped retinal area extending beyond zone II to the ora serrata
Severity	**Stage of ROP** (most severe ROP lesion)	**Stage 0:** incomplete vascularization (no ROP) **Stage 1:** a thin, flat, sharp white line of demarcation between the vascular and avascular retina **Stage 2:** an intraretinal elevation (ridge with height and width) at the junction between vascularized and avascular retina **Stage 3:** a ridge with extraretinal neovascular extension into the vitreous (can be flat) **Stage 4:** partial retinal detachment; ***stage 4A***, does not involve the fovea; ***stage 4B***, involves the fovea **Stage 5:** total retinal detachment; ***stage 5A***, optic disc is visible by ophthalmoscope (open-funnel); ***stage 5B***, optic disc is not visible (retrolental fibrovascular tissue or closed-funnel); ***stage 5C***, 5B findings plus anterior segment abnormalities (lens displacement, shallowing of the anterior chamber, corneal opacification, etc.)
Tempo of progression and appearance of vascular abnormalities	**Aggressive ROP* (A-ROP)**	Rapid development of neovascularization and severe plus disease without progression through typical stages of ROP; atypical stage 3 (flat neovascularization), dilated vascular loops, arteriovenous shunts, ill-defined vascular-avascular junction
Extent		Number of clock hours (30° sectors) of ROP along the circumference of the vascularized retina (no longer used for treatment decisions)
Posterior pole (Zone I) vascular abnormalities	**Plus disease spectrum**	Presence of dilated and tortuous vessels in the posterior (Zone I) retina
	Pre–plus disease spectrum	Abnormal vascular dilation and tortuosity that are insufficient for diagnosis of plus disease
Late phases	**Spontaneous or after treatment**	**Regression:** disease involution and resolution with complete vascularization or incomplete with persistent avascular retina (PAR) **Reactivation:** recurrence of acute phase features (vascular dilation, tortuosity, extraretinal neovascularization, etc.) **Long-term sequelae:** late retinal detachment, retinoschisis, persistent avascular retina, lattice-like changes, retinal holes, macular abnormalities, retinal vascular changes, abnormal foveal development

*The term *aggressive posterior-ROP* is no longer used because these abnormalities are not necessarily confined to the posterior retina.
ROP, Retinopathy of prematurity.
From Chiang MF, Quinn GE, Fielder AR, et al. International classification of retinopathy of prematurity. *Ophthalmology*. 2021;128(10):e51–68.



• **Fig. 96.10** Retina of the right eye *(RE)* and left eye *(LE)* showing zone borders and clock hours used to describe the location and extent of retinopathy of prematurity. (From Chiang MF, Quinn GE, Fielder AR, et al. International classification of retinopathy of prematurity. *Ophthalmology*. 2021;128:e51–68.)



• **Fig. 96.11** Fundus photograph showing the ridge between vascularized and avascular retina characteristic of stage 2 retinopathy of prematurity *(large arrow)*. Stage 3 retinopathy of prematurity is present (left portion of photograph, *small arrow)*.

to be designated as *plus disease*. Other signs that frequently accompany Plus disease include vitreous haze, iris vascular engorgement, and pupillary rigidity. Identification of plus disease can vary, even when performed by expert graders, who can have a tendency to systematically over-call or under-call plus disease.[101] This has led to the reframing of the diagnosis of plus disease as a spectrum of vascular change rather than a discrete classification in the most recent version of ICROP.[99] The inherently subjective nature of identifying plus disease demonstrates a need for careful consideration in using this designation in both clinical care and research.

In 2005, the designation aggressive posterior ROP (APROP) was added to ICROP. APROP was added as a separate entity based on the observation of a particularly aggressive form of ROP observed with increasing frequency in the smallest premature babies. This aggressive variant is seen more commonly in countries with developing neonatal care systems. Important hallmarks of the disease are marked posterior pole vascular abnormalities of dilation and tortuosity with what at first may appear to be mild peripheral retinopathy but on closer look is flat, subtle fine neovascularization, making this entity more difficult to recognize.[99,102]

Prevalence and Incidence of Retinopathy of Prematurity

Large natural history studies have shown that, in most cases, ROP begins at 31 to 33 weeks' PMA[100,103,104] with progression during the next 2 to 5 weeks. Spontaneous regression occurs in the majority of eyes that develop stage 1 to 3 ROP.[105,106] Blindness or severe visual impairment commonly results from progression of the retinopathy to retinal detachment or severe distortion of the posterior retina.

In the United States, ROP occurs in less than half of babies with birth weights less than 1500 g (very low birth weight [VLBW]).[104] A greater proportion of babies with birth weight less than 1000 g (extremely low birth weight, ELBW) or less than 750 g develop ROP.[100,103] As neonatal services continue to improve, a greater proportion of VLBW and ELBW babies survive, with a resultant increase in the population of babies at risk.[91] Based on a cohort of more than 7,400 neonates in the United States, the incidence of ROP was 43%, only 12.5% developed severe ROP, which was almost exclusively among those with low birth weight (less than 1251 g), and only about 6.5% met criteria for treatment.[104]

Detection of Serious Disease

Because the benefit of treatment of serious ROP has been shown in randomized clinical trials,[107–110] it is essential to identify the at-risk baby so that timely examinations can be performed to prevent blindness or at least decrease its likelihood. In the United States, the recommended guidelines for detection of serious ROP indicate that diagnostic examinations should be performed on infants with birth weights less than or equal to 1500 g or gestational age of less than or equal to 30 weeks 0 days, along with babies in the 1501- to 2000-g birth weight group thought to be at high risk by the neonatologist.[111] In Latin American countries and in urban centers of newly industrializing countries in Asia and eastern Europe, the same screening criteria do not apply, because evidence suggests that larger, older babies are also at risk in these settings, and national or regional guidelines need to be developed.[112–116]

The first examination should generally occur at 31 weeks' PMA or chronologic age of 4 weeks, whichever occurs later.[111] Examinations usually continue on an every-other-week basis unless ROP develops, at which time examination frequency may be increased depending on the severity of the disease. In general, examinations continue until ROP is observed to regress, ROP progresses to treatment severity, or vessels are observed on at least

two occasions to have progressed into zone III in the absence of prior development of ROP.

Prediction of Retinopathy of Prematurity

Following from the pathologic sequence described previously, important risk factors for ROP include the degree of prematurity (early gestational age at birth and low birth weight); hyperoxia early in the postnatal course, especially from excessive oxygen supplementation; and low postnatal serum IGF-1 level in the postnatal period up to 33 weeks' PMA.[117,118] Slow postnatal weight gain is a reliable surrogate measure for serum IGF-1 levels.[93,119,120] Numerous other factors have been associated with ROP, such as sepsis, acidosis, nutritional deficiencies, and necrotizing enterocolitis; however, many of these factors may influence serum IGF-1 levels in a common mechanistic pathway.[121,122] Newer research has suggested that the relationship between postnatal weight gain and the development of severe ROP is likely more complex than previously understood. A large study of neonates assessing postnatal weight gain found that a low weight gain rate during the 29- to 33-week postmenstrual period is consistently associated with severe ROP, but if weight gain during this period is moderate or high, subsequent rapid rises in weight gain are also associated with increased risk of developing severe ROP.[123]

Current screening guidelines focus solely on gestational age at birth and birthweight as predictors of ROP. Current guidelines have low specificity, as only 5% to 7% of infants require treatment, and very high but not 100% sensitivity for severe ROP because some infants with birth weight and gestational age above the screening thresholds develop disease severe enough to require treatment. Therefore, a third, subjective criterion, a poor postnatal course, must also be used.[122] Because slow postnatal weight gain is highly correlated with IGF-1 levels and has been shown to be a reliable predictor of subsequent ROP development,[121,124–126] multiple postnatal weight gain predictive models have been developed in an effort to improve the efficiency of ROP screening in highly developed NICU systems. These models include WINROP (Weight, Insulin-like growth factor-I, Neonatal, ROP),[127,128] ROPScore,[129] PINT-ROP,[126] the Children's Hospital of Philadelphia (CHOP) ROP model,[130] and the Colorado ROP model.[131] All of these models initially exhibited very high sensitivity for predicting severe ROP, while greatly reducing the number of infants who would have required examinations, in model development studies. However, in each case, the model was developed using too small of a cohort, resulting in overfitting and decreased sensitivity in validation studies.[122]

To address the limitations of prior models, the Postnatal Growth and Retinopathy of Prematurity (G-ROP) study group used data from 7483 infants to develop new screening criteria that led to a 30% reduction of infants requiring examinations while maintaining 100% sensitivity for detecting high-risk ROP without the need for a subjective criterion.[122] A premature infant meeting any one of the following six G-ROP criteria would receive examinations; gestational age less than 28 weeks; birth weight less than 1051 g; weight gain of less than 120 g, 180 g, or 170 g during ages 10 to 19 days, 20 to 29 days, or 30 to 39 days, respectively; or hydrocephalus. These criteria maintained 100% sensitivity in a prospective validation study of nearly 4,000 infants, and the G-ROP criteria were the first postnatal weight gain criteria to be successfully validated in this fashion.[132]

In addition to improving the specificity of ROP screening and eliminating potentially unnecessary examinations, newer risk stratification models such a G-ROP can also result in cost savings. A microsimulation model analyzing resource utilization and ROP outcomes found that instituting the more stringent screening G-ROP guidelines in the United States could result in more than 36,000 fewer examinations, with an annual cost savings of nearly $3,000,000 USD through hospital discharge.[133] Moreover, the criteria are also more sensitive than the current, conventional criteria, so the G-ROP criteria are a dominant cost-effective strategy.[133]

Based on these studies, postnatal weight gain could soon be incorporated into current screening guidelines given their high sensitivity for predicting and detecting severe ROP.[134]

Prevention of Retinopathy of Prematurity

Medical treatments designed to prevent ROP hold promise to decrease the development of serious ROP. The most obvious action is to control excessive oxygen supplementation. Strict regulation of the use of supplemental oxygen in the first few weeks of life has been recommended as a strategy to reduce the incidence of severe ROP; however, this can result in increased nonocular morbidity and mortality. In a multinational effort to determine the optimal oxygen saturation targets for premature infants, a group of harmonized randomized clinical controlled trials were conducted: the Surfactant, Positive Pressure, and Oxygenation Randomized Trial (SUPPORT) in the United States, the Canadian Oxygen Trial (COT), and the Benefits of Oxygen Saturation Targeting II (BOOST II) trial conducted in the United Kingdom, Australia, and New Zealand. In a meta-analysis of these three trials, the risk of developing severe ROP was not significantly different between the restricted oxygen group (Spo_2 targets of 85% to 89%) and the liberal oxygen group (Spo_2 targets of 91% to 95%) (risk ratio [RR] 0.72, 95% confidence interval [CI] 0.50 to 1.04); however, sensitivity analysis using a fixed-effect model (where BOOST II had a larger weight assigned) moved the estimated effect in favor of restricting oxygen use (RR 0.75, 95% CI 0.63 to 0.88). Higher rates of necrotizing enterocolitis and death before hospital discharge were, however, found in the restricted oxygen group. The study authors concluded that the level of certainty for the ROP outcome is low, and there is still significant uncertainty about the optimal target range for Spo_2.[135]

Currently, there are no other medical interventions that have been proven to prevent the development of severe ROP in VLBW infants. Interventions such as steroids given to expectant mothers immediately before preterm birth and surfactant administered to the neonate shortly after birth decrease the incidence of respiratory distress syndrome. However, these medical advances have also increased the survival rate of VLBW babies.[136] Vitamin E supplementation, dietary supplementation with omega-3 polyunsaturated fatty acids, systemic and topical beta-blockers, and intravenously administered IGF-1 and IGF-binding protein remain under investigation.[137–139]

Establishment of a Retinopathy of Prematurity Program

Robust ROP programs are essential to prevent the visual morbidity associated with failing to detect or treat severe ROP, and these programs must be tailored to each specific clinical environment. The main goals associated with establishing an ROP program are primary prevention (i.e., preventing ROP through overall care of the neonate) and secondary prevention (i.e., screening, case detection, and treatment).[140] Primary prevention, specifically with regard to supplemental oxygen administration, was discussed

earlier. The establishment of a robust screening system with the aim of secondary prevention requires a collaborative relationship between neonatologists, ophthalmologists, nurses, social workers, and discharge coordinators. An essential component is an ROP coordinator, working with the ophthalmologist and in communication with the NICU and involved staff.

Strong lines of communication improve workflows with regard to the frequency and timing of eye examinations and treatments. Maintaining a regular day and time each week for screening may improve buy-in from nursing staff who may plan to hold feedings prior to examinations. Unilateral cancellations of examinations by the neonatal team or nurse should never be made if a child is thought to be too unstable for an eye examination; rather, a conversation should occur between the neonatologist and the ophthalmologist, weighing the risks of the exam with the risks of postponing the exam in terms of the ROP risk for that specific infant at that postmenstrual age. Note that because plus disease plays a key role in most treatment decisions, and plus disease diagnosis is based upon the appearance of the more easily visualized vessels in the posterior pole of the eye, a quick, few-second look at each eye without an eyelid speculum can often be accomplished without adverse effects even in a sick infant, as a compromise partial exam, followed with another examination a week later.

An ROP coordinator can communicate with nurses to ensure that neonates are given dilating drops 30 to 60 minutes prior to their eye examination. These drops often include a combination of cyclopentolate 0.5% and phenylephrine 2.5% given 2 to 3 times 5 minutes apart; the precise agent and frequency may vary depending on provider preference and medication availability.[140] During the eye examination, pain can be addressed by administration of topical anesthetic eye drops such proparacaine and oral sucrose. Nurses can swaddle the infants and monitor oxygen saturation and heart rate. A sterile eyelid speculum and scleral depressor may be used to improve visualization of the retina. A re-usable indirect ophthalmoscope and 28 diopter indirect lens are typically used to examine the retina but should be cleaned in between patients with a disinfectant such as bleach and then rinsed well with water to avoid bleach contacting the eye and deposits on the equipment. An adenoviral outbreak involving 23 hospitalized neonates, as well as NICU staff and parents, was traced back to screening ROP examination equipment.[141] This epidemiological case study emphasized the importance of infection control measured including hand hygiene, glove use, standard and isolation precautions, and enhanced cleaning of lenses and ophthalmoscopes between examinations.[141] Given the possibility of severe health complications from adenovirus in vulnerable populations, strict hygienic standards must be prioritized.

An ROP coordinator can help not only with the logistics of inpatient examinations but also with ensuring that outpatient screening appointments are confirmed prior to discharge and kept by parents after discharge, to avoid a loss to follow-up and the risk of severe disease not being treated. A small study of follow-up for infants with or at risk for ROP found that nearly 50% did not attend timely follow-up, though the rate of follow-up was much higher when appointments were scheduled by hospital personnel prior to discharge.[142]

Coordination of this schedule among neonatal care providers, ophthalmologists, nursing staff, and parents is essential. If outpatient appointments are not kept or proper information is not conveyed with regards to the risk of blindness when the baby is transferred to another facility, potentially treatable disease may be missed, with disastrous consequences.[143] Even a week delay can be significant, as a treatment for ROP should occur within 72 hours once treatment criteria are met. From a medicolegal perspective, ROP claims represent anywhere from 0.5% to 4% of all ophthalmology-related lawsuits.[144,145] Causes for lawsuits have included inappropriately long periods between follow-up examinations, failure of outpatient referral from screening to treatment, and unsupervised resident provision of ROP care.[145] Most, if not all, of these preventable factors can be addressed through the establishment of a well-considered ROP program.

Telemedicine Screening for Retinopathy of Prematurity

Although direct examination by an ophthalmologist experienced in examining babies at risk of ROP continues to be the optimal standard of care, retinal digital imaging has emerged as a promising modality for ROP screening. The increased workload due to the increase in survival of premature infants worldwide[91] coupled with the reduced availability of ophthalmologists with sufficient ROP experience has required the development of alternative screening approaches. The published sensitivity of digital fundus imaging as a screening technique for ROP ranges from 57% to 100% (with wide 95% CIs) when compared with the reference standard (indirect ophthalmoscopy). In 2015, the American Academy of Pediatrics and the American Academy of Ophthalmology published a review of the current status of telemedicine in ROP[146] and concluded that retinal digital imaging is a reasonable alternative to but not a substitute for direct examination by an on-site ophthalmologist. However, more studies are needed to determine the overall validity of the approach.

Notwithstanding the lack of consensus, ROP telemedicine is becoming increasingly available in the United States and other countries. The Stanford University Network for Diagnosis of Retinopathy of Prematurity (SUNDROP) has reported its experience evaluating infants meeting ROP screening criteria with images taken in the NICU by trained nurses and remotely evaluated by an ophthalmologist. In this program, all infants undergo one ophthalmologist examination after discharge. In a recent report of 608 infants examined during a 6-year period, sensitivity was reported as 100%, with specificity and a negative predictive value of 99.8% and 100%, respectively.[147]

Other reports have focused on the logistics of the implementation of ROP telemedicine in various settings throughout the world. The Karnataka Internet Assisted Diagnosis of Retinopathy of Prematurity model provides ROP screening in more than 80 units in the Karnataka region of India, using technicians who obtain and grade the images at the bedside and report the need for an evaluation to an ophthalmologist, who can then give treatment if needed.[148] This model has demonstrated that ROP screening services can be provided in regions of the world with limited access, but it is still highly dependent on the availability of ophthalmologists to provide treatment and high levels of training and technical expertise. Gilbert et al.[149] have stressed the need to shift the emphasis on examination to remote imaging in low- and middle-income regions of the world where ROP remains a leading cause of childhood blindness yet the expertise, availability, and physical resources for adequate ROP screening are quite limited. In these countries, this approach has the potential to expand services dramatically as increasingly small birthweight and low gestational age infants survive worldwide. In more developed healthcare systems, the potential role of telemedicine as a stand-alone screening tool is currently undergoing intensive scrutiny, highlighting a number

of complex issues for implementation (limited number of available imaging systems, high cost of equipment, personnel training requirements, quality control issues, coordination of services, accountability, etc.) that should be resolved before a successful and safe implementation of such a screening modality.

Future directions in this domain include the use of artificial intelligence and deep learning to identify ROP. Research on these technologies has already begun with early yet encouraging results. One specific algorithm called DEEP-ROP performed well in differentiating plus disease based on wide-angle posterior pole retinal images.[150] Another deep learning system, i-ROP, was able to accurately detect a vessel severity score, which offers the potential advantage of providing objective data about ROP status based on imaging.[151] Further research and validation are required to determine the extent to which these technologies will be incorporated into ROP screening.[152]

Treatment of Retinopathy of Prematurity

Laser ablation of the peripheral avascular retina can prevent progression to blinding disease in patients with severe ROP and is currently the standard of care for treatment. The evidence of treatment effectiveness and the indications for treatment (severity level) have evolved through the last 30 years as multiple multicenter clinical trials have been conducted to evaluate different treatment modalities and preventative strategies. Landmark studies include the Cryotherapy for Retinopathy of Prematurity (CRYO-ROP) study, the Early Treatment for Retinopathy of Prematurity (ET-ROP) study, the Supplemental Therapeutic Oxygen for Prethreshold Retinopathy of Prematurity (STOP-ROP) study, the Effects of Light Reduction on Retinopathy of Prematurity study, and the Bevacizumab Eliminates the Angiogenic Threat for Retinopathy of Prematurity (BEAT-ROP) study.

Current treatment guidelines are based on the findings of the ET-ROP study, so treatment intervention is recommended on the development of "type 1" pre-threshold ROP, which is defined as *any stage with plus disease in zone I, stage 3 without plus disease in zone I, or stage 2 or 3 with plus disease in zone II*. It is important to highlight that the number of clock hours of disease previously used in the CRYO-ROP definition of "threshold" is not considered anymore, and treatment should not be delayed until "threshold" ROP is reached.

Today, laser photocoagulation is universally preferred over cryotherapy because it causes less intra- and postoperative pain and allows direct visualization of the area during treatment. For advanced disease (i.e., retinal detachment, stages 4 and 5), studies have shown mixed results, but treatment is in general much less successful.[153–156] For eyes with total retinal detachment, a good functional visual outcome is not likely to be achieved.[154,157,158] Concerns associated with laser treatment include the risk of intubation and sedation frequently required for the procedure, the prevalence of high myopia and peripheral visual field reduction, and poor visual acuity outcomes in patients with posterior or aggressive ROP.

In the past decade, there has been increased off-label use of intravitreal anti-VEGF drugs for the treatment of ROP. The effectiveness of these drugs, since reported in multiple observational studies, was demonstrated in a multicenter randomized controlled trial,[159] BEAT-ROP, in which intraocular bevacizumab injection was shown to result in a lower retreatment rate by 54 weeks' PMA than laser photocoagulation in babies with zone I and posterior zone II type 1 ROP (6% vs. 42% in the zone I subgroup). Although the BEAT-ROP study had limitations, including a subjective outcome measure based on treatment decisions made by ophthalmologists who were unmasked to the treatment group and an unusually high re-treatment rate following laser compared to common clinical experience, the study did clearly demonstrate the short-term effectiveness of anti-VEGF. As a result, intravitreal injection of bevacizumab and other anti-VEGF agents (e.g., ranibizumab, aflibercept) is increasingly being used as a first-line treatment for eyes with zone I or posterior zone II ROP. Historically, laser photoablation has been less effective at preventing progression to retinal detachment in zone I eyes compared to zone II eyes. At the 6-year outcome of the ET-ROP study, more than 30% of lasered zone I, type 1 eyes had visual acuity of 20/200 or less, and more than 20% had a macular fold or retinal detachment.

The advantage of anti-VEGF over laser for posterior disease is due to the speed of action of each treatment modality. Both modalities work by blocking VEGF-induced pathologic neovascularization. Laser results in the destruction of the source of VEGF (hypoxic avascular retina), so it takes a week or more to begin to see the effects of laser because free VEGF remains active until it is gradually broken down in the eye. In contrast, anti-VEGF directly binds free VEGF, so that its effect is seen rapidly, usually within 12 to 24 hours. Therefore, there is a benefit to anti-VEGF when ROP is relatively more aggressive. Using early postmenstrual age as a marker of more aggressive disease, Barry et al. reported a lower rate of short-term retinal detachment with anti-VEGF versus with laser when treatment occurred before 36 weeks post-menstrual age in a non-randomized but large study.[160] Another consequence of each modality's mechanism of action is that because anti-VEGF does not destroy the source of VEGF, ROP may recur and require retreatment following anti-VEGF, the effects of which typically last 3 to 6 weeks depending on the drug. Such recurrence has been reported as late as even 2 or 3 years of age and would not have been captured in the BEAT-ROP study.[161] In contrast, when an adequate laser is performed early enough to result in full regression of ROP, recurrences generally do not subsequently occur.

With regards to long-term refractive error, The BEAT-ROP study reported that children treated with bevacizumab had a significantly lower incidence of high myopia at 2.5 years post-treatment than infants treated with laser ablation. High myopia occurred in 3.8% of zone I eyes and 1.7% of zone II eyes that received bevacizumab, compared with 36.4% of zone I eyes and 51.4% of zone II eyes that received laser treatment ($P < .001$).[162] Intravitreal injection is faster to perform and typically requires minimal sedation without intubation. There is a faster recovery to baseline respiratory status following sedation and intravitreal bevacizumab compared to infants treated with laser under general anesthesia.[160]

The long-term ocular and systemic safety of anti-VEGF agents is unknown. Retinal vascularization after anti-VEGF is not normal. Lepore et al. reported peripheral and macular vascular abnormalities detected on retinal fluorescein angiogram 9 months after ROP treatment with bevacizumab that persisted 4 years after treatment and were not present in lasered eyes.[163,164] Moreover, systemic absorption of the anti-VEGF medications after intraocular injection is significant and leads to a reduction in systemic VEGF levels, lasting at least 60 days after injection, and raises concerns about systemic anti-angiogenic consequences of these drugs. VEGF is a critical factor in neurodevelopment and vasculogenesis in other developing organ systems, including the brain, lungs, and kidneys. Long-term safety data are limited. The BEAT-ROP study was too small to evaluate short or long-term safety effects on the

development of brain and other tissues. More recently, observational reports from Canada and Taiwan described an increased risk of motor impairment in ROP patients treated with bevacizumab.[165–167] In the Canadian study, the authors attempted to control for many possible factors that could affect neurocognitive development, such as SNAP II scores, bronchopulmonary dysplasia, sepsis, intraventricular hemorrhage, gestational age, and maternal education and found that at age 18 months, bevacizumab-treated infants were two to three times more likely to display unfavorable developmental outcomes compared with those that underwent laser ablation. However, these non-randomized studies may not adequately control for other unknown confounders. Severe ROP is an independent predictor of adverse long-term neurodevelopment, and the indication for anti-VEGF treatment over laser ablation may be confounded by the severity of the disease or the underlying systemic condition of the infant (confounding by indication). Nevertheless, this early evidence increases the concern regarding the long-term safety of anti-VEGF treatment and calls for more research in this area. When treatment is required, careful consideration and a thorough discussion with the parents and neonatology team are recommended. None of the anti-VEGF drugs have been approved by the US Food and Drug Administration for the treatment of ROP, and therefore explicit consent for their off-label use must be obtained. Premature infants treated with anti-VEGF injections require close follow-up for extended periods of time, at least until complete retinal vascularization is observed or recurrence occurs. As infants get older, it becomes more challenging to obtain an adequate retinal examination, and many ophthalmologists recommend laser treatment if vascularization has not occurred by a certain postmenstrual age, typically 50 to 60 weeks. Therefore, many infants initially treated with anti-VEGF ultimately also receive laser, and parental expectations should be set accordingly.

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Ear and Hearing Disorders

BETTY VOHR AND SUSAN WILEY

KEY POINTS

- Hearing loss is one of the most common congenital abnormalities, occurring in approximately 1.5 to 2 per 1000 newborns.
- Hearing loss may be present at birth or have delayed onset.
- A comprehensive work-up is indicated to identify if the etiology is related to genetic or nongenetic causes, or associated with other abnormalities.
- Early diagnosis, intervention, and access to language within the first 6 months of life provide the best opportunity for typical language development.

Normal Hearing

To understand the spectrum of permanent and transient hearing disorders that can be identified, it is necessary to understand the normal hearing pathway, which consists of transmission of sound energy from the environment via the outer ear through the middle ear, inner ear, auditory nerve, brainstem, and, finally the temporal lobe cortex, where the sound's electrical energy is interpreted as language (Table 97.1). Dysgenesis, disruption, or injury at any point in the pathway may result in a hearing loss that can have its onset in fetal life or during childhood. The auditory pathway develops embryologically very early in gestation. The inner ear develops first, at about 22 days, and is derived from the otic placodes on either side of the developing head. The middle ear and ossicles derive from the first and third pharyngeal pouches and begin to develop at about day 33. The outer ear develops from the dorsal portion of the first pharyngeal cleft. As a result, congenital hearing loss is often seen in conjunction with structural abnormalities of the head, eyes, ears, nose, and throat. In fact, the odds of having a hearing loss is significantly increased if a preauricular skin tag or pit is present.[1] Atresia or microtia of the pinna are also strongly associated with a permanent hearing loss. Table 97.2 shows the area of pathology associated with the types of hearing loss. A standard medical assessment for every newborn/child should include both a family history of hearing loss and a comprehensive physical examination for minor or major congenital abnormalities and syndromes associated with hearing loss.[2] The primary provider should be familiar with risk factors associated with hearing loss, particularly a family history of permanent hearing loss. Approximately 50% of children with permanent hearing loss, however, will not have associated stigmata. Every physical examination in childhood should include an evaluation for the presence of transient middle ear fluid. Once a child is diagnosed with a permanent hearing loss by an audiologist, additional consultation with ophthalmology, otolaryngology, genetics, and developmental behavioral specialists and a parent support group are recommended to ensure that both the hearing loss and associated diagnoses and challenges are addressed.[3]

Permanent Hearing Loss—The Challenge

Hearing loss is one of the most common congenital abnormalities, occurring in approximately 1.5 to 2 per 1000 newborns screened. Subgroups of infants with risk factors for hearing loss, especially those requiring neonatal intensive care unit (NICU) care are at increased risk of hearing loss.[4–7] Delayed identification of permanent hearing loss in infants is associated with significant delays in language development, literacy, and academic success.[8–11] Prior to newborn screening, children in the United States in the 1980s who were deaf or hard of hearing achieved reading comprehension skills at the third grade level at the time of graduation from high school (Deaf Children in America, 1986). A method of identification trialed in the 1980s was the high-risk register. Physicians would be informed by the birthing hospital of infants who were considered at high risk of hearing loss. However, approximately 50% of infants with a permanent hearing loss identified in the newborn period do not have a known risk factor for hearing loss.[12] Newborns were not routinely screened with technology for hearing loss at the time, because there was no effective physiologic method. This changed after a demonstration project[13] in Rhode Island funded by the Department of Education and Health and Human Services in 1990–1991, which demonstrated the feasibility of newborn hearing screening with a new objective physiologic technique, called oto-acoustic emissions (OAEs). This was followed in 1993 by an NIH consensus conference[14] ("NIH Consensus Statement. Early identification of hearing impairment in infants and young children") recommending universal newborn hearing screening. Data from CDC indicates that in 2018 more than 98% of newborns were screened in the United States (https://www.cdc.gov/ncbddd/hearingloss/2018-data/01-data-summary.html).

Hearing Disorders

A hearing loss is secondary to interference in the transmission of sound from the outer ear (pinna and ear canal) to the middle ear (tympanic membrane, ossicles, middle ear space and Eustachian tube opening), the inner ear (cochlea and vestibular system), the central auditory pathway (nerve pathways that transmit to the

TABLE 97.1 Anatomy of the Normal Hearing Pathway

Structure	Components	Function
Outer ear	Pinna/auricle, ear canal and the outer layer of the tympanic membrane	Sound waves travel through the air and are conducted through the ear canal to the tympanic membrane where vibrations enter the middle ear.
Middle ear	Three ossicles (malleus, incus, and stapes)	Vibrations enter the middle ear and are amplified and transmitted via the ossicles to the fluid within the cochlea (inner ear). Acoustic energy in air is converted to compression waves in fluid in the cochlea.
Inner ear	Cochlea Vestibular system	The cochlea converts sound pressure patterns into electrochemical impulses that are passed on to the auditory nerve. A part of the cochlea, the organ of Corti, consists of sensory epithelium and hair cells, which transform fluid waves to nerve signals that regulate balance.
Eighth cranial nerve Vestibulocochlear nerve contains both the auditory nerve and the vestibular nerve	Auditory nerve is also called the acoustic nerve or cochlear nerve	The auditory nerve carries auditory sensory information from the cochlea to the brainstem. The vestibular nerve transmits sensory information.
Brainstem	Superior olive, to inferior colliculus, to thalamus	Continued transmission of electrical energy on journey to temporal lobes
Temporal lobes	Superior temporal gyrus and transverse temporal gyrus (Heschl gyrus)	Sounds are processed and interpreted as language.

brainstem and cortex, and the auditory cortex (temporal lobes) where sound information is processed. There are anatomical or neural problems that can develop at any point in this pathway that may interfere with the transmission of sound energy and result in a change in the hearing threshold. There are three types of permanent hearing loss that may be present in the neonate including sensorineural hearing loss, auditory neuropathy (neural hearing loss), and permanent conductive hearing loss, in addition to transient conductive hearing loss (middle ear fluid or debris in the ear canal). Some infants may have a mixed hearing loss, which is a combination of two or more types of hearing loss. Types of permanent and transient hearing loss are shown in Table 97.2.

Degrees of hearing loss are determined using clinical audiograms, which evaluate the softest hearing level (HL) thresholds, in decibels (dB). Thresholds between –10 and +20 dB HL are considered to be in the normal range, while thresholds above 20 dB are considered diagnostic for mild (20 to 34 dB), moderate (35 to 49 dB), moderately severe (50 to 64 dB), severe (65 to 79 dB), and profound (>80 dB) hearing loss. Ear and frequency-specific behavioral response hearing testing using a visual reinforcement audiometry (VRA) protocol (conditioned response) can be accomplished beginning at approximately 5 to 6 months of age if this is consistent with the infant's development status. Behavioral testing using conditioned play audiometry can be used in children with developmental ages of 3 to 5 years. The official definition of deafness from the Individuals with Disabilities Education Act is "a hearing impairment that is so severe that the child is impaired in processing linguistic information through hearing, with or without amplification." *Hard of hearing* refers to a hearing loss where there may be enough residual hearing that an auditory device provides adequate assistance to process speech.

TABLE 97.2 Types of Hearing Loss

Type	Characteristics
Sensorineural	Pathology involving the eighth nerve, outer hair cells, and inner hair cells of the cochlea that impairs neuroconduction of sound energy to the brain stem
Permanent conductive	An anatomic obstruction of the outer (atresia) or middle ear (fusion of ossicles) that blocks transmission of sound
Neural or auditory neuropathy or auditory dyssynchrony	Pathology of the myelinated fibers of the eighth cranial nerve or the inner hair cells that impairs neuroconduction of sound energy to the brainstem. The function of the outer hair cells remains intact.
Transient conductive	Debris in the ear canal or fluid in the middle ear that blocks the passage of sound waves to the inner ear
Mixed hearing loss	A combination of sensorineural or neural hearing loss with transient or permanent conductive hearing loss

Methods for Newborn Hearing Screening

The current methods readily available for newborn screening do not independently differentiate permanent hearing loss from transient conductive hearing loss. Since the primary objective of newborn hearing screening is to identify permanent hearing loss, this limitation results in an ongoing challenge of false positive newborn screens, and at times, a delay in the diagnosis of a permanent hearing loss. There are currently two objective, non-invasive physiologic measures, OAEs, either transient or distortion product, and automated auditory brainstem response (AABR) that are available and reliable for screening newborns and young infants.[15–19]

There are important differences between the two measures as shown in Table 97.3. OAE measures a physiologic response from the cochlear outer hair cells, while AABR measurements reflect both cochlear status, as well as auditory neural function beyond the cochlea to the brainstem. Thus, the AABR response reflects

activity from a greater portion of the auditory pathway than OAE and will detect auditory neuropathy, whereas OAE screening will not. Therefore, because of the higher incidence of auditory neuropathy among NICU infants AABR is recommended for screening in the NICU.[3,20] Both methods will screen positive for sensorineural hearing loss and permanent conductive hearing loss. Permanent conductive hearing loss is caused by abnormalities of the ossicular chain or eustachian tube anatomy and is more often associated with unilateral hearing loss.[12] Relative to screen fail rates, AABR is more likely to miss borderline or mild hearing loss because of the higher threshold for a pass resulting.[21,22]

In addition, both methods screen false positive for permanent hearing loss in the presence of transient retained fluid in the ear canal or middle ear fluid with transient conductive hearing loss.[23] It is important to note that there is an increased incidence of transient middle ear fluid among high-risk infants cared for in the NICU.[24] Uncertainty also may arise with mixed hearing loss which is a combination of sensorineural or neural hearing loss in conjunction with transient conductive hearing loss. Therefore, although the methods continue to improve, limitations remain, supporting the need for ongoing surveillance in the medical home. Hearing loss missed by both methods may include mild hearing loss, hearing loss in an isolated frequency range, progressive hearing loss and late onset hearing loss.[16,25] A study[23] of a large longitudinal cohort in the United Kingdom of over 17,000 neonates born from 2013 to 2014 reported that 24% of infants who did not pass an TEOAE screen but passed an AABR screen were subsequently diagnosed with a hearing loss greater than 45 dB HL using diagnostic auditory brainstem response (ABR). Although current screen methods have limitations, tremendous headway has been made in lowering the age of diagnosis, and newborn hearing screening is recommended for all infants prior to discharge from the birthing hospital. In an effort to facilitate diagnosis of the highest risk in a timely fashion, a current recommendation is for NICU infants who do not pass their hearing screen to have a diagnostic ABR performed prior to discharge. This approach facilitates the current JCIH early hearing detection and intervention (EHDI) recommendation to screen by 1 month, diagnose by 3 months and provide intervention services by 6 months of age.[3,22]

TABLE 97.3 Comparison of Oto-Acoustic Emissions and Automated Auditory Brainstem Response Screens

	PHYSIOLOGIC SCREENING METHODS	
Characteristics	**OAE**	**AABR**
Measurement	Physiologic response of outer hair cells of cochlea	Response in cochlear and auditory neural function to brainstem
Detects sensorineural HL	Yes	Yes
Detects auditory neuropathy	No	Yes
Detects transient and permanent conductive HL	Yes	Yes
OAE threshold for fail	30–35 dB HL	
AABR threshold for fail		40–45 dB HL
Recommended for NICU screens	No	Yes
Recommended for well baby screens	Yes	Yes

AABR, Automated auditory brainstem response; *HL*, hearing level; *NICU*, neonatal intensive care unit; *OAE*, oto-acoustic emissions.

Risk Factors for Permanent Hearing Loss in Infants and Children

Although approximately 50% of infants with an identified hearing loss have a risk factor for hearing loss (Table 97.4), 50% do not. Therefore, ongoing surveillance and rescreening of all infants/toddlers is recommended with added diligence to those who pass the screen but have a risk factor. Most birthing hospitals currently report risk factors to the primary care provider. However, it may be incomplete since family history is often reported after discharge, and diagnosis of a congenital syndrome may occur postdischarge. Therefore a complete physical and comprehensive review of history of hearing loss with the family after discharge is recommended for all infants.

TABLE 97.4 Risk Factors for Permanent Hearing Loss

Family factors	1. Caregiver concern regarding hearing, speech, language, or developmental delay[30] 2. Family history of permanent childhood hearing loss[15,31,33]
Neonatal	3. Neonatal intensive care of more than 5 days or any of the following regardless of length of stay including ECMO[111] 4. In utero infections, such as CMV, herpes, rubella, syphilis, and toxoplasmosis[5,39,112]
Stigmata, syndromes, and neurodegenerative disorders	5. Craniofacial anomalies, including those that involve the pinna, ear canal, ear tags, ear pits, and temporal bone anomalies[43] 6. Physical findings, such as white forelock, that are associated with a syndrome known to include a sensorineural hearing loss or permanent conductive hearing loss[32,43,44] 7. Syndromes associated with hearing loss or progressive or late-onset hearing loss, such as neurofibromatosis, osteopetrosis, and Usher syndrome[113]; other frequently identified syndromes include Waardenburg, Alport, Pendred, and Jervell and Lange-Nielson[33,44,113] 8. Neurodegenerative disorders, such as Hunter syndrome, or sensory motor neuropathies, such as Friedreich ataxia and Charcot-Marie-Tooth syndrome[43]
Neonatal or post-neonatal	9. Culture-positive postnatal infections associated with bacterial and viral (especially herpes viruses and varicella) meningitis[53,91]
Post-neonatal	10. Head trauma, especially basal skull/temporal bone fracture that requires hospitalization[42,114] 11. Chemotherapy[52]

Identifying risk factors among all newborns and structured monitoring for late onset or progressive hearing loss allows early recognition of children who will not be identified through robust newborn hearing screening programs. As many as 1 in 5 children who are ultimately diagnosed with hearing loss may pass their hearing screening.[26,27] It is important to perform a complete family history and risk factor query in addition to a comprehensive physical examination. Having a monitoring system for risk factors, including developmental delays within an electronic health record can provide a reminder to the medical home provider to ensure earlier recognition of childhood hearing needs.

Table 97.4 shows the list of risk factors for permanent hearing loss. Although all infants require ongoing surveillance of auditory and speech language skills, infants who pass the newborn screen and have a risk factor will benefit from enhanced surveillance. Within the 2019 Joint Committee on Infant Hearing (JCIH) position statement, monitoring for hearing status noted specific intervals by risk factor. For many risk factors, completing an audiology evaluation with an audiologist experienced in assessing children by 9 months of age is recommended. Children who have been on ECMO and those with congenital cytomegalovirus (CMV) should have a hearing evaluation by 3 months of age. For children with culture positive infections (meningitis), ototoxic medications (such as chemotherapy) and head trauma should receive a hearing assessment no later than 3 months from the event. Most importantly, any time a family or caregiver has a concern about a child's hearing or speech/language development, referral for hearing testing should be immediate. It is important to remember that passing a newborn hearing screen does not protect a child from later onset hearing loss. Surveillance is especially important since it is well documented that the rate of identified childhood deafness and hearing loss increases from approximately 1.2/1000 in newborns to 3/1000 in early school age. In a 2015 report[28] the prevalence of children confirmed as deaf or hard of hearing by school age was 3.65 per 1000.[3,15,29–52]

Family

Risk factor 1 Parent or caregiver concern of child hearing, speech, language, or developmental delay has consistently been shown to be a reliable predictor of hearing loss.[30]

Risk factor 2 A history of family members who are deaf or hard of hearing with onset in childhood,[15,31–33] has high predictive value and is associated with known genetic causes including Connexin 26 mutations. It has been shown that young parents may not be informed of a family history until after their infant is diagnosed with hearing loss. Monitoring is based on etiology with a diagnostic evaluation recommended by 9 months of age.[34–36]

Neonatal

Risk factor 3 includes infants who require care in the NICU or special care nursery for more than 5 days, which is used as an indicator of illness severity.[3] This broad category encompasses most other medical risk factors including prematurity, perinatal asphyxia, hyperbilirubinemia requiring an exchange transfusion, perinatal asphyxia, regardless of length of stay, and aminoglycosides for 5 days or more.[37,38] Use of aminoglycosides for less than 5 days is not a risk factor. It is recommended that children in these categories of risk should have a diagnostic audiology evaluation by 9 months of age.

Risk factor 3 also includes infants who have required extracorporeal membrane oxygenation (ECMO). Children who have been on ECMO should have a diagnostic audiology evaluation no later than 3 months following ECMO and every 12 months to school age. If families have concerns about hearing, testing should occur at the time of concern.

Risk factor 4 includes in utero infections such as herpes, rubella, syphilis, and toxoplasmosis, which require audiological evaluation by 9 months of age. Due to a high rate of progressive loss in infants with congenital CMV, recommendations include an audiological evaluation by 3 months of age and yearly through 3 years of age. CMV is a common cause of nongenetic unilateral or bilateral early, progressive, and delayed-onset sensorineural hearing loss.[39–41] For infants with laboratory evidence of Zika, an AABR by 1 month of age is indicated.

Risk factors 5 and 6 Craniofacial anomalies, including those that involve the pinna, ear canal, ear tags, ear pits, and temporal bone anomalies,[42,43] and physical findings, such as cleft lip/palate, white forelock, or micro-ophthalmia can be indicators of a possible syndrome known to include a sensorineural hearing loss or permanent conductive hearing loss.

Risk factors 7 and 8 include syndromes associated with hearing loss or progressive or late-onset hearing loss, such as neurofibromatosis, osteopetrosis, and Usher syndrome;[53] other frequently identified syndromes include Waardenburg, Alport, Pendred, and Jervell and Lange-Nielson. There are more than 400 syndromes and genetic disorders associated with hearing loss.[31,35,45,46] Neurodegenerative disorders, such as Hunter syndrome, or sensory motor neuropathies, such as Friedreich ataxia and Charcot-Marie-Tooth syndrome, are strongly associated with permanent hearing loss.[53] Consultation with a geneticist is indicated for all infants diagnosed with congenital hearing loss for whom there is not a clear etiology.

Risk factors 9, 10, and 11 may occur in the neonatal period or postdischarge. Risk factor 9 includes culture-positive bacterial and viral (especially herpes viruses and varicella) postnatal infections (including meningitis) which may result in rapid onset hearing loss.[47–50] In these events, an audiological evaluation should be completed no later than 3 months from the onset of the illness. Although, widespread administration of conjugate vaccines has resulted in decreased rates of *Haemophilus influenza* type B infection, pneumonia, measles, mumps, rubella, and childhood meningitis, encephalitis viral infections especially herpes viruses and varicella remain a serious risk factor.[50] Children with cochlear implants remain at increased risk of postnatal infection.[51]

Risk factors 10 and 11. Head trauma, especially basal skull/temporal bone fractures that require hospitalization, as well as injury to the mastoid, is associated with hearing loss.[42] Chemotherapy[52] should also prompt early referrals for audiological assessment.

After Newborn Hearing Screening: Audiology Diagnostic Protocols

Since 1999, the American Academy of Pediatrics has endorsed early hearing detection and intervention (EHDI) defined as screening by 1 month, diagnosis by 3 months, and appropriate intervention services by 6 months of age. The recommended

audiology diagnostic assessment for infants who do not pass the newborn screen includes a battery of tests.[3]

1. Click-evoked ABR testing using both condensation and rarefaction single-polarity stimulus is recommended for all NICU graduates and, if there are risk indicators for neural hearing loss (auditory neuropathy), is considered the gold standard. Testing is done to quantify frequency-specific thresholds for air- and bone-conduction, and to determine the ear-specific type and degree of hearing loss.
2. OAE either distortion product or transient evoked emissions assess the integrity of the outer hair cells of the cochlea.
3. Tympanometry using a 1000-Hz probe tone is done to assess middle ear function.[24]
4. Acoustic reflexes are an important component of middle ear function. The acoustic reflex is absent in auditory neuropathy.
5. VRA is the gold standard for estimation of hearing thresholds and for confirmation of hearing level. It cannot be done until the infant reaches a developmental level of 7 to 9 months of age.
6. Clinician observation of the infant's auditory behavior may be used as a cross-check for the electrophysiologic measures. Behavioral observation alone is not adequate for determining whether hearing loss is present and is not adequate for the fitting of amplification devices.

Identification of an audiologist with skills in pediatric diagnostic assessment can be obtained from the roster of Pediatric Board–certified audiologists on the ABA website (https://www.boardofaudiology.org/) and the EHDI-PALS website (http://www.ehdipals.org/). The report from the audiologist will include the type of hearing loss, whether the loss is unilateral or bilateral, and the degree of hearing loss.

Medical Work-Up for Hearing Loss

A visit should be scheduled with the family as soon as a diagnosis of hearing loss is made by the audiologist to discuss the audiologist's report, provide information on community supports and intervention resources, and provide support for the family during a period of stress. During the visit with the family, the physician reviews the pregnancy, neonatal, and family history for hearing loss, and completes a comprehensive physical examination. The examination should focus on identifying findings which may suggest craniofacial abnormalities or a syndrome associated with hearing loss. An examination of the head, eyes, ears, nose and throat, and middle ear status are particularly important.

A thorough evaluation of middle ear status is important as the presence of fluid in the middle ear space can delay clarity regarding a child's hearing status.[27] It is not uncommon for newborns and NICU graduates to have middle ear effusion or retained amniotic fluid, which can cause transient conductive loss and a false positive screen. Persistence of middle ear fluid in the infant should be monitored and managed in the medical home as this can delay the definitive identification of a permanent hearing loss.[27] In cases of persistent middle ear fluid, myringotomy tube placement will be necessary in order to complete a valid diagnostic to rule out a concurrent permanent hearing loss. There is evidence that persistent middle ear fluid identified in the NICU is a risk factor for chronic otitis media with effusion in the first year of life.[54] Once the diagnosis of a permanent hearing loss is established it is necessary to discuss the benefits of early intervention services and amplification. The primary care physician needs to stay up to date and aware of community resources and support the family choice of early intervention program and approaches to communication development.

Multidisciplinary Care

For children identified with a permanent hearing loss, there are three medical specialties which are important for care and proactive identification of other conditions. These include an otolaryngologist with knowledge of pediatric hearing loss, a geneticist or genetic counselor, and an ophthalmologist with expertise in pediatric eye conditions.[22] The primary care provider facilitates referrals for these evaluations and can help explain to families why they are needed. The otolaryngologist conducts a comprehensive assessment to gain an understanding of etiologies of hearing loss, assesses risk for progression of hearing loss, evaluates factors which may impact an approach to management, and guides the identification of co-existing conditions,[55] and provides recommendations and information to the family, audiologist, and primary care provider. The pediatric otolaryngologist provides guidance on candidacy for amplification, assistive devices, and surgical intervention, including reconstruction, bone-anchored hearing aids, and cochlear implantation. The work-up may include genetic testing, evaluation for congenital cytomegalovirus (cCMV) infection, and imaging.

Genetics: Because of the prevalence of hereditary hearing loss, all families of children with confirmed hearing loss should be offered a genetics evaluation and counseling. This evaluation can provide families with information on etiology, prognosis, associated disorders, and recurrence in offspring. The geneticist will review a three-generation family history for specific genetic disorders or syndromes and complete genetic testing for syndromes or gene mutations for non-syndromic hearing loss such as *GJB2* (Connexin 26).[32,46,56,57] The field of genetics is rapidly evolving. While gene panels for hearing loss are commercially available, it is important for families to have accurate and up-to-date information.[58] The Hereditary Hearing Loss homepage (https://hereditaryhearingloss.org/) is a good resource for up-to-date information.

Imaging

The otolaryngologist will order radiographic imaging as appropriate. Temporal bone imaging is often indicated as part of the work-up to identify the etiology, any malformations that may need surgical intervention and appropriateness of amplification or cochlear implantation. The two modalities for imaging include high-resolution computed tomography (HRCT) and magnetic resonance imaging (MRI). These modalities have different benefits and considerations, such as visualization of bony structures (better with HRCT) and visualization of cranial nerves and soft tissue (MRI). The need for sedation and radiation exposure (HRCT) are also considerations.

Ophthalmology: It is recommended that each child with a permanent hearing loss have at least one examination by an ophthalmologist experienced in evaluating infants because of the association of hearing loss with vision impairments and the importance of vision for children with hearing loss. In some situations, an ophthalmologist may be the first to identify a finding associated with syndromic hearing loss.

Electrocardiogram: In children with profound sensorineural hearing loss without an identified etiology, it is appropriate to obtain an electrocardiogram to identify prolonged QT interval.[59]

This condition is associated with Jervell and Lange-Nielson syndrome and can result in sudden death.[59–61]

As 30% to 40% of children with confirmed hearing loss have developmental comorbidities, developmental milestones should be closely monitored and referrals initiated to developmental specialists for suspected disabilities as appropriate.

Communication Options

Early intervention providers including teachers of the deaf, the speech language pathologist and audiologist will provide information to the family on communication options consistent with the child's diagnosis. The family needs to decide on the communication mode that will work optimally for the child and family. There are five options described in Table 97.5: auditory oral communication (speech reading), auditory verbal communication, cued speech, total communication, and American Sign Language. Parents may initiate with total communication and change mode subsequently, depending on the child's progress with communication. For example, the family of a child with profound hearing loss may initially choose total communication but, after a cochlear implant at 12 months of age, may use predominantly auditory verbal or auditory oral communication.

Assistive Technologies

Hearing Aids: Infants as young as 1 month of age can be fitted with hearing aids. Hearing aids are compact and worn either in-the-ear (ITE) or behind-the-ear (BTE). Components are the microphone that picks up sounds and the amplifier. The audiologist uses computer programming to adjust the sound for an individual child's needs. If the child has different degrees of hearing loss at different frequencies, the audiologist adjusts the gain (loudness). The young growing infant needs to be seen by the audiologist approximately every 6 weeks to replace outgrown molds. Regular surveillance of hearing status is important to ensure that amplification is appropriately fitted and programmed as both progression and fluctuation of thresholds may occur.

TABLE 97.5 Characteristics of Modes of Communication

Mode	Mechanism	Language Goal
Auditory oral communication	The use of residual hearing and amplification with visual support (speech reading)	Spoken
Auditory verbal communication	Based on listening skills alone	Spoken
Cued speech	Uses visual communication and combines listening with 8 hand shapes in 4 placements near the face	Spoken
Total communication	Combines all means of communication and encourages simultaneous use of speech and sign	Spoken and sign
American Sign Language (ASL)	Visual and manual	ASL, English learned as a second language

Frequency modulated (FM) systems: FM systems were developed to enhance hearing in noisy environments. An FM system consists of a microphone and a receiver. A small radio transmitter is attached to a microphone and a small radio receiver. A parent, early intervention provider, or teacher wears the FM transmitter and microphone while the infant/child wears the FM receiver. The FM transmitter sends a low-power radio signal to the FM receiver that needs to be within 50 feet of the transmitter. The FM receiver gets the signal from the microphone and sends it to a personal hearing aid or cochlear implant. Listening to the FM signal is similar to listening to speech only inches away. Systems can be used in the home, while shopping, or at school.

Cortical Auditory Evoked Potential (CAEP): This is a relatively new assessment utilized in hearing aid fitting. CAEPs, are reported to confirm audibility of speech sounds at the cortical level.[62]

Cochlear Implants: Cochlear implants are FDA approved for children 12 months and older with severe to profound sensorineural hearing loss. As of December 2019, roughly 118,100 devices have been implanted in adults and 65,000 in children in the United States (https://www.nidcd.nih.gov/health/cochlear-implants). It is standard practice to recommend that children be adequately fit with hearing aids for 3 to 6 months before determining implant candidacy. A lack of benefit in the development of auditory skills with amplification with hearing aids needs to be demonstrated for eligibility for an implant. In 2019, the FDA expanded approval of cochlear implants to single-sided deafness (SSD) and asymmetric hearing loss. It is recognized that when children receive a cochlear implant at early ages, they have a higher likelihood of better speech and language development.[63] Early development of speech and language skills following cochlear implantation support sustained benefits in language into adolescence and beyond.[64]

Because of an increased risk for bacterial meningitis, it is recommended that physicians monitor all patients with cochlear implants.[65–67] *Streptococcus pneumoniae* is the most common pathogen causing meningitis in cochlear implant recipients.[51,68–70] All children with cochlear implants should be vaccinated according to the American Academy of Pediatrics high-risk schedule. Preferably, children under the age of 2 will have received the PCV13 at usual childhood interval. Children who have a cochlear implant should receive a Pneumovax-23 valent immunization after the age of 2 years (meningococcal cochlear implants and vaccination recommendations: information: https://www.cdc.gov/vaccines/vpd/mening/public/dis-cochlear-faq-gen.html).

Comprehensive Early Intervention

All infants diagnosed with a permanent hearing loss should be referred and enrolled in early intervention by 6 months of age to minimize to deleterious effects of communication deprivation.[71–74] The JCIH[75] recommends that states should have a single point of entry into intervention specific for children with hearing loss to ensure that, regardless of geographic location, all families who have infants or children with hearing loss receive information about a full range of options regarding amplification and technology, communication and intervention, and accessing appropriate counseling services.[3,75] The JCIH[75] published 12 best practice guidelines for EI programs that include the following:

- The provision of timely referral to EI services with providers who have knowledge and skills in early childhood deafness and hearing loss.

- Infusion within the system of partnerships with parents as well as professionals who are deaf/hard of hearing.
- Longitudinal developmental assessments for monitoring the child's development.
- Data management systems that include developmental outcomes.
- A process to monitor the fidelity of the intervention.
- Appropriate services for children with additional disabilities, those from non-English-speaking families, and those from special populations, including unilateral hearing loss and auditory neuropathy/dyssynchrony.

As the number of bilingual children with hearing loss continues to increase, the question has arisen regarding the most optimal way for early intervention providers to teach speech to bilingual children with HL. A number of investigators have reported that children with hearing loss supported in both their home language and the majority culture language compared to children with HL exposed to the majority culture language alone acquired English and their home language at similar rates.[76,77] Two studies[78,79] investigated the effects of supporting both the home language (Spanish) and the language of the majority culture (English) on language outcomes in bilingual children with HL who use CIs and HAs compared to bilingual peers who received English-only support. Bilingual children who received dual-language support outperformed peers who received English-only support as measured by Total Language and Expressive Communication language age scores of the Preschool Language Scales-4. Because of the high incidence of hearing loss among Hispanics in the US, it is likely there will be an increased need for provision of dual language Early Intervention services.[80]

In addition, a total communication approach including ASL and speech is recommended at the initiation of early intervention services to ensure access to language. An expert opinion summary proposes consideration of use of both sign and spoken language for all young children who are deaf to ensure access to language and linguistic competence. The report[81] reviews findings demonstrating that sign language development is associated with both written and spoken language development.[82,83]

Neonatal Intensive Care Unit Infants, Risk Factors, and Hearing Loss

The majority of infants cared for in an NICU have a risk factor for hearing loss. The relationship between risk factors and hearing loss in several studies of NICU infants is reviewed, and summaries

TABLE 97.6 Risk Factors and Rates of Hearing Loss Among Infants Cared for in a Neonatal Intensive Care Unit

Author	Dates of Birth	Sample	Screen Diagnostic	Rates of HL	Risk Factors
Coenraad et al., 2010[115]	2004–2009	3366 NICU infants		Screen refer 3%; diagnosed 1.7%; 17.2/1000	Dysmorphism, low 1 min Apgar, sepsis, meningitis, cerebral bleed and infarction
Schmidt et al., 2015[86]	1996–1998 at 36 centers assessed effects of brain injury, BPD and severe ROP	500–1250 g followed at 5 years	Primarily AABR and Sound Field	HL 2%	↑ Rate of HL by # morbidities None 1.3% 1 risk factor 3.6% 2 risk factors 5.4% 3 risk factors 25.0%
Van Dommelen et al., 2015[87]	1998–2012	18,564 preterms Dutch NICU	Two-stage AABR followed by diagnosis at 2–4 m	24.0–24.9 wk, 7.5% 25.0–25.9 wk, 5.2% 26.0–26.9 wk, 4.6% 27.0–27.9 wk, 2.8% 28.0–28.9 wk, 2.0% 29.0–29.9 wk, 1.6% 30.0–30.9 wk, 2.0% 31.0–31.9 wk, 1.2%	Analysis: Risk increases with decreasing gestation; small for gestation infants <27 weeks also at increased risk of HL
Kraft et al., 2014[116]	2001–2007	26,341 infants, 25,400 with completed screen	Screen not described; postdischarge, ABR, OAE, and tympanometry	Refer rate 1.3% 90 infants diagnosed including 16 with delayed onset	All JCIH risk indicators significant; however, ototoxic medications and >5 days in NICU provide small contribution; authors report congenital diaphragmatic hernia NS. Increased risk with increased number of risk factors
Goderis, 2014[117]		Review of 37 studies: 10 population, 14 longitudinal, 13 retrospective	1/3 symptomatic and 1/10 asymptomatic with HL	12.6% HL (CI: 10.2–16.5)	Delayed onset HL of 0%–50%. Calculated risk of 18% in symptomatic group, 9% in asymptomatic group

AABR, Automated auditory brainstem response; *BPD*, bronchopulmonary dysplasia; *CMV*, cytomegalovirus; *HL*, hearing level; *JCIH*, Joint Committee on Infant Hearing; *NICU*, neonatal intensive care unit; *OAE*, oto-acoustic emissions; *ROP*, retinopathy of prematurity.

are shown in Table 97.6. A study by Robertson et al.[84] included a cohort of 1279 infants weighing 1250 g and below or at 28 weeks' gestation screened with AABR followed by audiology diagnostic assessment and followed to 5 years of age. The rate of permanent hearing loss was 3.1%, rate of severe to profound was 1.9%, 28% had progressive hearing loss, and 73% had other disabilities. Risk factors included prolonged oxygen use, gastrointestinal surgery, patent ductus arteriosus ligation, and low socioeconomic status (SES).

Schmidt et al.[85] published two studies examining the effects of the major neonatal morbidities of bronchopulmonary dysplasia (BPD), major brain injury, and severe retinopathy of prematurity (ROP) on rates of hearing loss at 18 months in very low-birth-weight infants weighing less than 1250 g. Rates of hearing loss compared with not having the risk factor were 3.5% versus 1.4% for BPD, 2.9% versus 2.2% for brain injury, and 3.8% versus 2.2% for severe ROP. In the second study, children were followed to 5 years of age.[86] Overall, 2% of the 1514 infants weighing between 500 and 1250 g were described as deaf. Rates of deafness were related to the risk factors of BPD, serious brain injury, and severe ROP. In this report, the investigators identified a relationship of rate of hearing loss with an increasing number of risk factors (no risk factors: 1.3%, one: 3.6%, two: 5.4%, and three: 25%).

Van Dommelen et al.[87] reported on the relationship between gestational age and hearing loss in a large cohort of 18,564 preterm infants. The prevalence of hearing loss consistently increased with decreasing week of gestation from 1.25% at 31 weeks to 7.55% at 24 weeks. A study by Morris et al.[88] examined the effects of two protocols to provide phototherapy to very preterm infants with hyperbilirubinemia. Compared with the conservative-phototherapy group, the aggressive-phototherapy group had significant reductions in the rate of severe hearing loss, from 3% to 1%. The conclusion was that the benefit to decreased impairment may be offset by the increased mortality in the smallest infants. All of the studies indicate that there are increased rates of hearing loss among NICU infants who are the most preterm and have the highest rates of neonatal morbidities. Using a readily identifiable event (such as NICU stay longer than 5 days), allows clinicians and monitoring systems to flag and follow an individual child, thus simplifying the number of factors required to identify infants at risk of hearing loss. Among NICU cohorts described in the literature, these children have a higher prevalence rate of hearing loss than the general population, with reports as high as 7.5/1000 for preterm infants born at 24 weeks' gestation in the Netherlands (see Table 97.6). Various studies have controlled for risk factors to try to refine our understanding of individual risk. Factors which have maintained statistical significance when controlling for various other factors include dysmorphic features, low APGAR scores, sepsis, cerebral injury (bleeding/infarction),[89,90] severe asphyxia,[91,92] ventilation ≥5 days,[91] and prolonged oxygen use ECMO.[49,93]

The Brain, Language Outcomes, and Access to Language

The most important predictor of improved language outcomes is early age of identification. Age of identification reported in the 1980s and 1990s ranged from 19 months to 31–35 months of age.[94,95] Since 1997, age of identification in some reports has dropped to 2 to 4 months of age.[16,96] The National CDC EHDI data for 2013 indicate 69% of newborns who did not pass the newborn screen are diagnosed with a permanent hearing loss by 3 months of age. This rate may in part be due to incomplete reporting but indicates some parts of the country continue to struggle in achieving the EHDI 1-3-6 goal (https://www.cdc.gov/ncbddd/hearingloss/2013-data/2013_ehdi_hsfs_summary_e.pdf). Early identification remains a critical EHDI goal as earlier age is consistently associated with improved outcomes. Yoshinaga-Itano et al.[8,97] have shown that early quality intervention services in the first year of life which provide access to language (either speech or visual [ASL]) improve the language and developmental outcomes for children who are deaf or hard of hearing. The longitudinal study by Moeller[74] showed, in adjusted analyses, that enrollment in early intervention services and greater family involvement predicted higher vocabulary scores on the Peabody Picture Vocabulary test at 5 years of age among DHH children in Nebraska.[74] The 2018 CDC report indicates that 70.1% of infants with a diagnosed permanent HL are receiving EI services by 6 months of age (https://www.cdc.gov/ncbddd/hearingloss/2018-data/01-data-summary.html).

The first 3 years after birth are particularly critical for access to language since this period is a time of both rapid language and rapid brain growth. In addition, it is not the ears, but the brain where language is processed and understood. During the first 3 years, the brain grows and matures in complexity and connectivity at an incredible rate which is, in part, mediated by the infant's exposure to language. Sharma et al.[98,99] have shown that earlier implantation of a cochlear implant is also associated with more optimal development of the auditory pathway to the auditory cortex (temporal lobes). Children who are deaf and born to deaf parents have natural early exposure to visual language (ASL). However, more than 90% of children born DHH are born to hearing parents and do not have immediate access to either visual language (ASL) or auditory language (speech). Therefore, early access to language and early intervention that meets the communication needs of the family is the key. The studies of Hart and Risley[100] have shown that the amount and quality of language that all children are exposed to in the home in the first 3 years of life varies significantly, and the greater the input of child directed quality language, the more optimal the child's language and cognitive outcomes. Further evidence of the importance of language access comes from the studies of the neuronal maturation of the central auditory pathway.[98,99] In the absence of typical auditory stimulation the auditory pathway and cortex remain maximally sensitive and plastic for development for about 3.5 years in response to placement of a cochlear implant. From 3.5 to 7 years response to a cochlear implant drops to 7% to 30% and after 7 years to less than 5%. These data identify the window of opportunity and plasticity for development of the auditory pathway and speech for a child with a severe to profound HL is the first 3.5 years of life. In addition, MRI studies suggest that cross-modal reorganization of the auditory cortex occurs in DHH children without auditory stimulation, in response to visual and somatosensory stimulation. It is suggested that this modification in brain development may facilitate enhanced processing of skills in these domains for DHH.[98] Evidence of very early impact has been shown in infants who are DHH exposed to visual language who develop sign babbling at a rate that hearing infants develop verbal babbling.[101] A study by Tomblin et al.[102] of the usage characteristics of amplification identified that a combination of factors including earlier age of fitting, higher daily duration of amplification, and greater audibility of the device were all significantly associated with higher early language skills in a cohort of children with mild to severe hearing loss. Another important finding, however,

was that children fit with hearing aids after 18 months of age were able to improve their language abilities as a function of increased duration of hearing aid use. This finding again suggests that brain plasticity and the language learning system remain open to experience provided by increased access to linguistic input during the preschool years. Data from Tomblin et al.[102] also indicate that the development of language for children with a more severe HL is more challenging than children with a mild loss. Within a cohort of children divided into mild (25 to 45 dB HL), moderate (45 to 60 dB HL), and moderate to severe HL (>60 dB HL) compared to age- and SES-matched controls, children with moderate to severe HL consistently had the lowest scores at 2 to 5 years of age. For all of the HL groups, greater degree of HL was associated with lower language abilities. Additional factors that have been shown to contribute to language success in children include enhanced maternal communicative intent,[103] higher SES, higher parent education level,[100] less parental stress,[104] a more optimal home language environment,[105] and access to deaf role models.[75]

The Walker et al.[106] study clearly demonstrated the importance of wearing hearing aids among 5- to 7-year-old children with slight or mild hearing loss (≤25 dB HL). Children were divided into three groups of hearing aid use including none, part-time (2 to 8.3 hours) and full-time (>8.7 hours). Full-time users had higher Peabody receptive vocabulary scores and CELF grammar scores after adjusting for age of diagnosis, best ear speech intelligibility, and early intervention. In addition, the audibility of hearing aids has been shown to moderate school-age academic outcomes of children with all degrees of HL.[107] Finally, an important language skill is pragmatic language which forms the foundation for conversation competence. This is a particularly challenging skill for children who are deaf or hard of hearing.

In a recent report,[108] the pragmatic language of children with bilateral HL was followed longitudinally to 7 years of age. In a multivariable analysis, meeting the 1/3/6 EHDI guidelines, greater quantity of parent-child directed talk during a 25-minute recorded session at a mean age of 32.2 months, higher nonverbal intelligence, lesser degree of HL and higher maternal education were significantly associated with higher levels of pragmatic language ability at age 7. Increased parent-to-child-directed talk has also been reported in a number of studies to be associated with improved language outcomes.[109]

In summary, EHDI systems of care continue to improve access to timely screening, diagnosis, and early intervention services to ensure successful outcomes. It is expected that ongoing surveillance and facilitation of individualized coordinated care with the use of the electronic medical record, multidisciplinary partnering, implementation of both local and telemedicine services,[110] and coordination with state EHDI programs will continue to contribute to improved hearing outcomes.

Suggested Readings

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